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RESEARCHES ON FUNGI  
VOLUME V



RESEARCHES  
ON FUNGI

VOLUME V

HYPHAL FUSIONS AND PROTOPLASMIC STREAM-  
ING IN THE HIGHER FUNGI, TOGETHER WITH AN  
ACCOUNT OF THE PRODUCTION AND LIBERATION  
OF SPORES IN SPOROBOLOMYCES, TILLETIA, AND  
SPHAEROBOLUS

BY

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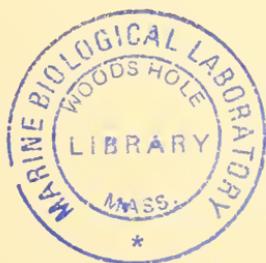
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WITH ONE HUNDRED AND SEVENTY-FOUR FIGURES IN THE TEXT



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TO

*E. C. STAKMAN*

A DISTINGUISHED PHYTOPATHOLOGIST,  
AN INSPIRING TEACHER, AND  
A VALUED FRIEND



## PREFACE

IN Volume IV of this work it was shown that a diploid mycelium  $(Ab)+(aB)$  of *Coprinus lagopus* will not only diploidise a haploid mycelium  $(aB)$  or  $(Ab)$  in what was called a *legitimate* combination, but will also diploidise a haploid mycelium  $(AB)$  or  $(ab)$  in what was called an *illegitimate* combination. How diploidisation in an illegitimate combination of a diploid and a haploid mycelium is accomplished I was unable to explain. Recently, however, in a review of Volume IV, Felix Rawitscher<sup>1</sup> has suggested what seems to be a very simple solution of the problem. According to him, during the diploidisation process in both of the illegitimate combinations  $(AB) \times (Ab)+(aB)$  and  $(ab) \times (Ab)+(aB)$  the haploid mycelium receives from the diploid mycelium both  $(Ab)$  nuclei and  $(aB)$  nuclei. These nuclei pass out of the diploid mycelium into the haploid mycelium, divide there conjugately, displace the  $(AB)$  or  $(ab)$  nuclei of the  $(AB)$  or  $(ab)$  mycelium, and take possession of the growing hyphae. Later on, when the transformed  $(AB)$  or  $(ab)$  mycelium produces a fruit-body, in each young basidium an  $(Ab)$  nucleus fuses with an  $(aB)$  nucleus and thus makes it possible for the fruit-body to produce (as was found by me) all the four kinds of spores :  $(AB)$ ,  $(ab)$ ,  $(Ab)$ , and  $(aB)$ .

Another explanation of illegitimate diploidisation has just been offered by Quintanilha.<sup>2</sup> He has suggested that, when a haploid mycelium  $(Ab)$  is mated with a diploid mycelium  $(AB)+(ab)$ , under the influence of an  $(Ab)$  nucleus the two nuclei of a conjugate pair  $(AB)+(ab)$ , whilst dividing parallel to, and close to, one another,

<sup>1</sup> F. Rawitscher, *Zeitschrift für Botanik*, 1933, p. 136.

<sup>2</sup> A. Quintanilha, "Le problème de la sexualité chez les Champignons. Recherches sur le genre *Coprinus*," *Bol. da Soc. Brot.*, II sér., Vol. VIII, Coimbra.

exchange chromosomes, so that the daughter nuclei at the poles remote from the (*Ab*) nucleus have the constitution (*AB*)+(ab) and the daughter nuclei at the poles near to the (*Ab*) nucleus have the constitution (*Ab*)+(a*B*). The (a*B*) nucleus which has thus come into existence thereupon begins to diploidise the (*Ab*) mycelium. This theory, as Quintanilha has pointed out, would serve to explain not only the phenomenon observed by myself but also an allied phenomenon observed by Brunswik. However, it does not seem to be so simple as the theory of Rawitscher, nor does it account for the "patchiness" of the diploid mycelium resulting from illegitimate diploidisation.

Part I of this volume treats of the structure and physiology of the mycelium of the Higher Fungi, and it may therefore be considered as a continuation of Part II of Volume IV.

The first Chapter of Part I is devoted to the manner in which hyphal fusions are made. Four types of hyphal fusions have been recognised, and an appropriate terminology for them has been introduced. New light has been thrown upon the mode of formation of clamp-connexions.

The second Chapter of Part I is concerned with the translocation of protoplasm through the septate mycelium of certain Pyrenomycetes, Discomycetes, and Hymenomycetes and, in addition, it contains a number of new observations on protoplasmic streaming in the Mucorineae. The existence of a central pore in each septum of the mycelium of an Ascomycete, a Basidiomycete, or a Fungus Imperfectus was established by Wahrlich in 1893, but his work has been very generally overlooked or forgotten. On this account a number of his illustrations showing pores in septa have been reproduced. The author has been able to convince himself that in many of the Higher Fungi the pore in each cross-wall is open and that protoplasm flows freely through it from cell to cell. The formation of pore-plugs and the healing of wounds in a mycelium have been described and illustrated.

Part II is devoted to studies in the mode of reproduction of certain non-hymenomycetous Basidiomycetes, namely, *Sporobolomyces*, *Tilletia*, and *Sphaerobolus*.

The first Chapter of Part II treats of *Sporobolomyces roseus* and,

on the basis of the mode of spore-production and spore-discharge, an attempt has been made to show that *Sporobolomyces* is to be regarded as a basidiomycetous Yeast-genus.

The second Chapter of Part II, of which A. H. R. Buller and T. C. Vanterpool are joint authors, gives an account of the production and violent discharge of the so-called secondary conidia of *Tilletia tritici*. The authors regard these secondary conidia as the true basidiospores which correspond to the similarly constructed and similarly discharged basidiospores of the Hymenomycetes and the Uredineae.

In the final Chapter of Part II, the structure and mechanism of the *Sphaerobolus stellatus* gun has been redescribed in detail. Attention has been called to the fact that *Sphaerobolus* is coprophilous as well as xylophilous, and the mode of dissemination of the fungus has been discussed.

This volume contains one hundred and seventy-four illustrations in the text, including one hundred and thirty drawings and forty-four photographs. Thirty-three 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 or T. C. Vanterpool. For copying the drawings reproduced in Figs. 21 and 23 my thanks are due to Dr. Nellie Carter. The source of each borrowed illustration is acknowledged in the text.

Of the forty-four photographs in the text twenty-six were made under my direction, and the others were kindly contributed by friends and correspondents: one by Somerville Hastings, two by H. T. Güssow, four by I. L. Conners, ten by Leva B. Walker, and one by the Manitoba Department of Agriculture.

My best thanks are due to the Canadian National Research Council for grants in aid of the work. These grants have enabled me to employ in succession three research assistants, Miss Ruth Macrae, M.Sc. (McGill), Miss Eleanor S. Dowding, Ph.D. (Manitoba), and C. C. Neufeld. The investigations on *Sporobolomyces* and *Sphaerobolus* were accomplished in conjunction with Miss Macrae, those on hyphal fusions and protoplasmic streaming in the Higher Fungi in conjunction with Dr. Dowding, and those on protoplasmic streaming in *Rhizopus nigricans* in conjunction with C. C. Neufeld.

I here desire to express my indebtedness to these helpers for their valuable services. My colleague, J. N. Finlayson, Professor of Civil Engineering, kindly came to my aid in making the calculations concerned with the kinetics of the Sphaerobolus gun. Once again Mr. W. B. Grove, M.A., has been good enough to give me the benefit of his assistance in reading the proofs.

A. H. REGINALD BULLER.

Kew, *October 31, 1933.*

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PART I

HYPHAL FUSIONS AND PROTOPLASMIC STREAMING  
IN THE HIGHER FUNGI



# RESEARCHES ON FUNGI

## CHAPTER I

### THE FORMATION OF HYPHAL FUSIONS IN THE MYCELIUM OF THE HIGHER FUNGI

Introduction—Investigations by the Author—Methods and Materials—A Generalisation on Hyphal Fusions—The Four Kinds of Hyphal Fusions—Hypha-to-Hypha Fusions—Hypha-to-Peg Fusions—Peg-to-Peg Fusions—Hook-to-Peg or Clamp-connexion Fusions—The Function of a Clamp-connexion—Clamp-connexions and the Hooks of Ascogenous Hyphae—Biological Significance of the Hook of a Clamp-connexion growing Backwards instead of Forwards—Mlle Bensaude's Second Mode of Formation of a Clamp-connexion unconfirmed—*Pleurogaster curvicolle*—*Pleurogaster anserina*—*Fimietaria fimicola*—*Pyronema confluens*—*Coprinus sterquilinus*—*Coprinus lagopus*—*Sphaerobolus stellatus*—*Microsporon audouini*—Critical Remarks on Supposed End-to-Side and Side-to-Side Fusions—Action at a Distance in Vegetative Hyphal Fusions and its Theoretical Explanation—Passage of Nuclei through Hyphal Fusions.

**Introduction.**—It is well known that, in many species of Ascomycetes, Basidiomycetes, and Fungi Imperfecti, the mycelium, owing to the formation of numerous anastomoses between neighbouring hyphae, becomes converted in an early state of its development into a three-dimensional network.<sup>1</sup> In Volume IV of this work it was shown that these anastomoses—there called *hyphal fusions*—are of considerable functional importance. Thus, in the Hymenomycetes, hyphal anastomoses make possible sexual co-operation, facilitate the passage of nuclei through a haploid mycelium whilst it is becoming diploidised, aid the flow of food materials to fruit-bodies and sclerotia whilst these are developing, diminish the deleterious effects of small wounds in a mycelium and, in any one

<sup>1</sup> Cf. these *Researches*, Vol. IV, 1931, pp. 152–180.

species, make possible the co-operation of more or less numerous monosporous mycelia in the formation of one or more fruit-bodies.<sup>1</sup> Since Volume IV was written, the author has realised that there are two other special functions associated with hyphal fusions: (1), in *Arthrobotrys oligospora*, the formation of numerous mycelial loops which function in the trapping and killing of the larvae of Nematode worms<sup>2</sup>; and (2), in *Pyronema confluens* and other Discomycetes, the repair of wounds made by the killing of one or more cells in the series of cells making up a long hypha. The repair of these wounds will be treated of in Chapter II.

Hyphal fusions occur not only in the mycelia but also in the fruit-bodies of the Higher Fungi, e.g. in the very young fruit-body rudiments of *Pleuroge anserina* and in the pileus-flesh of *Marasmius oreades*. In the larger fruit-bodies of the Hymenomycetes, the hyphal fusions which there develop no doubt: (1) aid the flow of nutrient substances to the growing hyphae and spores during fruit-body development; (2) connect the living hyphae together so that these may the more readily serve as a unit in the development of a fruit-body as a whole; and (3) increase the mechanical stability of the fruit-body flesh.

The pileus-flesh of *Marasmius oreades* and of many other species of Agaricaceae is made up of a three-dimensional network of hyphae in which, collectively, the air-spaces between the hyphae are much greater in volume than the hyphae themselves. If one cuts through the pileus of a Fairy-Ring fungus and looks at the cut surface, one sees that it is white. This whiteness, like that of snow and of white petals, is due indirectly to the presence of air-spaces between the units of structure—here the hyphae. If one places a drop of water on the cut surface of the pileus, the water is at once absorbed: by capillarity it is drawn into the spaces between the hyphae. Notwithstanding its great porosity, the pileus-flesh of *M. oreades* is decidedly tough. This toughness is due in large measure to the existence of numerous hyphal fusions between adjacent hyphae, which fusions during their formation served to convert the hyphae as a whole into a fine-meshed coherent tissue.

<sup>1</sup> These *Researches*, Vol. IV, 1931, pp. 181-184.

<sup>2</sup> Cf. W. Zopf, *Die Pilze*, Breslau, 1890, pp. 17-18.

It is important to distinguish between (1) a hyphal contact, (2) a hyphal adhesion, and (3) a hyphal fusion or hyphal anastomosis. A *hyphal contact* may be said to exist where two hyphae come into contact with one another but do not adhere to one another, so that they can be readily separated. In a *hyphal adhesion* two hyphae come into contact with one another, end-to-end, end-to-side, or side-to-side, and adhere to one another without fusing; while, in a *hyphal fusion*, two hyphae come into contact end-to-end, adhere to and flatten out against one another, and then the double partition-wall breaks down and disappears, so that the two masses of protoplasm become confluent.

Fusions or anastomoses may be divided into (1) vegetative and (2) sexual. *Vegetative fusions* are those fusions which promote vegetative processes, such as the conduction of food materials, the conveyance of stimuli, etc., while *sexual fusions* are those which assist the coming together of nuclei destined to co-operate in a sexual process. Typical vegetative fusions are those which occur between ordinary hyphae in most mycelia, while typical sexual fusions are those which take place between two morphologically distinguishable sexual organs, e.g. the antheridium and the trichogyne of the oogonium in *Pyronema confluens*.

In the Hymenomycetes, e.g. *Coprinus lagopus*, when two complementary haploid mycelia, such as (*AB*) and (*ab*), are mated on a dung-agar plate, many hyphal fusions take place between the two mycelia. Doubtless some of the passage-ways thus formed are used for the exchange of nuclei during the initiation of the diploidisation process, while others are not. In this case it is a matter of chance which of the fusions are strictly vegetative and which assist the sexual process, and the attempt to distinguish sharply between vegetative and sexual fusions here breaks down.

In the Mucorineae, the parasitic species attack their relatives in various ways. Thus *Syncephalis nodosa* bores into and produces a mycelium within the sporangiophores of *Pilobolus longipes*; *Piptocephalis Freseniana* attacks the hyphae of *Mucor Mucedo* by means of fine haustoria sent out in groups from appressoria; while *Parasitella simplex* and *Chaetocladium Brefeldii* form hyphal fusions

with *Absidia glauca*, *Mucor hiemalis*, etc.<sup>1</sup> These fusions (Figs. 5 and 6, pp. 13 and 14), which bring the protoplasm of the parasite and host into continuity with one another, are used by the parasite for abstracting nutriment from the host and are therefore *vegetative* in function. However, if we wish to distinguish them from ordinary vegetative fusions such as occur in the mycelium of most of the Higher Fungi, we may refer to them as *parasitic fusions*.

In a mycelium of one of the Higher Fungi, e.g. *Coprinus lagopus* or *Pyronema confluens*, growing in a dung-agar medium, the individual hyphae are not at all times in the right physiological condition for fusion. Young hyphae growing rapidly at the periphery of a mycelium often grow close together or cross one another, and yet they show no tendency to fuse with one another. Fusion first sets in in the older parts of a mycelium where the culture medium is becoming exhausted and, in general, the process is promoted by conditions of starvation.

In *Pleuroge curvicolla* and other Pyrenomycetes, where two hyphae run near to one another and more or less parallel to one another for some distance, bridging hyphae are usually formed between them at intervals along their length, so that they become converted into a scalariform structure (cf. Fig. 1). Any one hypha may fuse with two or more neighbouring hyphae by means of such bridges. The nearer the parallel hyphae are together, the more numerous, as a rule, are the bridges which come to connect them.

The formation of one hyphal fusion at a particular place in a mycelium appears to prevent the formation of other hyphal fusions close by; and, when a certain density of hyphal fusions per unit of mycelium has been attained (cf. Fig. 1), no more hyphal fusions are formed. As will be shown in Chapter II, there is protoplasmic continuity between all the cells in a mycelium of a Higher Fungus or a Fungus Imperfectus. Such a mycelium, however much branched, is therefore a morphological and physiological unit, and as such it should be thought of in connexion with the formation of hyphal fusions. The mycelium as a whole strives, as it were, to become a three-dimensional network, but a network of not too fine

<sup>1</sup> For more details of the parasitism of *Parasitella* and *Chaetocladium*, as determined by Burgeff, *vide infra*.

a mesh ; and such a structural condition is attained by the formation of a certain number of hyphal fusions, but not too many. The actual

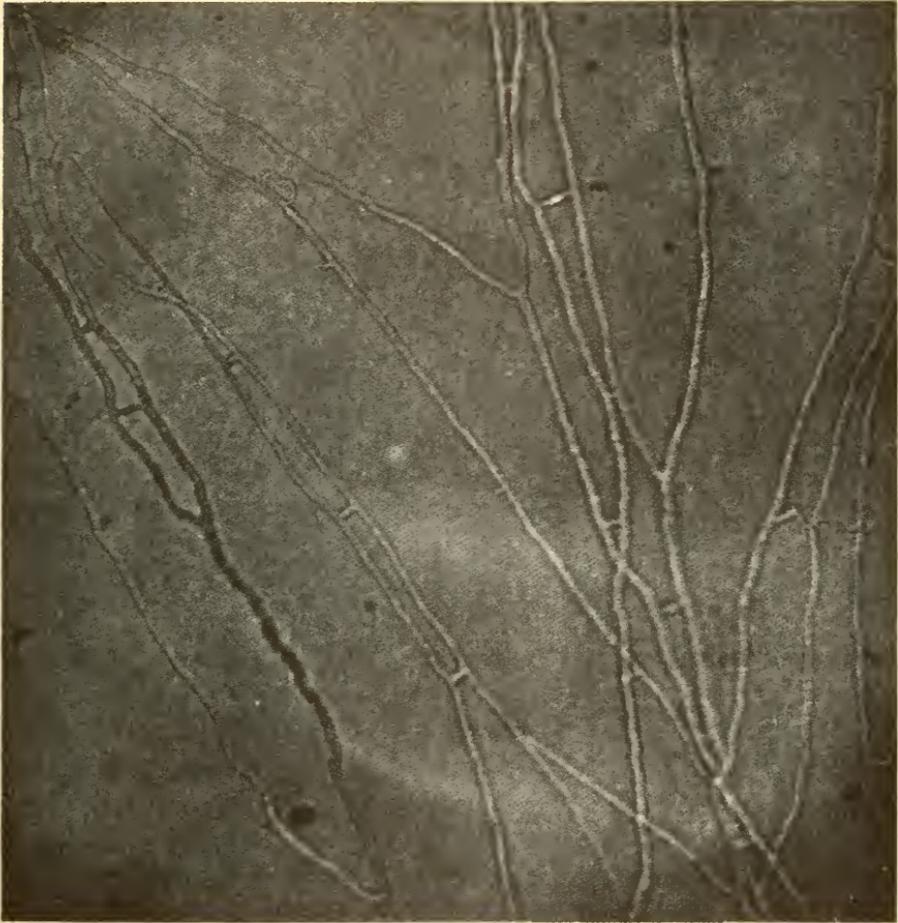


FIG. 1.—*Pleuroge curvicolli*. Photomicrograph of a mycelium produced from spores sown in a hanging drop of cleared dung-agar on the previous day. To show bridging hyphae, formed by peg-to-peg fusions, connecting the ordinary hyphae into a network. The number of hyphal fusions in the field of the photograph has about reached its maximum. The scalariform structure formed when two parallel hyphae become united at intervals along their length by several bridging hyphae is well shown on the left. Magnification, 600.

formation of hyphal fusions seems to alter the physiological condition of a mycelium in such a way that the mycelium in the end ceases to produce hyphal fusions.

It is characteristic of both vegetative and sexual fusions that hyphae or sexual organs destined to fuse act upon one another *at a distance*.

Action at a distance between fusion-elements appears to have been first noticed by de Bary.<sup>1</sup> In 1881, he pointed out that, in *Pythium de Baryanum*, the oogonium is formed first and that then the oogonium stimulates neighbouring hyphae to send out antheridial branches which grow toward it and eventually bring about the fertilisation of the oosphere.

Action at a distance in respect to sexual fusions was also observed by Blakeslee<sup>2</sup> in the Mucorineae. In 1904, he remarked that in paired cultures of *Mucor Mucedo* two conjugating zygomorphs, one derived from a (+) mycelium and one derived from a (—) mycelium, grow out into the air, stimulate one another, and grow toward one another until they meet (*cf.* Vol. IV, Fig. 84, p. 153). and he referred to the mutual attraction of the zygomorphs as being *zygotactic*.

Action at a distance in respect to ordinary vegetative fusions was recognised and clearly analysed by Marshall Ward<sup>3</sup> in 1888 in his account of a Lily disease caused by Botrytis. He rightly maintained that in certain fusions (*cf.* Fig. 14, p. 29) the action at a distance is of two kinds: (1) an action by one hypha causing another hypha to send out a side branch toward it and (2) an action which causes the first hypha and the branch to grow toward one another until they meet. Said Ward<sup>4</sup>: "It seems to me, after observing numerous cases of these fusions in this and other fungi, that we must distinguish between two steps in the process. In the first place there is some cause at work which determines the formation of a branch, and then, in the second place, we have to assume that some other cause determines the direction in which the branch grows."

In 1892, Rothert<sup>5</sup> described the formation of hyphal fusions in

<sup>1</sup> A. de Bary, "Untersuchungen über die Peronosporen und Saprolegnien und die Grundlage eines natürlichen Systems der Pilze," *Beiträge zur Morphologie und Physiologie der Pilze*, Frankfurt, Reihe IV, 1881, p. 85.

<sup>2</sup> A. F. Blakeslee, "Sexual Reproduction in the Mucorineae," *Proc. Amer. Acad. of Arts and Sciences*, Vol. XL, 1904, p. 274, Plate II, Figs. 25-27.

<sup>3</sup> Marshall Ward, "A Lily-Disease," *Annals of Botany*, Vol. II, 1888, pp. 319-382.

<sup>4</sup> *Ibid.*, p. 330.

<sup>5</sup> W. Rothert, "Ueber *Sclerotium hydrophilum* Sacc., einen sporenlosen Pilz," *Bot. Zeit.*, Jahrg. L, 1892, pp. 358-359.

*Sclerotium hydrophilum* which he said "give the impression that the hyphae which unite are bent out of their direction of growth and are, so to speak, drawn together as if by the excretion of some stimulating substance."

In 1892, Reinhardt,<sup>1</sup> in the course of his study of the growth of the hyphae of *Sclerotinia sclerotiorum* (his *Peziza sclerotiorum*), observed and illustrated the formation of fusions in which there was action at a distance. He clearly saw the growth of a younger hypha to the side of an older hypha, the formation of an opposing side-branch (Nebenast), the growth of the younger hypha and of the side-branch toward one another, and the ultimate meeting and fusion of these elements.

Reinhardt<sup>2</sup> grew different species of fungi together in pairs in the same culture medium and observed the manner in which they influence one another at a distance and in contact in their struggle for existence. Among his observations were the following. *Penicillium glaucum*, by means of excretions, checks the growth of *Aspergillus flavus* and *A. niger* and also stops the growth and causes the death of species of *Sclerotinia* and of *Mucorineae*. *Aspergillus flavus* and *A. niger* act on species of *Sclerotinia* and of *Mucorineae* like *Penicillium glaucum*, but not so strongly. *Sclerotinia sclerotiorum* attacks and kills the hyphae of *Phycomyces nitens*, *Mucor Mucedo*, *M. racemosus*, *Rhizopus nigricans*, *Acrostalagmus cinnabarinus* and *Trichothecium roseum*: it winds its hyphae around, and lays the ends of its hyphae upon, the hyphae of its victims, so that they become almost or entirely enveloped in a mycelial sheath. *Sclerotinia* also lays its hyphae upon the sprout-cells of *Dematium pullulans* and of *Fumago salicina*.

Reinhardt<sup>3</sup> observed that, when a mycelium of *Sclerotinia sclerotiorum* and of a *Mucor* are grown together, the *Mucor* acts upon the *Sclerotinia* at a distance (Fig. 2), probably by means of an excretion: it (1) causes the hyphae of the *Sclerotinia* to branch in a curious manner and (2) causes many of the branch-hyphae of

<sup>1</sup> M. O. Reinhardt, "Das Wachstum der Pilzhyphen. Ein Beitrag zur Kenntniss des Flächenwachsthums vegetabilischer Zellmembranen." *Jahrb. f. wiss. Bot.*, Bd. XXIII, 1892, pp. 500-502, Taf. XXIII, Fig. 13.

<sup>2</sup> *Ibid.*, pp. 502-519.

<sup>3</sup> *Ibid.*, pp. 502-505.

the Sclerotinia to grow toward the Mucor hyphae, to wind about them so as eventually to hide them in hyphal cylinders, to lay their ends upon them, to kill them, and to absorb their contents. No hyphal fusions were formed between the Sclerotinia and the

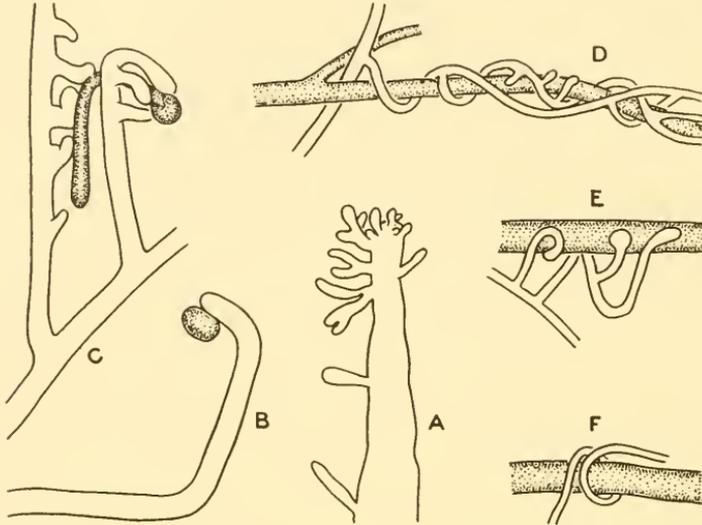


FIG. 2.—Reactions of a mycelium of *Sclerotinia sclerotiorum* (unshaded) to a *Mucor* mycelium (shaded). A, irregular branching of the end of a *Sclerotinia* hypha after stimulation by an excretion of a neighbouring *Mucor* mycelium. B, growth of a *Sclerotinia* hypha toward a *Mucor* spore. C, a spore and germ-tube of *Mucor* attacked by short hyphae put out by a *Sclerotinia* mycelium. D, E, and F, early stages in the envelopment of *Mucor* hyphae by *Sclerotinia* hyphae. The *Sclerotinia* hyphae apply themselves to the *Mucor* hyphae, but do not fuse with them. Eventually, the *Sclerotinia* hyphae kill the enveloped *Mucor* hyphae and absorb their contents osmotically. The drawings copied by the author from Reinhardt's *Das Wachstum der Pilzhyphen*.

*Mucor*, so that, at the points of contact, the former could only absorb the contents of the latter by osmosis.

Reinhardt,<sup>1</sup> working with *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. tuberosa*, observed: that, when two mycelia of the same species are paired in a culture, they grow toward each other, intermingle, and form hyphal fusions with each other quite smoothly;

<sup>1</sup> M. O. Reinhardt, "Das Wachstum der Pilzhyphen. Ein Beitrag zur Kenntniss des Flächenwachstums vegetabilischer Zellmembranen," *Jahrb. f. wiss. Bot.*, Bd. XXIII, 1892, pp. 509-513.

but that, when two mycelia of two *different species* are paired, they interfere with each other's growth, grow toward, attack, and kill individual interpenetrating hyphae, and never form hyphal fusions with each other (Fig. 3). Reinhardt considered that all these signs of antagonism support the view that *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. tuberosa* are three distinct species.

In 1919, Laibach,<sup>1</sup> whilst studying the genus *Septoria*, isolated

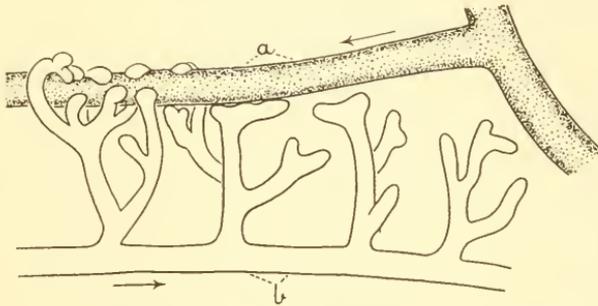


FIG. 3.—Struggle for existence between a mycelium of *Sclerotinia tuberosa* (shaded) and a mycelium of *Sclerotinia trifoliorum* (unshaded) in an artificial culture where the two mycelia were grown side by side. A hypha of *S. tuberosa*, *a*, has grown into the mycelium of *S. trifoliorum*, *b*, and is now being attacked by the latter. The hypha *b*, stimulated morphogenically by the hypha *a*, has sent out short branched branches toward *a*, and these branches, stimulated tropically by *a*, have grown toward *a* and are now applying their ends to it and are beginning to envelop it. In the end the *S. trifoliorum* hyphae will kill the *S. tuberosa* hypha. The drawing copied by the author from Reinhardt's *Das Wachstum der Pilzhyphen*.

two races (Stämme) of *Septoria apii* and observed that, whereas the germ-tubes of conidia of one and the same race readily fuse with one another, a germ-tube of one race and a germ-tube of the other race, while exhibiting a tendency to fuse with each other, never fuse in such a way as to leave no doubt that complete fusion has taken place. A tendency to fuse, without fusion becoming

<sup>1</sup> F. Laibach, "Zur Kenntnis der Gattung *Septoria*." *Ber. d. D. bot. Gesell.*, Bd. XXXVII, 1919, pp. 247-248; also "Untersuchungen über einige *Septoria*-Arten," *Zeitschr. f. Pflanzenkr.*, Bd. XXXI, 1921, p. 189. In the latter communication he mentions *Septoria humuli* and *S. oenotherae* as species which show no tendency to fuse with one another; and he also states that he thought of obtaining a chimaera by isolating fused hyphae of the two forms of *Septoria apii*, but did not succeed in doing so.

undoubtedly complete, was also observed in mixed cultures of *Septoria apii* and *S. petroselini*, but not in mixed cultures of other *Septoria* species. In consequence of having made these observations Laibach suggested that fusion phenomena may possibly be of assistance in deciding upon the affinity of doubtful forms.

In 1902, Arthur Meyer<sup>1</sup> gave a list of fungi in which hyphal fusions had been found and discussed the physiological significance of the channels which hyphal fusions provide. He illustrated a tip-to-tip fusion of two hyphae of *Hypomyces rosellus* in a series of four drawings much resembling similar drawings made by Marshall Ward. He thought that a side-branch which took part in a fusion was produced only after a younger hypha had touched the side of an older hypha, but this may have been due to insufficiently close observation at the moment when the side-branch first began to appear.

In 1924, Burgeff,<sup>2</sup> in an important paper, analysed the reactions which take place between (+) and (−) mycelia of the Mucorineae at the time when the sexual process is initiated and zygophores and zygospores are being formed. He confirmed Blakeslee's discovery that (+) and (−) zygophores grow toward one another through the air (*cf.* Volume IV, Fig. 84, p. 153). Thus, in an experiment with *Mucor hiemalis*, he<sup>3</sup> set two strips of agar, one containing a (+) mycelium and the other a (−) mycelium, 1.5–2.0 mm. apart and observed that the two mycelia produced (+) and (−) zygophores which grew toward one another through the air and across the gap between the two pieces of agar until they met. In another experiment with *Mucor Mucedo* he<sup>4</sup> separated two pieces of agar, one containing a (+) mycelium and the other a (−) mycelium, by a celluloid membrane, and he observed that the mycelia mutually stimulated one another through the membrane in such a way that they both produced zygophores and so that the (+) and (−) zygophores grew toward one another, although prevented by the membrane from coming into contact.

<sup>1</sup> Arthur Meyer, "Die Plasmaverbindungen und die Fusionen der Pilze der Florideenreihe," *Bot. Zeit.*, Jahrg. LX, pp. 162–163, Taf. VI, Figs. 29–31.

<sup>2</sup> H. Burgeff, "Untersuchungen über Sexualität und Parasitismus bei Mucorineen. I." Goebel's *Botanische Abhandlungen*, Heft IV, 1924, pp. 1–135.

<sup>3</sup> *Ibid.*, pp. 14–15.

<sup>4</sup> *Ibid.*, pp. 20–23.

Burgeff introduced a new terminology for the phenomena which he had observed. He designated: as *telemorphosis*<sup>1</sup> the induction of sexual hyphae (zygophores) by action at a distance through the air or a substratum; as *zygotropism*<sup>2</sup> the growth of a (+) zygophore and of a (—) zygophore toward each other until they meet; and as *thigmomorphosis*<sup>3</sup> the swelling of the ends of the (+) and (—) zygophores when these come into contact with one another.

Burgeff<sup>4</sup> sought to explain the telemorphic, zygotropic, and thigmomorphic reactions which take place between a (+) and a (—) mycelium of *Mucor Mucedo* and other Mucorineae by supposing that they are induced by volatile, soluble, sexual substances, one substance for each sex, which are excreted by the mycelia and diffuse readily through air or a solid substratum from a (+) mycelium to a (—) mycelium, and *vice versa*. The sexual substances, on this theory, act *chemomorphically*<sup>5</sup> and *chemotropically*<sup>6</sup> and, if we wish to emphasise the part which chemical substances play in the sexual process, instead of employing the terms telemorphosis, thigmomorphosis, and zygotropism to designate the phenomena concerned, we may speak of *telechemomorphosis*,<sup>7</sup> *thigmochemomorphosis*<sup>8</sup> and *chemotropism*.<sup>9</sup>

According to Burgeff,<sup>10</sup> in heterothallic Mucorineae, *e.g.* *Mucor Mucedo*, *Rhizopus nigricans*, etc., every growing mycelium forms its sexual chemical substance not merely when confronted with a mycelium of opposite sex but also when it is growing by itself. As evidence supporting this statement he cites the following experiment. Atmospheric air was passed through a tube one metre long containing a (+) culture of *Rhizopus nigricans* and then over a (—) culture of *Mucor Mucedo*, with the result that, in the *M. Mucedo* culture, the young sporangiophores were transformed into branched zygophores. Where a (+) mycelium and a (—) mycelium of *Mucor Mucedo*, etc., enter upon the sexual process, the telemorphic effect

<sup>1</sup> H. Burgeff, "Untersuchungen über Sexualität und Parasitismus bei Mucorineen. I," Goebel's *Botanische Abhandlungen*, Heft IV, 1924, p. 15.

<sup>2</sup> *Ibid.*, p. 12.

<sup>3</sup> *Ibid.*, p. 13.

<sup>4</sup> *Ibid.*, pp. 12–13, 17, 19, 23, 63–64.

<sup>5</sup> *Ibid.*, p. 15.

<sup>6</sup> H. Kniep, *Die Sexualität der niederen Pflanzen*, Jena, 1928, p. 312.

<sup>7</sup> H. Burgeff, *loc. cit.*, p. 15.

<sup>8</sup> *Ibid.*, p. 13.

<sup>9</sup> H. Kniep, *loc. cit.*, p. 456.

<sup>10</sup> H. Burgeff, *loc. cit.*, p. 76.

resulting in the production of zygophores must be caused by excretions of the sexual substances from the ordinary vegetative hyphae, while the zygotropic effect which results in (+) and (-) zygophores growing toward one another must be caused by excretions of the

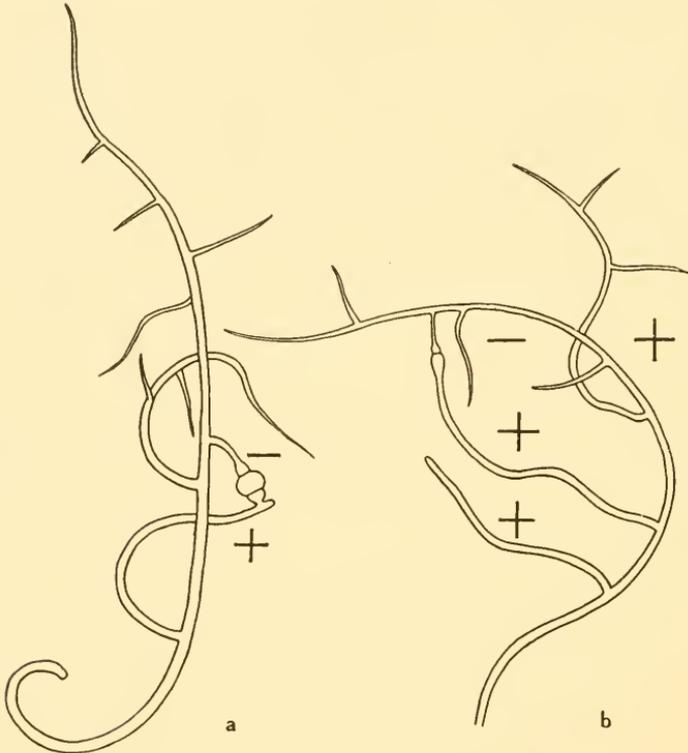


FIG. 4.—*Zygorhynchus exponens*, one of the homothallic Mucorineae. Zygophores with conjugating branches. Conjugation takes place only between a (+) branch and a (-) branch. In this species the (+) branches and the (-) branches are different not only physiologically but also morphologically. From Burgeff's *Untersuchungen über Sexualität und Parasitismus bei Mucorineen*. Magnification, 80.

sexual substances from the zygophores themselves.<sup>1</sup> Whether or not a single pair of sexual substances is sufficient to cause all the three kinds of reactions—telemorphic, zygotropic, and thigmomorphic—remains to be determined.

In a homothallic species, e.g. *Zygorhynchus exponens*, *Absidia*

<sup>1</sup> H. Burgeff, *loc. cit.*, pp. 22-23.

*spinosa*, and *Sporodinia grandis*, according to Burgeff,<sup>1</sup> in a single mycelium some branches become (+) and others (−) in sex, and conjugation occurs only between a (+) hypha and a (−) hypha

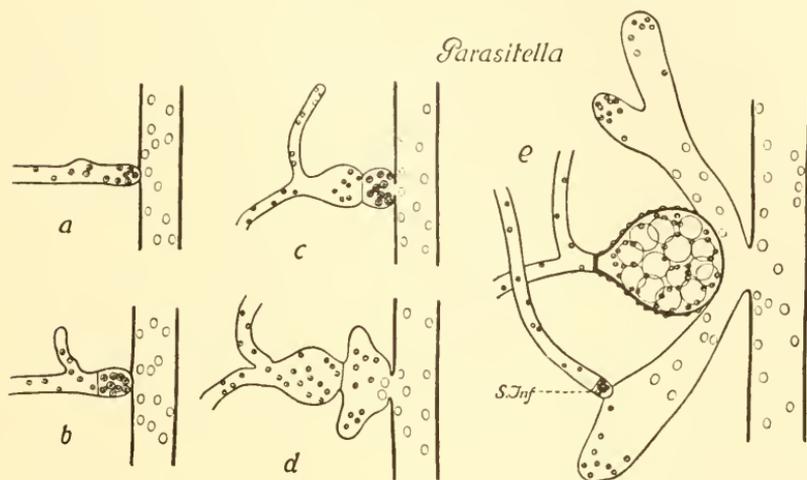


FIG. 5.—Diagram showing in one plane stages in the union of *Parasitella simplex* with one of its host Mucorineae (*Absidia glauca*, *Rhizopus nigricans*, etc.). *a*, a hypha of *Parasitella* has grown toward, and has applied its tip to, the side of a relatively thick host-hypha. The nuclei of the parasite are shaded, and those of the host left clear. Nuclei are collecting at the end of the *Parasitella* hypha. *b*, a septum has been formed near the end of the *Parasitella* hypha; the cell so formed contains about ten *Parasitella* nuclei. These nuclei are enlarging, and the *Parasitella* cell is about to fuse with the host-hypha. *c*, the *Parasitella* cell has now fused with the host-hypha and is enlarging, while the adjacent part of the *Parasitella* hypha is also enlarging as a preliminary stage in the formation of a spore. *d*, the *Parasitella* cell (Schröpfkopfzelle = cupping cell) is sending out lateral processes, its own nuclei have diminished in size, and host-nuclei are wandering into it, while the future spore is still enlarging. *e*, the *Parasitella* cell has now sent out a number of radiating processes which partially envelop the spore. The cell with its processes constitutes a gall (the Schröpfkopf). The gall-branches contain *Parasitella* nuclei at their ends and host-nuclei in their middle and basal parts. One of the gall-branches is becoming secondarily infected by a *Parasitella* hypha at *s. inf.* The *Parasitella* spore, known as a *sicyospore*, is now fully developed; it is cut off from the hypha which bears it by a septum, its wall is thickened and covered with calcium-oxalate crystals, and it contains numerous nuclei and many oil-drops. From Burgeff's *Untersuchungen über Sexualität und Parasitismus bei Mucorineen*.

(Fig. 4). He also holds that under certain conditions a (+) hypha can change into a (−) hypha, and *vice versa*.

Burgeff<sup>2</sup> described in detail the relations between the parasites

<sup>1</sup> H. Burgeff, *loc. cit.*, pp. 32–43.

<sup>2</sup> *Ibid.*, pp. 91–135.

*Parasitella simplex* and *Chaetocladium Brefeldii* var. *macrosporum* and their hosts *Absidia*, *Mucor*, etc. (Figs. 5 and 6). He showed : that hyphae of the parasites and hosts "attract" one another ;

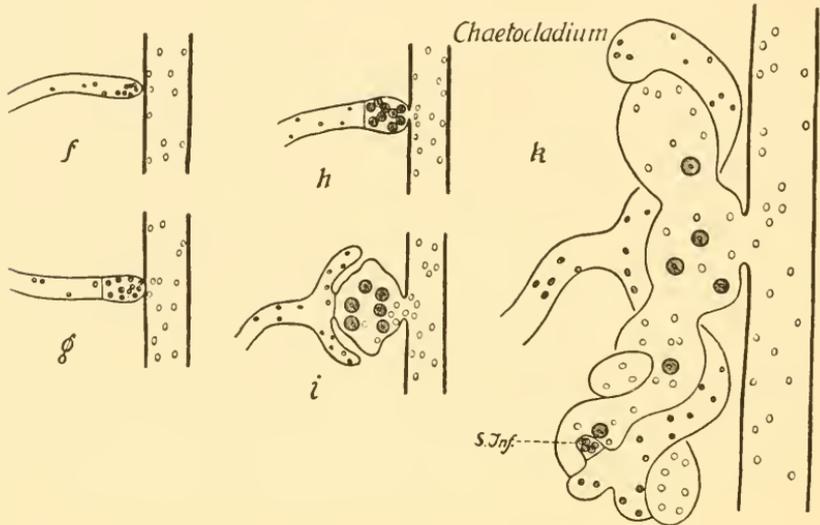


FIG. 6.—Diagram showing in one plane stages in the union of *Chaetocladium Brefeldii* var. *macrosporum* with one of its host Mucorineae (*Absidia glauca*, *Rhizopus nigricans*, etc.). *f*, a hypha of *Chaetocladium* has grown toward, and has applied its tip to, the side of a relatively thick host-hypha. The nuclei of the parasite are shaded, and those of the host left clear. Nuclei are collecting at the end of the *Chaetocladium* hypha. *g*, a septum has been formed near the end of the *Chaetocladium* hypha ; the cell so formed contains a number of *Chaetocladium* nuclei. These nuclei are about to enlarge, and the *Chaetocladium* cell is about to fuse with the host-hypha. *h*, the *Chaetocladium* cell has now fused with the host-hypha ; it is expanding and its nuclei are growing larger. *i*, the *Chaetocladium* cell (*Schröpfkopfzelle* = cupping cell) is sending out lateral processes, its own nuclei are still enlarging, and host-nuclei are wandering into it, while the adjacent part of the *Chaetocladium* hypha is also sending out processes. The *Chaetocladium* cell with its processes is becoming a gall (the *Schröpfkopf*). *k*, the gall-cell is now larger and its branches have grown in length. The *Chaetocladium* nuclei in the gall have now attained their maximum size and are much larger than the host-nuclei. The processes from the adjacent part of the *Chaetocladium* hypha have grown around the gall, are branching, and the tip of one of the branches is causing a secondary infection of the gall at *s. inf.* Later, *Chaetocladium* sporophores arise from the gall and, as they form spores, completely empty it. From Burgeff's *Untersuchungen über Sexualität und Parasitismus bei Mucorineen*.

that a hypha of a parasite which has grown toward, and has come into contact with, a host-hypha fuses with the latter ; and that a curious gall, surrounded by a wall derived from the parasite but containing nuclei of both host and parasite, is formed close to the

point of fusion. The processes in the formation of a gall are reminiscent of plasmogamy. Both *Parasitella* and *Chaetocladium* successfully attack the homothallic species *Zygorhynchus exponens*. *Parasitella* parasitises, and *Chaetocladium* reacts with, both (+) and (−) mycelia of *Mucor hiemalis*. However, the parasitism of *Parasitella* and *Chaetocladium* in respect to *Absidia glauca* is strictly *sex-limited* in that *Parasitella* (+) parasitises only *A. glauca* (−) and *Parasitella* (−) parasitises only *A. glauca* (+), while *Chaetocladium* (+) parasitises only *A. glauca* (−).<sup>1</sup> In view of all these and other related facts Burgeff<sup>2</sup> came to conclusions which may be summarised as follows. The parasitism of *Parasitella* and *Chaetocladium* probably originated by these fungi making use of their sexual reactions to attack their hosts. A parasite mycelium, (+) or (−), shows an affinity for both sexes of most of its heterothallic hosts; and, where this occurs, the parasite must react not to one or other of the two sexual substances but to some third substance excreted by both the (+) and the (−) mycelia of the hosts. Where the parasitism is sex-limited, as it is in respect to the heterothallic *Absidia*, this third substance is not produced and the parasites react to the (+) and (−) sexual substances.

From the foregoing summary of Burgeff's investigations we see that Burgeff, in attempting to throw light on the sexual and parasitic fusions in the Mucorineae, has assumed as causal agents at least three volatile soluble substances—a substance excreted by (+) mycelia, a substance excreted by (−) mycelia, and a third substance excreted by both (+) and (−) mycelia which is not connected with sex. It is probable that, if chemical stimuli are responsible for the telemorphic and zygotropic phenomena concerned with the sexual and parasitic fusions of the Mucorineae, chemical stimuli are also responsible for the telemorphic and zygotropic phenomena concerned with the vegetative fusions which take place in the mycelia of Ascomycetes, Basidiomycetes, and Fungi Imperfecti. Since numerous hyphal fusions take place in every haploid mycelium of a Higher Fungus, e.g. *Coprinus lagopus*, if a pair of chemical substances

<sup>1</sup> *Chaetocladium* (−) was not obtained and therefore no experiments could be made with it.

<sup>2</sup> H. Burgeff, *loc. cit.*, p. 133.

are really the chemomorphic and zygotropic agents which bring about fusions of the peg-to-peg type (*cf.* Fig. 15, p. 31), these substances can have nothing to do with sex.

In 1928, Laibach<sup>1</sup> compared the vegetative hyphal fusions which are found in the mycelia of two Ascomycetes, *Leptosphaeria Coniothyrium* and *Monilia fructigena*, with the fusions that take place between the sporidia of species of Ustilago, *e.g.* *Ustilago bromivora*, and he came to the conclusion: "that important physiological differences between the fusions investigated in the Ascomycetes and those which occur in the Ustilagineae do not exist; and that from the physiology of the fusion of sporidia in the Smut Fungi no argument for the sexual nature of the phenomenon can be drawn;" and he accepted the view of Boss<sup>2</sup> that, when two Smut sporidia unite, one should not speak of the *copulation* of sporidia, but should employ Brefeld's non-committal term *fusion*. However, against Laibach's conclusion may be urged, in respect to fusions, that, whereas in *Leptosphaeria Coniothyrium*, *Monilia fructigena*, *Trichoderma lignorum*<sup>3</sup> and other Ascomycetes the germ-tubes or young mycelia derived from any two spores of the same species may unite, in *Ustilago violacea* and certain other Smuts there are two kinds of promycelial cells of such a nature that union takes place between a descendant (sporidium or sprout-cell) derived from one kind of cell and a descendant derived from the other kind of cell, but not between descendants of the same cell or of two cells of the same kind.<sup>4</sup> There seems no good reason why, with Kniep, we should not regard the fusions between sporidia in Smut Fungi as essentially sexual in their physiological nature rather than as vegetative.

Laibach<sup>5</sup> came to the conclusion that action at a distance between hyphae destined to fuse is exhibited by both Ascomycetes and Ustilagineae; and he remarked that "in both groups it appears

<sup>1</sup> F. Laibach, "Über Zellfusionen bei Pilzen," *Planta*, Bd. V, 1928, pp. 340-359.

<sup>2</sup> G. Boss, "Beiträge zur Zytologie der Ustilagineen," *Planta*, Bd. III, 1927, p. 642.

<sup>3</sup> *Vide these Researches*, Vol. IV, 1931, Figs. 98 and 100, pp. 173 and 176.

<sup>4</sup> H. Kniep, "Untersuchungen über den Antherenbrand," *Zeitschrift für Botanik*, Bd. XI, 1919, pp. 257-284; also *Die Sexualität der niederen Pflanzen*, Jena, 1928, pp. 429-430.

<sup>5</sup> F. Laibach, *loc. cit.*, pp. 356-357.

that chemomorphic influences are exerted by the fusion hyphae, which come to expression in that these hyphae stimulate a neighbouring hypha or cell to send out a fusion-branch."

In his investigations on *Leptosphaeria Coniothyrium* and *Monilia fructigena* Laibach<sup>1</sup> noticed that in water and in weak nutrient media hyphal fusions are far more frequent than in strong nutrient media and that fusion-formation begins when the rate of growth of the germ-tubes begins to slow down. He<sup>2</sup> also observed that, when conidia of *Monilia fructigena* are germinated in a film of apple-juice between a slide and a cover-glass, the germ-tubes of the conidia near the edge of the cover-glass grow toward the middle of the cover-glass and never show any inclination to form anastomoses with one another, and he concluded that in this phenomenon an important part is played by the pressure of oxygen.

In 1929, Köhler,<sup>3</sup> as a result of watching the formation of fusions between hyphae in the mycelium of *Sclerotium solani*, *Sclerotinia fructigena*, and *Hypochnus (Rhizoctonia) solani*, confirmed Marshall Ward's observations on action-at-a-distance between hyphae about to fuse and, with the exception of clamp-connexion fusions, he observed and illustrated fusions corresponding to all those types which I myself have observed and which will shortly be described and classified.

Köhler<sup>4</sup> also suggested that the terms *telemorphosis* and *zygotropism* which Burgeff<sup>5</sup> had employed in his analysis of the "attractive processes" concerned in the conjugation of certain Mucorineae should be employed for the essentially similar "attractive processes" which take place in connexion with hyphal fusions in the Higher Fungi. Thus he would use the term *telemorphosis* for the phenomenon in which one hypha, acting at a distance, stimulates another hypha to alter its form by sending out an opposing fusion hypha; and he would designate as *zygotropism* the phenomenon in which

<sup>1</sup> F. Laibach, *loc. cit.*, pp. 348-349, 352-353.

<sup>2</sup> *Ibid.*, pp. 253-356.

<sup>3</sup> E. Köhler, "Beiträge zur Kenntnis der vegetativen Anastomosen der Pilze, I.," *Planta*, Bd. VIII, 1929, pp. 140-153.

<sup>4</sup> *Loc. cit.*, p. 153.

<sup>5</sup> H. Burgeff, "Untersuchungen über Sexualität und Parasitismus bei Mucorineen. I.," *Goebel's Botanische Abhandlungen*, Heft IV, 1924, pp. 12-14.

two hyphae, as a result of mutual stimulation, make growth curvatures toward one another and grow toward one another until they meet. I see no objection to the adoption of this terminology.

In 1930, Köhler<sup>1</sup> published the results of a series of experiments by means of which he sought to answer the question whether or not vegetative hyphal fusions can be formed between mycelia belonging to two different species. He chose for his material species in which young mycelia derived from conidia showed a strong inclination to form fusions. A sterile glass slide was dipped in melted nutrient or

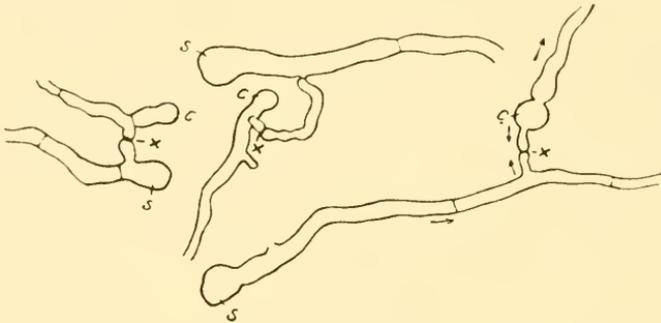


FIG. 7.—Result of a mixed sowing of conidia of *Neurospora sitophila*, *s*, and *Neurospora crassa*, *c*. There has been a fairly strong reaction between the two species. Their germ-tubes, by means of fusion-branches which have grown toward one another, have initiated the process of union. After contact had been established at the places marked with a cross, the growth of the fusion-branches ceased, their ends did not flatten out against one another, and no open fusions were formed. From Köhler's *Zur Kenntnis der vegetativen Anastomosen der Pilze*.

plain agar, and the superfluous agar was allowed to drip away. When the remaining film of agar had become solid, a brush was dipped into water containing a mixture of conidia of two species and was then drawn over the agar film. The water so added to the agar film was soon absorbed by the agar. Sometimes it was necessary to sow the two kinds of conidia at successive intervals of time. In some of the experiments, in order to distinguish the young mycelia from one another, the two sets of conidia, before being sown, were treated with a mordant (1 per cent. tannin solution) and then were stained with distinctive basic dyes (methyl violet, fuchsin, malachite green, methyl green). The slides were kept on wet filter-

<sup>1</sup> E. Köhler, "Zur Kenntnis der vegetativen Anastomosen der Pilze, II.," *Planta*, Bd. X, 1930, pp. 495-522.

paper in Petri dishes. When a slide was ready for examination it was removed from the damp-chamber and then the agar film was covered with water and a cover-glass. The capacity for forming fusions in germ-mycelia was found to be dependent on various conditions. It is especially influenced by the state of nourishment of the conidia used for sowing and also by the composition and thickness of the substratum.

In the following combinations Köhler observed that the two mycelia did not form fusions with one another, nor did they exhibit signs of telemorphosis or zygotropism :

*Sclerotinia fructigena* with *Sclerotinia cinerea*,

*Neurospora tetrasperma* (homothallic) with *N. crassa* (B-haplonts),

*Fusarium coeruleum* with *Neurospora crassa*,

*Fusarium coeruleum* with *Botrytis cinerea*,

*Fusarium aurantiacum* with *Neurospora crassa*,

*Sclerotinia fructigena* with *Botrytis narcissicola*.

On the other hand telemorphosis and zygotropism, but no undoubted plasma fusions, were observed in a more or less marked degree in the following combinations :

*Botrytis allii* with *Botrytis narcissicola*,

*Neurospora crassa* (B-haplont) with *N. sitophila* (A-haplont) (Fig. 7),

*Neurospora tetrasperma* (homothallic) with *N. sitophila* (A-haplont),

*Neurospora sitophila* (A-haplont) with *N. tetrasperma* (B-haplont),

*Fusarium coeruleum* with *Fusarium sarcochromum*,

*Fusarium coeruleum* with *Fusarium aurantiacum*,

*Botrytis allii* with *Neurospora sitophila* (Fig. 8).

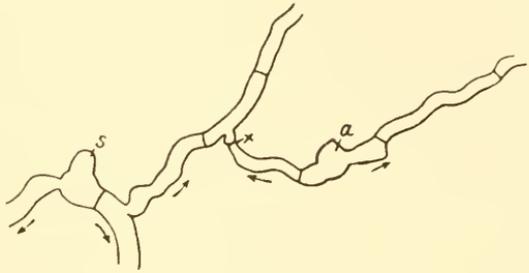


FIG. 8.—Result of a mixed sowing of conidia of *Neurospora sitophila*, *s*, and *Botrytis allii*, *a*. A hypha of *B. allii* has grown toward a hypha of *N. sitophila* which has sent out an opposing branch-hypha. The two have met at the place marked by a cross, but have not fused with one another. From Köhler's *Zur Kenntnis der vegetativen Anastomosen der Pilze*.

*Botrytis allii* with *Neurospora crassa*,  
*Sclerotinia fructigena* with *Neurospora crassa* (Fig. 9),  
*Sclerotinia fructigena* with *Neurospora tetrasperma*,  
*Sclerotinia cinerea* with *Neurospora sitophila*,  
*Sclerotinia cinerea* with *Neurospora crassa*,  
*Sclerotinia fructigena* with *Neurospora sitophila*,  
*Sclerotinia fructigena* with *Botrytis allii*.

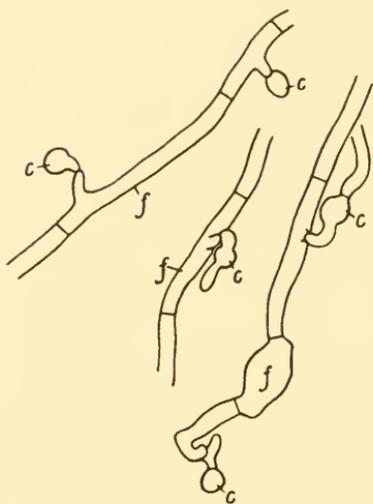


FIG. 9.—Result of a mixed sowing of conidia of *Sclerotinia fructigena*, *f*, and *Neurospora crassa*, *c*. The *S. fructigena* hyphae have sent out fusion-branches toward approaching germ-tubes of *N. crassa*. The fusion-branches and germ-tubes have come into contact with one another but, so far as can be seen, have not fused with one another. From Köhler's *Zur Kenntnis der vegetativen Anastomosen der Pilze*.

Köhler found that the teleomorphic and zygotropic reactions observed in the combinations of species just given are similar to, but always very much weaker than, the teleomorphic and zygotropic reactions which take place between two mycelia of one and the same species. In no combination of species was it possible to observe the broad plasma-fusions that are so characteristically formed between mycelia of a single species. If any such fusions do occur, the fusion-canals must be extremely small.

In 1931, in the fourth volume of these *Researches*,<sup>1</sup> I discussed the functional value of fusion-channels in connexion with sex and social organisation in the Higher Fungi.

In 1931, Forsteneichner<sup>2</sup> and, in 1932, Matsumoto, Yamamoto,

and Hirane<sup>3</sup> reported the results of hyphal-fusion studies made on

<sup>1</sup> These *Researches*, Vol. IV, pp. 152-186, 231-232, 243-245, 300-301.

<sup>2</sup> F. Forsteneichner, "Die Jugendkrankheiten der Baumwolle in der Türkei," *Phytopath. Zeitschr.*, III, 1931, pp. 367-419. Cited from Matsumoto.

<sup>3</sup> T. Matsumoto, W. Yamamoto, and S. Hirane, "Physiology and Parasitology of the Fungi generally referred to as *Hypochnus Sasakii* Shirai: I. Differentiation of the Strains by means of Hyphal Fusion and Culture in Differential Media," *Journ. Soc. Tropical Agriculture*, Vol. IV, 1932, pp. 370-388.

strains of *Rhizoctonia*. Forsteneichner found that in a *Rhizoctonia* growing on cotton plants in Turkey fusions were common, but that the mycelium would not form fusions with the mycelium of *R.*

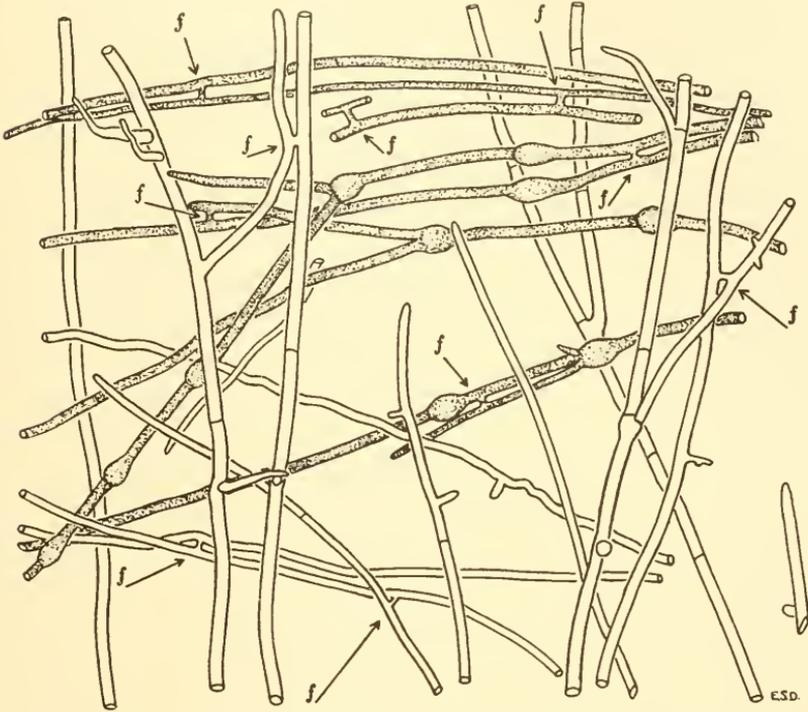


FIG. 10.—Absence of hyphal fusions between two species of *Microsporon*. The mycelium of *Microsporon audouini* from patient C, left unshaded, and the mycelium of *Microsporon lanosum* from patient E, shaded, which have been growing side by side in a hanging drop of Sabouraud's medium for three weeks, have come into contact with one another, have become interlaced, but have not fused with one another. Hyphal fusions may be observed between two hyphae of *M. audouini* or between two hyphae of *M. lanosum*: *ff*, numerous hyphal fusions, but none of them between a hypha of *M. audouini* and a hypha of *M. lanosum*. Drawn by E. S. Dowding. From the paper of Davidson, Dowding, and Buller on *Hyphal Fusions in Dermatophytes*. Magnification, 347.

*Solani* and two other *Rhizoctonia* strains. Matsumoto and his co-workers found that, in *Hypochnus Sasakii*, perfect fusions take place between hyphae of one and the same strain, but that only hyphal contacts or imperfect fusions take place between strains less closely related as judged morphologically. They also found that the mycelia of *Hypochnus Sasakii* and *Rhizoctonia Solani* do not

fuse with one another and that they grow together without any inhibitory effects being observable.

In 1932, in a paper on *Hyphal Fusions in Dermatophytes*, Davidson, Dowding, and Buller<sup>1</sup> recognised that hyphal fusions are an important character of the mycelium of fungi which parasitise the human skin, and they came to the following conclusions. In *Microsporion audouini*,<sup>2</sup> *M. lanosum*,<sup>3</sup> and *Trichophyton gypseum*<sup>4</sup> hyphal fusions: (1) are formed between hyphae of one and the same mycelium isolated from a single patient, (2) are formed between any two mycelia of the same species isolated from two different

patients, and (3) are *not* formed between a mycelium of one species and a mycelium of another species (cf. Fig. 10). The occurrence or non-occurrence of hyphal fusions between hyphae of two mycelia of different origin may be applied as a criterion for identifying species of dermatophytic fungi whose specific nature is uncertain.

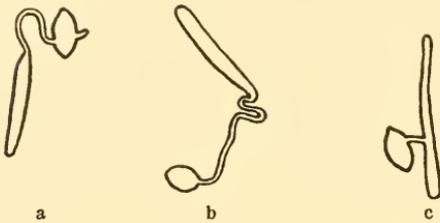


FIG. 11.—Fusions between sporidia of two different species of *Ustilago*: *U. longissima* var. *macrospora*, sporidia elongated, with *U. bromivora*, sporidia oval or lemon-shaped; a, b, and c, different sexual combinations. From Kniep's *Über Artkreuzungen bei Brandpilzen*.

The problem whether or not two different allied species of fungi can fuse with one another, either sexually or vegetatively, is of considerable interest from the point of view both of genetics and of evolution.

Sexual fusions must take place where hybrids are formed. Naturally occurring hybrid fungi are at present unknown, but certain hybrids have been obtained in artificial cultures. Thus Saito and Naganiski<sup>5</sup> obtained zygosporidia in crosses between

<sup>1</sup> A. M. Davidson, Eleanor S. Dowding, and A. H. R. Buller, "Hyphal Fusions in Dermatophytes," *Canadian Journal of Research*, Vol. VI, 1932, pp. 1-20, 22 Text-figs. and Plates I-III.

<sup>2</sup> The commonest cause of ringworm of the scalp at Winnipeg.

<sup>3</sup> The cause of crusted lesions on the scalp and of circular red scaly lesions on the glabrous skin.

<sup>4</sup> It attacks hairs in the beard area and causes sycosis parasitaria (tinea barbae).

<sup>5</sup> K. Saito and J. Naganiski, "Bemerkungen zur Kreuzung zwischen verschiedenen *Mucor*-arten," *Bot. Mag. Tokio*, Vol. XXIX, 1915, pp. 149-154. Burgeff (next

closely related *Mucor* species or possibly different forms of a single *Mucor* species, and Burgeff<sup>1</sup> obtained zygospores when he crossed *Phycomyces nitens* and *P. Blakesleeanus*. Kniep<sup>2</sup> (Figs. 11 and 12), Dickinson,<sup>3</sup> and Bauch<sup>4</sup> all observed fusions between sporidia or sporidial hyphae of different species or races of *Ustilago*. Hanna and Popp<sup>5</sup> obtained smut spores as a result of crossing *Ustilago avenae* and *U. levis*; while Flor,<sup>6</sup> and subsequently Hanna,<sup>7</sup> obtained bunt balls as a result of crossing *Tilletia tritici* and *T. laevis*. The fact that B. O. Dodge<sup>8</sup> obtained hybrids by crossing *Neurospora sitophila* and *N. tetrasperma* seems to indicate that these species must fuse with one another, although such fusions were not definitely

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citation, p. 43) states that Blakeslee regards the supposed species as different forms of one and the same species.

<sup>1</sup> H. Burgeff, "Über Arten und Artkreuzung in der Gattung *Phycomyces* Kunze," *Flora*, Bd. CVIII and CIX (Goebel-Festschrift), 1925, pp. 40-46. The zygospores germinated and gave rise to sporangia.

<sup>2</sup> H. Kniep, "Über Artkreuzungen bei Brandpilzen," *Zeit. f. Pilzkunde*, Bd. X, 1926, pp. 217-251; also *Die Sexualität der niederen Pflanzen*, Jena, 1928, p. 431. In the genus *Ustilago*, as a result of pairing sporidia, Kniep found that he could obtain fusions: (1) between any two species with reticulate spores (*U. violacea*, *U. Scabiosae*, *U. Cardui*, *U. utriculosa*, *U. vinosa*, *U. anomala*, and *U. Tragopogonis*); (2) between any two species with smooth or punctate spores (*U. longissima*, *U. longissima* var. *macrospora*, *U. grandis*, *U. bromivora*, *U. Hordei*, and *U. perennans*); but (3) not between a species with reticulate spores and a species with smooth or punctate spores. The promycelia of *U. nuda* and *U. Tritici*, which do not produce sporidia, fused with sporidia of *U. Hordei*, and *U. bromivora*.

<sup>3</sup> S. Dickinson, "Experiments on the Physiology and Genetics of the Smut Fungi, Hyphal-fusion," *Proc. Roy. Soc. (B)*, Vol. CI, 1927, p. 133. Dickinson observed the fusion of a sporidial hypha of *Ustilago levis* (Covered Smut of Oats) with a sporidial hypha of *U. Hordei* (Covered Smut of Barley) and the passage of a nucleus through the open channel from one cell to the other.

<sup>4</sup> R. Bauch, "Rassenunterschiede und sekundäre Geschlechtsmerkmale beim Antherenbrand," *Biol. Centralbl.*, Bd. XLVII, 1927, pp. 370-383. Bauch found that strains of *Ustilago violacea* obtained from *Dianthus deltoides*, *Melandryum album*, and *Malachium aquaticum* can be crossed and that the conjugated sporidia give rise to germ-tubes.

<sup>5</sup> W. F. Hanna and W. Popp, "Relationship of the Oat Smuts," *Nature*, Nov. 29, 1930.

<sup>6</sup> H. H. Flor, "Heterothallism and Hybridization in *Tilletia tritici* and *T. levis*," *Journ. Agric. Research*, Vol. XLIV, 1932, pp. 49-58.

<sup>7</sup> W. F. Hanna, "The Odor of Bunt Spores," *Phytopathology*, Vol. XXII, 1932, pp. 978-979.

<sup>8</sup> B. O. Dodge, "Production of Fertile Hybrids in the Ascomycete *Neurospora*," *Journ. Agric. Research*, Vol. XXXVI, 1928, pp. 1-14.

observed by Köhler<sup>1</sup> when he grew germ-mycelia of these fungi side by side.

So far as vegetative fusions between mycelia of different species are concerned, as we have seen, the special observations of Reinhardt, Laibach, Köhler, Forsteneichner, Matsumoto, and Davidson, Dowding, and Buller have yielded results which are essentially negative. A similar negative result was obtained by myself recently when I paired mycelia of *Pleurance conica* and *P. anserina* in a

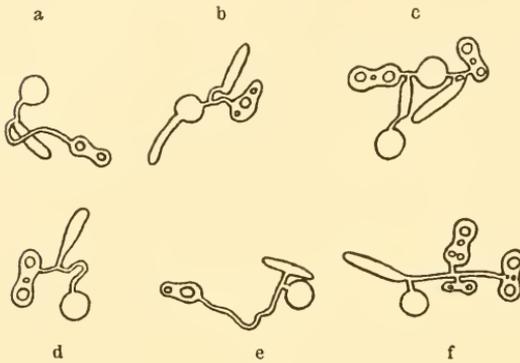


FIG. 12.—Fusions between sporidia of three different species of *Ustilago*: *U. Tragopogonis*, sporidia oblong; *U. anomala*, sporidia biscuit-shaped with oil-drops; *U. Cardui*, sporidia spherical. From Kniep's *Über Artkreuzungen bei Brandpilzen*.

hanging drop of cleared dung-agar; for, whereas numerous hyphal fusions could be readily observed between hyphae of *P. conica* or between hyphae of *P. anserina*, after a prolonged and careful search no hyphal fusions could be detected between a hypha of *P. conica* and a hypha of *P. anserina*.

In my field and laboratory studies of the Hymenomycetes, I have seen nothing to suggest that in this group species-hybrids exist. In general, the species of Hymenomycetes remain morphologically distinct from one another without intermediate forms. Thus on unsterilised horse dung in laboratory cultures several species of *Coprinus*, e.g. *C. sterquilinus*, *C. niveus*, *C. stercorarius*, *C. lagopus*, *C. curtus*, and *C. ephemerus*, often come up close together, yet their fruit-bodies remain specifically distinct and never show appearances which suggest that the haploid mycelia of two distinct species have fused with one another and have given rise to a hybrid diploid mycelium which in turn has given rise to a hybrid diploid fruit-body. Brunswik<sup>2</sup> failed in attempts to cross *Coprinus* species, and

<sup>1</sup> E. Köhler, *loc. cit.*, p. 511.

<sup>2</sup> H. Brunswik, "I. Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomycetengattung *Coprinus*," Goebel's *Botanische Abhandlungen*,

Kniep<sup>1</sup> reports that he made a great number of attempts to cross species in the genera *Hypholoma*, *Collybia*, and *Mycena*, but without success. Vandendries<sup>2</sup> states that, on pairing haploid mycelia of *Panaeolus campanulatus* and *P. fimicola*, he obtained in one instance a mycelium bearing clamp-connexions; but this mycelium did not grow well and did not fruit, so that it was impossible to obtain analytic evidence of its hybrid origin. From what we know at present it seems probable that, in the Hymenomycetes, hyphal fusions between haploid mycelia of distinct species are formed either not at all or but rarely.

**Investigations by the Author.**—Since hyphal fusions are of such common occurrence and of such high functional importance in the Higher Fungi, it seems well worth while to learn all we can about them. As a contribution to our knowledge of hyphal fusions, therefore, my own observations on the mode in which hyphal fusions are formed in certain representative Ascomycetes and Basidiomycetes will now be recorded.

**Methods and Materials.**—The mycelia employed for hyphal-fusion studies were grown in shallow drops of cleared dung-agar<sup>3</sup> suspended in van-Tieghem cells. The drops were inoculated either with spores or with pieces of mycelium removed from stock plate cultures. The species chiefly used for observation were six in number and they are classified in the following list.

*List of Fungi used in Hyphal Fusion Studies*

Ascomycetes	{	Pyrenomycetes	{	Pleurance curvicolla
			{	Pleurance anserina
			{	Fimetaria (Podospora) fimicola
		Discomycetes .		Pyronema confluens
Basidiomycetes	{	Hymenomycetes	{	Coprinus sterquilinus
			{	Coprinus lagopus
		Gastromycetes .		Sphaerobolus stellatus

Heft V, 1924, p. 145. Among the crosses which Brunswik tried were: *C. niveus* × *C. fimetarius*; *C. niveus* × *C. Friesii*; *C. lagopus* (not the *C. lagopus* of these volumes) × *C. fimetarius*; and *C. ephemerus* × *C. curtus*.

<sup>1</sup> H. Kniep, *Die Sexualität der niederen Pflanzen*, Jena, 1928, p. 411.

<sup>2</sup> R. Vandendries, "Recherches sur le déterminisme sexuel des Basidiomycètes, *Mém. Acad. Belg.*, Cl. des Sci., Sér. 2, T. V, 1923, p. 98.

<sup>3</sup> Details of the preparation of cleared dung-agar are given in these *Researches*, Vol. IV, pp. 195-197.

The principle involved in the mode of observation was that of continuous watching of a particular part of a living mycelium with a view to witnessing all those stages in growth leading up to, and culminating in, the actual union of two hyphae of one and the same mycelium.

A younger hypha growing toward the side of an older hypha may either pass the older hypha or fuse with a process which this sends out. Two branch-hyphae whose ends are growing toward one another may pass one another or fuse with one another. Passing is the rule in younger rapidly growing mycelia. Also hyphae or hyphal processes upon which one may fix one's attention as likely to take part in a hyphal fusion may cease to grow. Hence one's observations often yield negative results with consequent loss of time. In any one mycelial preparation it was not found possible to observe more than one or two fusions in a day.

**A Generalisation on Hyphal Fusions.**—As a result of comparative observations made on the species of Ascomycetes and Basidiomycetes listed in the previous Section, I have come to the important general conclusion that, in the mycelium of the Higher Fungi, *all hyphal fusions are essentially end-to-end ones, i.e.* that, when a fusion takes place, it takes place between the end of one hypha and the end of another hypha. The youngest parts of hyphae—the growing points—are alone involved in the final stages of the fusion process. The evidence upon which this conclusion is based will appear in what follows.

It was formerly held by myself, Mlle Bensaude, and others that hyphal fusions are often *end-to-side* ones, *i.e.* that often the end of a younger hypha fuses directly with the side of an older hypha. However, direct observations on the formation of hyphal fusions in living mycelia do not support the view that end-to-side fusions take place. A further discussion of this matter will be deferred until the facts yielded by my new investigations have been recorded.

**The Four Kinds of Hyphal Fusions.**—Granted that end-to-end fusions include all those occurring in mycelia, the various types of hyphal fusions may be classified as follows: (1) *hypha-to-hypha* fusions; (2) *hypha-to-peg* fusions, (3) *peg-to-peg* fusions, and (4) *hook-to-peg* or clamp-connexion fusions. In this terminology: by

the word *hypha* is meant an ordinary vegetative hypha of some length which has grown freely in the culture medium before becoming "attracted" by another hypha and undergoing growth changes leading to a hyphal fusion; by the word *peg* is meant a very short special fusion hypha which has never grown freely in the medium by itself but has been stimulated to come into existence by another hypha, another peg, or a hook with which it is destined to fuse; and by the word *hook* is meant a very short curved hypha such as is always produced during the formation of a clamp-connexion. A peg may be regarded as a fusion organ.

**Hypha-to-Hypha Fusions.**—A hypha-to-hypha fusion takes place between two hyphae of greater or less length which at first grow freely and independently of one another in the culture medium. As growth proceeds, these hyphae, by chance, come within a certain distance of one another (about 7–15  $\mu$ ). Then their ends change their direction of growth so that they grow directly toward one another. Soon, their apices meet and flatten out against one another. Next, the appressed walls at the ends of the two hyphae break down and disappear and, at the same time, the cylindrical side-walls of the hyphae become continuous with one another so as to form a compound tube. Finally, the two masses of protoplasm at the ends of the two hyphae meet and fuse, with the result that protoplasmic continuity from one hypha to the other becomes established.

Successive stages in the formation of a hypha-to-hypha fusion are shown diagrammatically in Fig. 13. At A, owing to apical growth, two long independent branch-hyphae happen to be approaching one another. At B, they have come near enough to one another for mutual stimulation to take place. At C, as a result of this stimulation, the ends of the two hyphae have altered their direction of growth and are growing toward one another. At D, the two hyphae are rapidly approaching one another. At E, they have met and their ends are touching. The ends of the hyphae now flatten against one another, the cylindrical walls fuse together, and the dividing double wall dissolves and disappears. Finally, as shown at F, with the disappearance of the double wall, a passage-way is formed from one hypha to the other, and the two masses of

protoplasm in the two fusing hyphae come into contact with one another, melt together, and thus establish protoplasmic continuity from one hypha to the other.

Whilst the mycelium is growing rapidly at its periphery, hypha-to-hypha fusions take place only between lateral hyphae in the

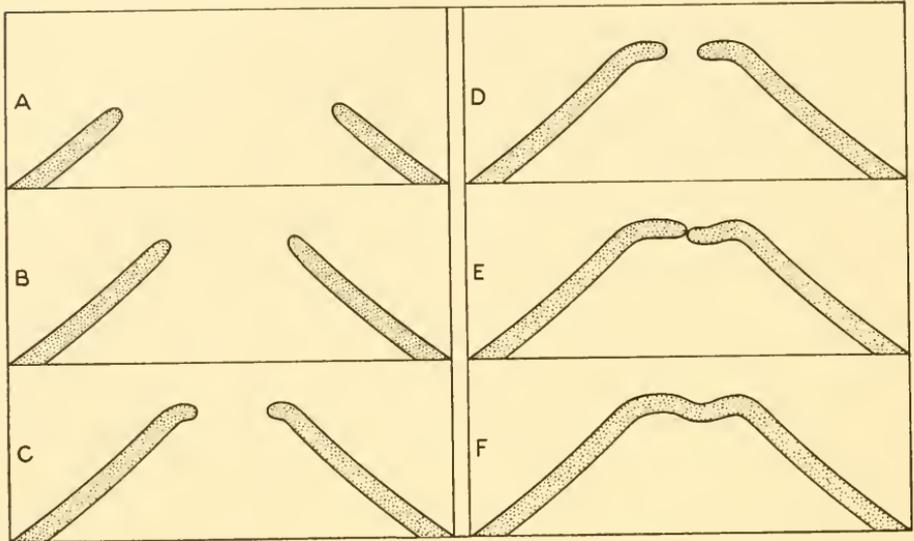


FIG. 13.—Diagram showing successive stages in a *hypha-to-hypha* fusion in one of the Higher Fungi (Pyrenomycetes, Discomycetes, Hymenomycetes, and Gastronomycetes). A, two ordinary lateral vegetative hyphae, elongating at their apices, by chance are approaching one another. B, growth has continued and the two hyphae have now come sufficiently close together to stimulate one another tropically. C, as a result of mutual stimulation, the ends of the hyphae have changed their direction of growth and are now growing toward one another. D, the ends of the two hyphae are rapidly approaching one another. E, the ends of the hyphae have met. F, fusion has taken place. Time which elapses between stage A and stage F varies with the rate of growth of the hyphae, but is often of the order of one hour.

older more internal parts of the mycelium where the medium is partially exhausted. The peripheral radial hyphae, so long as they are vigorously elongating, never fuse with one another. In media becoming exhausted as a whole hypha-to-hypha fusions may take place even between peripheral hyphae.

**Hypha-to-Peg Fusions.**—A hypha-to-peg fusion takes place between the end of a younger hypha which at first grows freely and independently in the culture medium and a special peg-like hypha

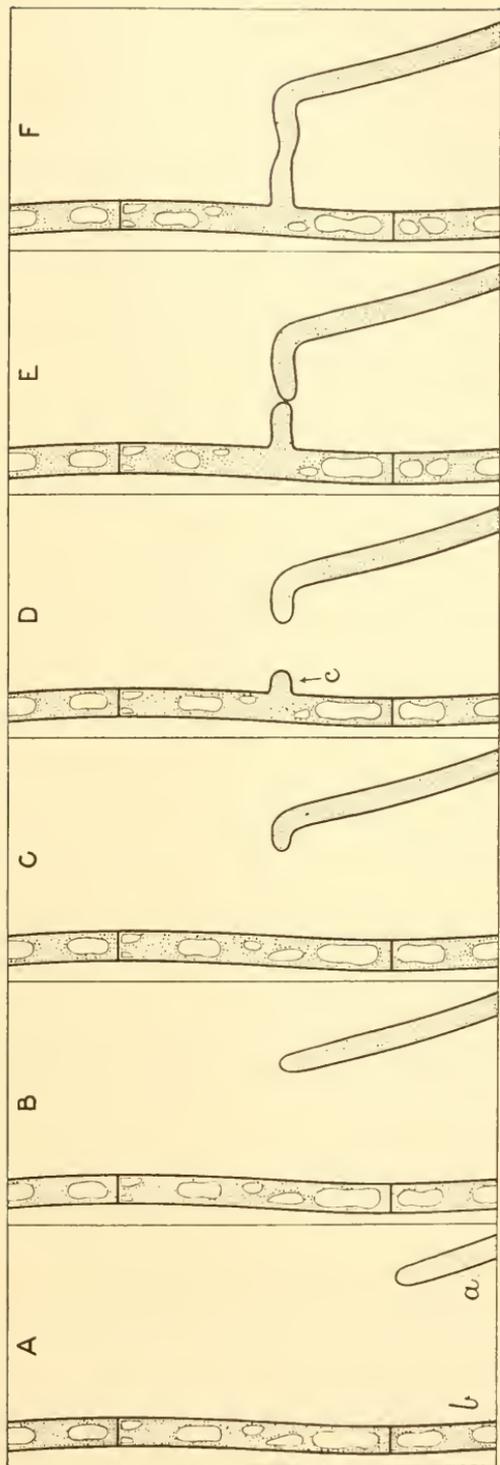


FIG. 14.—Diagram showing successive stages in a *hypha-to-peg* fusion in one of the Higher Fungi (Lyrenomyces, Discomycetes, Hymenomyces, and Gastromyces). A, a younger hypha *a*, elongating at its apex, by chance, by chance is approaching the side of an older hypha *b*. B, the end of the younger hypha has approached much nearer to the side of the older hypha, and the older hypha is now stimulating the younger hypha tropically. C: as a result of the stimulus, the end of the younger hypha has changed its direction of growth and is now growing directly toward the older hypha; the younger hypha is now stimulating the older hypha morphogenically. D: as a result of the morphogenic stimulus, the older hypha has sent out a peg *c* at a point opposite to the end of the younger hypha; the end of the younger hypha and the end of the peg are rapidly growing toward one another. E, the ends of the younger hypha and of the peg have fused together. F, the ends of the younger hypha and of the peg have fused together. Time which elapses between stage A and stage F varies with the rate of growth of the hyphae, but is often of the order of one hour.

developed on the side of an older hypha in response to a stimulus sent out by the younger hypha.

Successive stages in the formation of a hypha-to-peg fusion are shown diagrammatically in Fig. 14. At A, the end of a younger hypha (*a*) is seen approaching by chance the side of an older hypha (*b*). At B, the end of the younger hypha has approached within a certain distance of the side of the older hypha. At this stage of development the older hypha, either chemotropically or possibly by the emission of radiations, causes the younger hypha to alter its direction of growth so that the younger hypha grows directly toward the older hypha. An early stage in this tropism is shown at C. In response to a morphogenic stimulus received from the end of the younger hypha, the older hypha now sends out a very short peg-like hypha (*c*), as shown at D. Next, the younger hypha and the peg grow toward one another until their apices meet, as shown at E. Finally, the end of the younger hypha and the end of the peg flatten out against one another, the cylindrical walls of the hypha and peg become continuous with one another, the two walls appressed together become dissolved, a compound cylindrical tube is thus formed, and the protoplasm of the younger hypha becomes continuous with that of the peg, as shown at F.

Very often, when the end of a hypha meets the end of a peg, their axes are inclined to one another at an obtuse angle (*cf.* Fig. 14, E), with the result that, after a hypha and a peg have fused with one another, a more or less S-shaped twist can be seen about the point of their union. This S-shaped twist frequently aids one in recognising as a hypha-to-peg fusion an anastomosis which one has found after it has been formed.

**Peg-to-Peg Fusions.**—A peg-to-peg fusion takes place between the ends of two very short peg-like hyphae produced opposite to one another on the sides of two older hyphae. We may call the compound hypha formed by the union of two pegs a *bridging hypha* or *bridge*.

Successive stages in the formation of a peg-to-peg fusion are shown diagrammatically in Fig. 15. At A are seen two older hyphae running more or less parallel to one another. These hyphae in some way stimulate one another morphogenically so that at

opposing points and simultaneously they send out hyphal pegs, as shown at B. At C and D the pegs are shown growing toward one another; at E the pegs have come into contact at their apices; and, finally, at F the pegs have already fused at their apices and have formed a compound bridging hypha. In Fig. 16 a series of

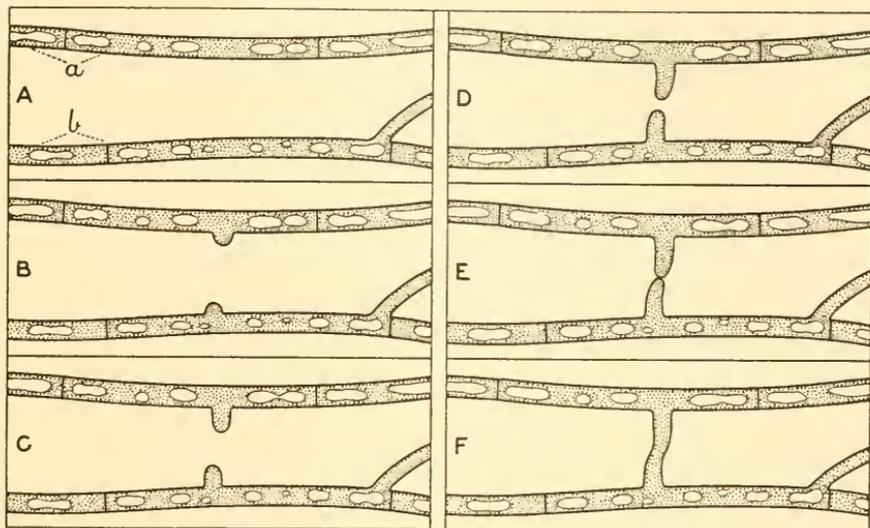


FIG. 15.—Diagram showing successive stages in a *peg-to-peg* fusion in one of the Higher Fungi (Pyrenomycetes, Discomycetes, Hymenomycetes, and Gymnomyces). A, two older hyphae, *a* and *b*, which happen to be more or less parallel to one another, are now stimulating one another morphogenetically where they have approached nearest to one another. B, as a result of this mutual stimulation, the two older hyphae have sent out opposing pegs. C, the pegs are growing in length. D, the ends of the two pegs are stimulating one another tropically, in consequence of which they are growing toward one another. E, the ends of the two pegs have met. F, the pegs have fused with one another and have thus formed a *bridging hypha* between the two older hyphae *a* and *b*. Time which elapses between stage A and stage F varies with the rate of growth of the pegs, but is often of the order of 30–40 minutes.

similar stages in a *peg-to-peg* fusion is shown, with the difference that the older hyphae are very much nearer together than those illustrated in Fig. 15.

The two older hyphae which give rise to a pair of pegs often run more or less parallel to one another (Figs. 15 and 16), but they may cross one another at any angle up to a right angle. When they cross one another, the two pegs are produced at the crossing, *i.e.* at those points in the two hyphae which are nearest to one another

(Fig. 17). The length of the pegs depends on the distance apart of the two older hyphae which produce them. This distance usually does not exceed  $25 \mu$ . Peg-to-peg fusions are often produced when

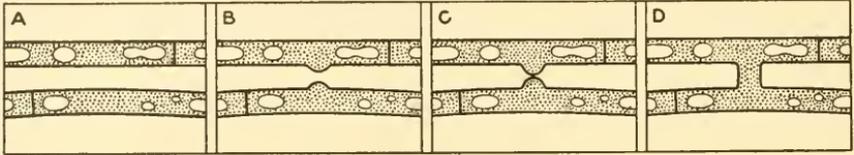


FIG. 16.—Diagram showing successive stages in a *peg-to-peg* fusion in one of the Higher Fungi (Pyrenomycetes, Discomycetes, Hymenomycetes, and Gastromycetes). In contrast with the diagram shown in Fig. 15, the two older hyphae are here very close together. A, parts of two long almost parallel older hyphae which lie very close together. B, a mutual morphogenic stimulation has resulted in the production of opposing pegs. C, the pegs have met. D, the pegs have fused and have formed a very short *bridging hypha* connecting the two older hyphae. Time which elapses between stage A and stage D is often of the order of 20 minutes.

the two older hyphae are  $7-15 \mu$  apart and still more frequently when the two older hyphae are only  $3-5 \mu$  apart. Even where two older hyphae happen to touch one another when running more or

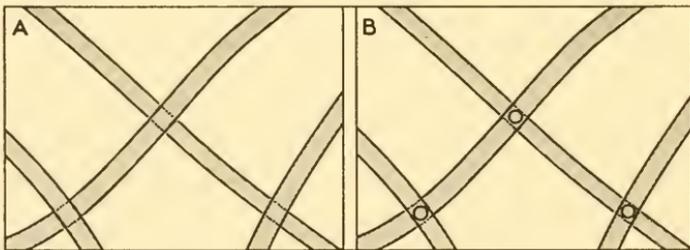


FIG. 17.—Diagram showing the formation of very short *bridging hyphae*, derived from *peg-to-peg* fusions, between older hyphae which have crossed one another. A, four older hyphae with three crossing places. B: the same, after the mycelium has increased in age and the culture medium has become exhausted; at each of the crossing places, a very short bridging hypha, seen in the axial view, has been formed. The construction of each bridging hypha might be accomplished in 10 minutes.

less parallel to or crossing one another, bridging hyphae may be formed. Under such conditions the two pegs, at the moment they begin to fuse with one another, are no more than convex processes which have flattened out against one another.

It sometimes happens that, where two older hyphae lie parallel

to one another, a *single peg* is produced by one of them and a *pair of pegs* facing the single peg by the other (Fig. 27, A, p. 53). As all the three pegs grow in length, both the pegs in the pair bend toward the opposing single peg, but the single peg bends towards one only of the pair of pegs and finally fuses with it alone (Fig. 27, B). As soon as fusion has taken place, the unpaired peg, as a rule, ceases to grow in length. In *Pleurance anserina*, a few triple-peg fusions have been found in which, apparently, the single peg produced by one older hypha fused with both of two pegs produced by the other older hypha (Figs. 30, D, and 31, B, p. 56).

When two hyphae in a mycelium run parallel to one another for some distance, they may become connected by several peg-to-peg fusions formed at intervals along their length and thus with their bridges they may come to have a scalariform appearance. Some scalariform hyphae in a mycelium of *Pleurance curvicolle* are to be seen in the photomicrograph reproduced in Fig. 1 (p. 5).

The two parallel hyphae about to form a scalariform structure stimulate one another *at a distance* from one another and at intervals along their length, and it is in response to this telemorphic stimulation that successive pairs of opposing pegs are produced. The two pegs of any pair then stimulate one another tropically, and it is in response to this zygotropic stimulation that they grow toward one another and meet. It used to be supposed that the scalariform structure which is formed during the process of scalariform conjugation in *Spirogyra* originates like the scalariform hyphae just described; but the recent observations of Czurda<sup>1</sup> and Saunders<sup>2</sup> have shown that this supposition is erroneous, for it has been found that conjugation in *Spirogyra* begins only after the two filaments concerned have come *into contact with one another side by side* and have become glued together by mucilage.

**Hook-to-Peg or Clamp-connexion Fusions.**—Clamp-connexions are characteristic of the secondary mycelium of the Hymenomycetes, Gastromycetes, Ustilaginaceae, and Tilletiaceae.<sup>3</sup> In certain species

<sup>1</sup> V. Czurda, "Zur Kenntnis der Copulationsvorgänge bei *Spirogyra*," *Archiv f. Protistenk.* Bd. LI, 1925. Cited from Saunders's paper, p. 233.

<sup>2</sup> Hazel Saunders, "Conjugation in *Spirogyra*," *Annals of Botany*, Vol. XLV, 1931, pp. 239-242, 255.

<sup>3</sup> Cf. these *Researches*, Vol. IV, 1931, p. 287.

of Hymenomycetes, e.g. *Coprinus curtus* and *Psalliota campestris*, which must be considered as exceptional, they are absent. Their formation has been described by Brefeld, Mlle Bensaude, Kniep, and others. Stages in the formation of a clamp-connexion in association with conjugate nuclear division in a diploid mycelium of *Coprinus fimetarius* (= *C. lagopus* of these volumes), as represented by Mlle Bensaude, are shown in Fig. 21 (p. 45). The final stage in the development of a clamp-connexion involves the formation of an anastomosis, and it is the mode in which this is accomplished that is of interest to us here. A clamp-connexion in a *Coprinus*, *Hypohoma*, *Collybia*, etc., is formed in the terminal cell of a growing hypha. From the middle of the cell a hook grows outwards, backwards, and then inwards toward the parent hypha; two cross-walls are formed, one across the base of the hook and the other across the parent hypha; and then the end of the hook fuses with the parent hypha, there being thus left a little air-space between the loop and the main hypha at the level of the septum which crosses the parent hypha.

Investigations on the details of clamp-connexion formation in two species of *Coprinus* have taught me that the hook of a clamp-connexion does not fuse directly with the main hypha as hitherto has been supposed, but with a little blunt process or peg sent out by the main hypha in response to a stimulus given by the apex of the hook.

Diploid mycelia of *Coprinus sterquilinus* and of *C. lagopus* were grown in hanging drops of cleared dung-agar, and the process of clamp-connexion formation was observed with the high power of the microscope. In *C. lagopus*, the clamp-connexions are small, but along each leading hypha they are produced at very regular distances apart<sup>1</sup> and at the rate of about one every fifty minutes.<sup>2</sup> In *C. sterquilinus*, on the other hand, the clamp-connexions are much larger; but they are not formed at every septum nor by every hypha<sup>3</sup> and, even on the leading hyphae, they are somewhat irregular in their distribution, are produced at longer intervals of time, and develop more slowly.<sup>4</sup>

<sup>1</sup> These *Researches*, Vol. IV, Fig. 118, p. 202.

<sup>3</sup> *Ibid.*, Fig. 89, p. 159.

<sup>2</sup> *Ibid.*, p. 243.

<sup>4</sup> *Ibid.*, Fig. 89, p. 159.

Some of the details in the formation of a clamp-connexion in *Coprinus sterquilinus* and *C. lagopus* are shown semi-diagrammatically in Fig. 18, where it will be seen : (1) that the hook grows backwards and comes almost or quite into contact with the main hypha (A and B); (2) that two septa are formed, one across the main hypha and the other across the base of the hook, and that both have a small central pore<sup>1</sup> such as is characteristic for the septa of

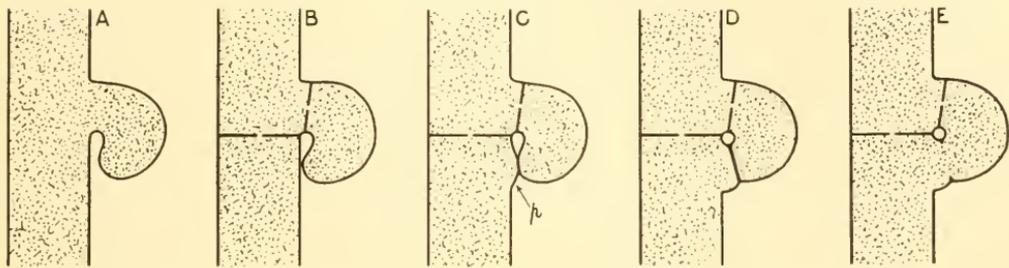


FIG. 18.—Semi-diagrammatic median longitudinal sections of a hypha of *Coprinus sterquilinus* or *C. lagopus* showing successive stages in a hook-to-peg fusion during the formation of a clamp-connexion. A, part of the terminal cell in an elongating hypha in which a clamp-connexion is being formed; already a hook has grown outwards and backwards from the main hypha. B, the hook has grown toward the main hypha and is almost or quite in contact with it; also two septa have been formed, one across the main hypha and the other across the base of the hook; each septum (as is usual for all septa in the Hymenomycetes) has a small central pore occupied by a protoplasmic bridge. C, the main hypha has sent out a peg, *p*, opposite the apex of the hook. D, the apex of the hook and the apex of the peg have now flattened out against one another. E, the double wall between the hook-cell and the peg has broken down and now there is protoplasmic continuity between the hook-cell and the peg. In a certain hypha of *C. lagopus* in which the formation of a clamp-connexion was observed, the time at which the hook began to be formed was taken as zero in the time-scale and the times at which the successive stages were attained were found to be as follows: A, 3 minutes; B, 15 minutes; C, 21 minutes; D, 22 minutes; E, 23 minutes. In *C. sterquilinus* and *C. lagopus* the thickness of the hyphae in which the formation of clamp-connexions was observed was about  $5.0\ \mu$  and  $2.5\ \mu$  respectively.

the mycelium of Hymenomycetes in general (B); (3) that the main hypha sends out a peg in the form of a blunt process opposite to the apex of the hook (C); (4) that the peg grows in size and that the end of the peg and the end of the hook press against, and flatten out against, one another (D); and, finally, (5) that the double wall between the hook and the peg breaks down and disappears so that

<sup>1</sup> For a full description and a treatment of the function of septal pores *vide infra*, Chapter II. On account of the small scale, pores have not been shown in the cell-walls in any illustration in this chapter other than Figs. 18, 19, and 22.

protoplasmic continuity between the peg and the hook becomes established (E).

In a completely formed clamp-connexion the part contributed by the peg can still be distinguished from that contributed by the hook, for where these parts became united there is usually a slight groove (Figs. 18, E, and 19. B. C. and D).

In one clamp-connexion of *Coprinus lagopus* the time which elapsed between the first formation of the hook as a tiny rudiment and complete fusion between the hook and the peg was 23 minutes. If the time at which the hook was first observed beginning its growth be taken as the zero of the time-scale, then the times at which the successive stages shown in Fig. 18 were attained were as follows: A, 3 minutes; B, 15 minutes; C, 21 minutes; D, 22 minutes; and E, 23 minutes. The first septum to be formed—that across the main hypha—was observed as a definite structure at the end of 14 minutes. Since, as we know from the cytological investigations of Mlle Bensaude, the conjugate division of the nuclei does not begin until the hook has become well advanced in growth, and since nuclear division must be over by the time the first septum has been formed, we may conclude that conjugate nuclear division in *C. lagopus* under laboratory conditions takes less than 14 minutes and is probably accomplished in about 12 minutes.

Before the hook and peg of a clamp-connexion have fused with one another, a nucleus is imprisoned in the clamp-cell (Fig. 21, p. 45), and protoplasmic continuity between the ultimate and penultimate cells of the main hypha is limited to the bridge of protoplasm stretching through the pore of the septum in the main hypha (Fig. 18, D). As soon as fusion has taken place, the imprisoned nucleus escapes from the hook into the penultimate cell of the hypha (Fig. 21); while, at the same time, the protoplasmic continuity between the ultimate and penultimate cells becomes strengthened, for protoplasm now extends without a break around the hook and through the pore of the septum situated at the base of the hook (*cf.* D and E in Fig. 18).

Nine successive stages in the formation of a clamp-connexion of *Coprinus sterquilinus* are shown semi-diagrammatically in Fig. 19, A. In a hanging drop of cleared dung-agar at room temperatures, all

the stages in the formation of two particular clamp-connexions were passed through in 40 and 45 minutes respectively. In both *C. sterquilinus* and *C. lagopus*, the hook-septum is always formed a minute or two after the septum across the main hypha (Fig. 19, A, cf. stages nos. 4, 5, and 6).

**The Function of a Clamp-connexion.**—The existence of clamp-

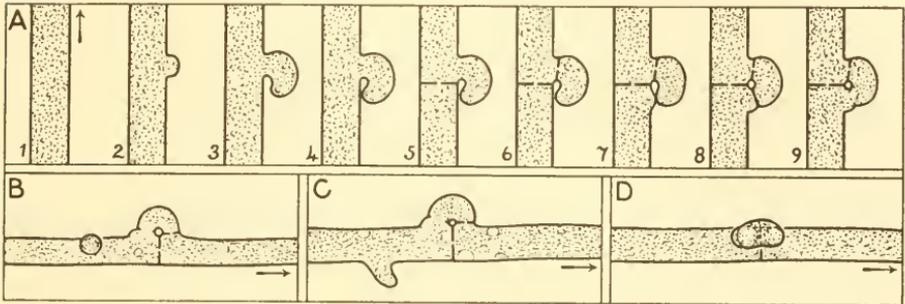


FIG. 19.—*Coprinus sterquilinus*. A, semi-diagrammatic representation of nine stages in the formation of a clamp-connexion on the side of a terminal cell of a hypha: no. 1, the middle part of the cell, the arrow indicates direction of growth; no. 2, a hook is growing outwards; no. 3, the hook has grown backwards and is now growing inwards; no. 4, the apex of the hook has touched the side of the main hypha; no. 5, a septum (with a central pore, characteristic of mycelial septa in general) has been formed across the main hypha; no. 6, a septum has been formed across the base of the hook, thus converting the hook into a clamp-cell; no. 7, a peg is growing outwards from the main hypha opposite the end of the hook; no. 8, the end of the hook and the end of the peg have flattened out against one another; and no. 9, the double wall between the hook and the peg has now broken down, the clamp-cell has thus fused with the penultimate cell, and the clamp-cell as such no longer has an individual existence. In a hanging drop of cleared dung-agar at room temperatures, all the stages in the formation of two clamp-connexions were passed through in 40 and 45 minutes respectively. B, C, and D, camera-lucida drawings of particular clamp-connexions: B and C, seen from the side; D, seen from above. The arrows indicate the direction of growth of the hyphae. On the left of each of the three clamp-connexions can be seen the part contributed by the peg. In B and C, the penultimate cell is sending out a lateral hypha. Magnification: A and C, 920: B and D, 860.

connexions in the diploid mycelium of the Hymenomycetes from the Thelephoraceae to the Polyporaceae, and in the Gastromycetes <sup>1</sup>

<sup>1</sup> Among Gastromycetes which develop clamp-connexions is *Sphaerobolus stellatus*. If one places a gleba in a hanging-drop of water, there soon grows out from it a diploid mycelium bearing clamp-connexions (*vide infra*).

In her recent Monograph "A Study of the Genus *Podaxis*" (*Mycologia*, Vol. XXV, 1933, pp. 1-33), Miss E. E. Morse has expressed the view that this genus, with its "unorganised hymenia," "may have arisen, *via* *Leucogaster* and *Alpova*, from the Ascomycetes." In material of *Podaxis pistillaris*, kindly sent to me by Miss Morse,

and Ustilaginales,<sup>1</sup> suggests that these structures assist the mycelium in some way in its physiological activities.

It has been supposed that clamp-connexions in Hymenomycetes and Gastromycetes have some sexual significance, but there does not seem to be any evidence of weight in favour of this view. It is true that the formation of the twin cross-walls of a clamp-connexion is preceded by conjugate nuclear division. Yet this does not indicate that the formation of clamp-connexions is a necessary accompaniment of conjugate nuclear division; for (1) in *Psalliota campestris*,<sup>2</sup> *Coprinus curtus*,<sup>3</sup> *Hypochnus solani* (*Rhizoctonia solani*),<sup>4</sup> and certain other Hymenomycetes, conjugate nuclear division goes on in the diploid mycelium without any clamp-connexions being constructed; and (2) in the pilei of many Agaricaceae, even in species in which the diploid mycelium produces clamp-connexions. *e.g.* *Coprinus lagopus*<sup>5</sup> and *Clitocybe expallens*,<sup>6</sup> clamp-connexions are not formed when the nuclei divide conjugately. Moreover, in the Uredineae, in which numerous conjugate nuclear divisions take place during the produc-

I have found numerous clamp-connexions in the mycelium attached to the base of the stipe. This new fact affords one more piece of evidence that *P. pistillaris* is a true Basidiomycete, and it also indicates that the genus *Podaxis* is not closely related to any known genera of Ascomycetes.

<sup>1</sup> Clamp-connexions occur in *Ustilago*, *Doassansia*, *Urocystis*, *Tilletia*, and *Entyloma*, and they develop by means of a backwardly-growing hook, just as in the Hymenomycetes. *Vide* R. Seyfert, "Über Schnallenbildung in Paarmyzel der Brandpilze," *Zeitschrift f. Botanik*, Bd. XIX, 1927, pp. 577-601.

<sup>2</sup> Max Hirmer, "Zur Kenntnis der Vielkernigkeit der Autobasidiomyeten. I.," *Zeitschrift f. Botanik*, Vol. XII, 1920, pp. 657-674.

<sup>3</sup> H. Brunswik, "Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyceten Gattung *Coprinus*," in Goebel's *Botanische Abhandlungen*, Heft V, 1924, pp. 124-125, 142.

<sup>4</sup> K. O. Müller, "Untersuchungen zur Entwicklungsgeschichte und Biologie von *Hypochnus solani* (*Rhizoctonia solani*)," *Arbeiten aus der Biologischen Reichsanstalt für Land- und Forstwirtschaft*, Bd. XIII, 1924, pp. 208-222.

<sup>5</sup> My own observations.

<sup>6</sup> Hans Kniep, "Über die Bedingungen der Schnallenbildung bei den Basidiomyzeten," *Flora*, Bd. XI, 1918, p. 380. Kniep distinguished three groups of these fungi: (1) those with clamp-connexions in all stages of development of the diploid mycelium and of the fruit-body, and with a clamp-connexion at each cross-wall; (2) those in which clamp-connexions are completely absent; and (3) those in which clamp-connexions occur more or less irregularly. He also found that, in certain species, but by no means all, when a diploid clamp-bearing mycelium is submerged in a culture medium, it ceases to produce clamp-connexions.

tion of the aecidiospores and the development of the diploid mycelium, clamp-connexions are unknown.

A clamp-connexion in a diploid mycelium, as we have seen, may be formed in a very few minutes, but it may persist and be active for many days, weeks, or months. Let us now turn our attention away from the clamp-connexion's mode of formation and endeavour to find in its location and in its structure some explanation of its function.

Among the facts which may bear upon the problem of the function of the clamp-connexion in the diploid mycelium of Hymenomycetes and Gastromycetes are the following : (1) in both haploid and diploid mycelia each septum has a small central open pore through which protoplasm can flow from cell to cell ; (2) under natural conditions in the open, the haplophase of a mycelium is in general of short duration and soon passes into the diplophase ; and (3) since, as a rule, wild fruit-bodies, as well as wild sclerotia and mycelial cords which act as magazines and subsequently produce fruit-bodies, are diploid, these structures are formed not by haploid mycelia but by diploid mycelia, so that it is the clamp-bearing diploid mycelium, and not the clamp-less haploid mycelium which has the task of evacuating its protoplasm and despatching it either directly to the fruit-body or to temporary storage organs.

The flow of labile protoplasm, driven by vacuolar pressure, through the hyphal pipes of a mycelium encounters a certain amount of resistance at each septum ; for the central pore of each septum, through which the protoplasm must pass, is relatively small (about  $1 \mu$  in diameter). If, therefore, we suppose that of two hyphae, where other things are equal, one has single septa between adjacent cells, as in a haploid mycelium, and the other has twin septa between adjacent cells, as in a diploid mycelium, it is clear that the flow of protoplasm would be faster from cell to cell in the second or diploid type of hypha than in the first or haploid type of hypha.

In view of the above discussion it may be concluded that, in the Hymenomycetes and Gastromycetes, the clamp-connexion may be regarded as a means for providing between any two adjacent cells of a diploid mycelium two septa instead of one and, therefore, *two passage-ways for the streaming of the protoplasm instead of one*. Thus,

where clamp-connexions exist in a diploid mycelium, they serve to facilitate the translocation of protoplasm from cell to cell and, in particular, from the vegetative diploid mycelium as a whole either directly into a fruit-body or into such temporary storage organs as sclerotia or mycelial cords which are destined eventually to produce fruit-bodies.

Where clamp-connexions occur in hymenomycetous fruit-bodies, e.g. in those of *Armillaria mucida*,<sup>1</sup> *Corticium petrophilum*,<sup>2</sup> and *Peniophora clavigera*,<sup>3</sup> they doubtless serve to facilitate the passage of protoplasm through the fruit-body tissues and into the basidia and other hymenial elements.

#### Clamp-connexions and the Hooks of Ascogenous Hyphae.—

The hooks produced by ascogenous hyphae in *Pyronema confluens* (Fig. 20) and certain other Discomycetes<sup>4</sup> have been regarded by Mlle Bensaude,<sup>5</sup> Kniep,<sup>6</sup> and Gäumann<sup>7</sup> as homologous with the clamp-connexions of the Hymenomycetes; but between hooks and clamp-connexions there are important differences, which may be summarised as follows: (1) whereas clamp-connexions occur in series along the hyphae of a vegetative mycelium, hooks occur not in a vegetative mycelium but in the terminal branches of ascogenous hyphae; (2) whereas a clamp-connexion is formed in the middle of a terminal cell of a hypha, a hook is formed at the extreme end of a terminal cell of a hypha; (3) whereas in a clamp-connexion the cell in which a single nucleus is temporarily imprisoned is formed from a lateral branch of the main hypha, in a hook the corresponding cell is cut off from the end of the main hypha after this has become bent backwards at its apex; and (4) whereas clamp-connexions

<sup>1</sup> Vide H. Kniep's illustration in Strasburger's *Text-book of Botany*, English ed. No. 6, London, 1930, Fig. 413, p. 458.

<sup>2</sup> H. Bourdot and A. Galzin, *Hyménomycètes de France*, 1927, Fig. 70, p. 230.

<sup>3</sup> *Ibid.*, Fig. 78, p. 280.

<sup>4</sup> Not all Discomycetes develop hooks at the ends of their ascogenous hyphae. Among discomycetous species in which the asci are formed from binucleate ascogenous cells, no hooks being formed, are: *Geopyxis cutinus* (Guillermont, 1905); and *Plicaria succosa* and *Acetabula leucomelas* (Maire, 1905). Vide Gäumann and Dodge, *Comparative Morphology of Fungi*, 1928, p. 131.

<sup>5</sup> Mathilde Bensaude, *Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes*, Nemours, 1918, pp. 117-123.

<sup>6</sup> Hans Kniep, *Die Sexualität der niederen Pflanzen*, Jena, 1928, pp. 392-393.

<sup>7</sup> E. Gäumann, *Vergleichende Morphologie der Pilze*, Jena, 1926, pp. 399-400.

connect two successive cells and, as a rule, are not points of departure for new lateral branches, the end-cell of each hook, after fusion with the third cell from the end of the hypha, immediately grows forward to form a new very short branch which in turn soon becomes hooked at its apex.

From a consideration of the facts just brought forward there seems to be but little doubt that the physiological significance of the hook in the Discomycetes is quite different from that of the clamp-connexion in the Hymenomyces. Whereas the clamp-connexion, as we have seen,

may be regarded as a means for facilitating the flow of protoplasm from cell to cell, the hook may be regarded as a means for constructing numerous ascus cells which shall be compactly arranged in a discoid hymenium. Each ascogenous hypha bends around at its tip to form a hook, then conjugate nuclear division takes place, after which twin septa are constructed. The result of these operations is that the penultimate cell of the hook is at the top of the hook and therefore in a good position to develop into an ascus. The terminal cell of the bent hypha, which has become directed backwards and so has taken up a lower position than the ascus cell above it, now fuses with the third cell from the end of the bent

hypha and immediately thereafter it grows outwards to form a short lateral branch which soon becomes hooked at the end and

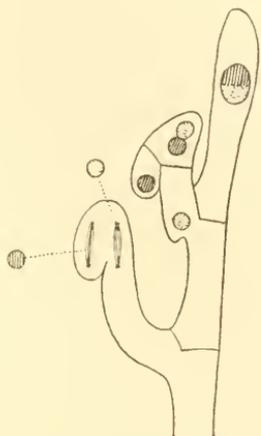


FIG. 20.—*Pyronema confluens*.

Diagram showing a branched ascogenous hypha forming hooks and asci. The end of the lower branch has become bent backwards and in it conjugate nuclear division is taking place. In the end of the upper branch conjugate nuclear division has been completed and two septa have been formed; the ultimate cell of the curved hypha, directed backwards, is the *hook-cell* which is destined to fuse with the adjacent *stalk-cell*; while the penultimate cell, which contains two nuclei, is destined to develop into an *ascus*. The uppermost erect cell, containing a fusion nucleus, is a young *ascus*; it originated as a penultimate cell in the same manner as the penultimate cell of the upper lateral branch, and the *hook-cell* below fused with the *stalk-cell* before it gave rise to the hooked lateral branch which is seen growing away from it. From Mlle Bensaude, after Claussen.

repeats the process of ascus-cell formation already described. It is evident that the formation of hooks is an important factor in arranging the asci in an advantageous manner in the hymenium.

Gäumann,<sup>1</sup> in his discussion of the phylogeny of the Hymenomyces, says "clamp formations would be incomprehensible if one could not explain them as a relic of the Ascomycetes." On the other hand, it seems to me not impossible that the hooks of the ascogenous hyphae of the Discomycetes and the clamp-connexions of the diploid mycelium of the Hymenomyces may, in response to two quite different physiological needs, have originated independently of one another.

In attempting to homologise the hooks of *Pyronema confluens* and other Discomycetes with the clamp-connexions of Hymenomyces, Mlle Bensaude, Kniep, and Gäumann have chosen as examples of clamp-connexions those which, in some species of Hymenomyces, are to be found at the base of the basidia and not those which occur in the diploid mycelium and in the general tissues of the fruit-bodies. It is true that, in the formation of a hook of an ascogenous hypha and of a clamp-connexion at the base of a basidium, conjugate nuclear division and the fusion of two uninucleate cells take place, but there the analogy ceases; for (1) a basidium arises from the *ultimate* cell of an *erect* hypha, whereas an ascus arises from the *penultimate* cell of a *semicircularly bent* hypha; and (2) the hypha which forms the clamp-connexion at the base of a basidium is a *special lateral hypha*, whereas the cell which in the hook of an ascogenous hypha forms the structure which is supposed by Mlle Bensaude, Kniep, and Gäumann to correspond with a clamp-connexion at the base of a basidium is the *terminal cell* of the ascogenous hypha.<sup>2</sup> These differences in detail in the mode of formation of the hooks in Discomycetes and of the clamp-connexions at the base of the basidia of Hymenomyces appear to me to greatly

<sup>1</sup> E. Gäumann, *loc. cit.*, p. 400; also E. Gäumann and C. W. Dodge, *Comparative Morphology of Fungi*, New York, 1928, p. 421.

<sup>2</sup> These morphological dissimilarities have previously been noted, among others by M. and Mme Moreau (*Rev. gén. de Bot.*, T. XXXVII, 1925, pp. 470-471) and by H. C. I. Gwynne-Vaughan and B. Barnes, *Structure and Development of the Fungi*, Cambridge, 1927, p. 133.

weaken the supposition that hooks and clamp-connexions are homologous structures.

According to M. and Mme Moreau,<sup>1</sup> the asci in species of the lichen genera *Parmelia*, *Physcia*, and *Anaptychia* are formed from terminal cells of ascogenous hyphae, and these hyphae resemble the hyphae of the diploid mycelium of the Hymenomycetes in that they consist of a *chain of binucleate cells with a clamp-connexion between every two successive cells*. M. and Mme Moreau used fixed and stained material and did not watch the "ascogenous clamp-connexions" actually being developed by the living hyphae. Therefore, there is the possibility that each "clamp-connexion" arises in the first place by the *Pyronema confluens* hooking method and that the binucleate penultimate cell of the hook, instead of developing into an ascus, grows forward as a hypha, becomes hooked at its end in its turn, repeats the process of "clamp-connexion" formation just described, and so on. In this way, by sympodial branching, there might be produced along a multicellular hypha a series of structures resembling in appearance the clamp-connexions of the Hymenomycetes but having a distinctly different mode of origin. If, on the other hand, the "ascogenous clamp-connexions" observed by M. and Mme Moreau are formed not from the curved end of a hypha but from a special branch-hypha which grows backwards from the middle of a straight terminal cell, there still remains the possibility that the clamp-connexions of the Hymenomycetes and of the Lichen-fungi under discussion may have arisen, not by inheritance from a common ancestor, but by parallel evolution.

**Biological Significance of the Hook of a Clamp-connexion growing Backwards instead of Forwards.**—When studying the development of the mycelium of *Coprinus stercorarius*, Brefeld<sup>2</sup> observed that, when a clamp-connexion is being formed, the hook always grows *backwards*. Subsequent observations have taught us that this rule holds for Hymenomycetes and Gastronomycetes in

<sup>1</sup> M. and Mme F. Moreau: (1) "Recherches sur quelques Lichens des genres *Parmelia*, *Physcia* et *Anaptychia*," *Rev. gén. de Bot.*, T. XXXVII, 1925, pp. 385-417; (2) "Le mycélium à boucles chez les Ascomycètes," *Compt. rend.*, 18 avril, 1922; and (3) "Crochets et anses ascogènes," *Bull. Soc. Myc. France*, T. XLI, 1926, pp. 469-471.

<sup>2</sup> O. Brefeld, *Untersuchungen über Pilze*, Heft III, Leipzig, 1877, pp. 17-18.

general. I myself have observed the backward growth of the hook in various Coprini, including *C. sterquilinus* and *C. lagopus*; and, from the disposition of the septa at the clamp connexions, I have made the deduction that backward growth of the hook occurs in *Omphalia flavida*, *Polyporus squamosus*, and *Sphaerobolus stellatus*.

In view of the fact that on leading hyphae of the mycelium of *Coprinus lagopus*, other Hymenomycetes, and Gastromycetes the ordinary vegetative lateral hyphae grow *forward*, so as to make an acute angle<sup>1</sup> with the parent hypha, it is of interest to ask the question: what biological advantage, if any, accrues to a mycelium by reason of the fact that a clamp-connexion hook, which originates as a lateral hypha, grows backwards instead of forwards?

For some years the problem of the backward growth of the hook of clamp-connexions puzzled me. At length, in November, 1932, I suddenly thought of a reasonable solution based on certain experimental data having to do with the relation of nuclei and cytoplasm.

In *Coprinus lagopus* (= *C. fimetarius* of Mlle Bensaude) Mlle Bensaude<sup>2</sup> found that the formation of a clamp-connexion is associated with a conjugate nuclear division, and in a diagrammatic illustration, reproduced here in Fig. 21, she represented five successive stages in the whole process. In the fourth stage (Fig. 21), one of the nuclei is imprisoned temporarily in the clamp-cell; while, in the fifth stage, fusion has just been completed and the imprisoned nucleus is escaping and joining its yoke-companion.

Mlle Bensaude's fourth stage of clamp-connexion development I have represented diagrammatically in Fig. 22 at A. Here are shown three complete cells: (1) the *ultimate* cell which at its apex<sup>3</sup> is growing rapidly in length, (2) the *penultimate* cell which has ceased to grow in length, and (3) the *clamp-cell* destined soon to fuse with the penultimate cell of the parent hypha. While in Fig. 22 at A

<sup>1</sup> In *Coprinus lagopus* this angle is 10°–45°. Vide these *Researches*, Vol. IV, 1931, p. 199 and Fig. 118, p. 202.

<sup>2</sup> Mathilde Bensaude, *Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes*, Nemours, 1918, pp. 1–156.

<sup>3</sup> In mycelia, as Reinhardt showed forty years ago ("Das Wachstum der Pilzhypphen." *Jahrb. f. wiss. Bot.*, Bd. XXIII, 1892), the hyphae elongate at their extreme tips only. Intercalary growth in length of mycelial hyphae is unknown.

is represented a *normal* hypha in which the hook is directed *backwards*, at B there is represented a *theoretical* hypha, in general similar to A but having the hook of the clamp-cell directed *forwards* instead of backwards.

Let us now compare the disposition of the nuclei in the normal hypha A and in the unnatural hypha B. At once we see that, whereas in A the ultimate cell contains two nuclei and the penultimate cell one, in B this is reversed, for here the ultimate cell contains one nucleus and the penultimate cell two.

The disposition of the nuclei in A and B in Fig. 22 is, of course, only temporary; for, as soon as the clamp-cell fuses with a cell of the main hypha, the penultimate cell in A and the ultimate cell in B both come to contain a pair of nuclei.

As growth continues and new clamp-connexions are formed, in such a normal hypha as A in Fig. 22 there are *always two nuclei* in the ultimate growing cell; whereas, in such an unnatural hypha as B, the ultimate growing cell would contain *sometimes two nuclei* and *sometimes one*, the reduction from two nuclei to one taking place during the completion of the last half of the development of each new clamp-connexion.

In *Coprinus lagopus*, in respect to the formation of clamp-connexions on a leading hypha, the following time-data have been determined: (1) a new clamp-connexion is formed about every 50 minutes; (2) the time required for the formation of a single clamp-connexion is about 23 minutes; (3) the time which elapses between the formation of the first septum across the

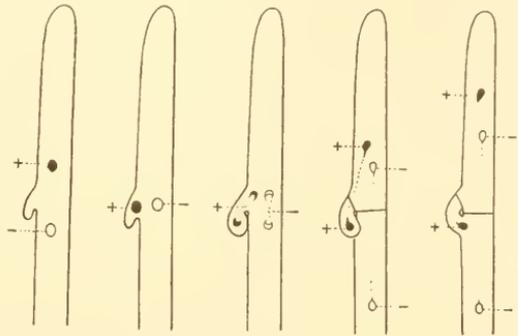


FIG. 21.—Diagram showing, from left to right, five successive stages in a conjugate nuclear division associated with the formation of a clamp-connexion in a hypha of a diploid mycelium of *Coprinus lagopus* (= *C. fimetarius* of Mlle Bensaude) as represented by Mlle Bensaude. For a detailed explanation *vide* Vol. IV, p. 155. Copied for the author by Dr. Nellie Carter.

main hypha and complete fusion of the clamp-cell and the penultimate cell is about 9 minutes; and (4) the time which

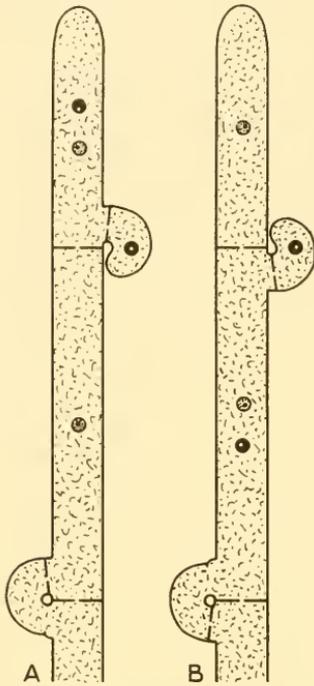


FIG. 22.—*Coprinus lagopus* and other Hymenomycetes. A, a diagram to show the disposition of the nuclei in a diploid hypha during the time a nucleus is imprisoned in the clamp-cell and just before a clamp-connexion has been fully formed. The hook is directed backwards and there are two nuclei in the apical cell. B, a diagram similar to A, but in which the hook is represented as growing forwards. Here there is only one nucleus in the apical cell.

elapses between the formation of the septum across the base of the hook and complete fusion of the clamp-cell and penultimate cell (time between stages B and E in Fig. 18, p. 35) is about 8 minutes.<sup>1</sup>

From the data just set forth it is evident that, in *Coprinus lagopus*, after the first-formed septum separating the ultimate from the penultimate cell has been formed, the penultimate cell of a normal hypha, such as A in Fig. 22, actually is, and the ultimate cell of such a theoretical hypha as B in Fig. 22 would be, without a second nucleus for about 9 minutes.

Therefore, in *Coprinus lagopus*, whereas in a normal hypha in which the hook grows backwards there are always two nuclei in the ultimate cell, in an unnatural hypha such as that of B in Fig. 22 where the hook is supposed to grow forward, if the growth-rate were the same as in a normal hypha, there would be in the ultimate cell: two nuclei for 41 minutes, then one nucleus for 9 minutes, then two nuclei for 41 minutes, then one nucleus for 9 minutes, and so on indefinitely.

There can be no doubt that the nucleus of a cell has a profound

<sup>1</sup> Records were made of the times at which all the externally visible events in the formation of a clamp-connexion were accomplished. In one instance, the hook took 4 minutes to grow outwards, backwards, and inwards until it touched or almost touched the main hypha. Then there was a pause for 10 minutes (during which conjugate nuclear division was going on) at the end of which period a septum could

influence on growth. Thus Gerassimoff,<sup>1</sup> on artificially increasing the size of the nucleus in *Spirogyra* filaments, observed that the cytosome increased in size correspondingly, so that the normal filaments developed into thicker 'giant' filaments. These experiments, as Wilson<sup>2</sup> has remarked, "seem to afford decisive proof that the nucleus is the *primary* agent in the constructive processes of cytoplasmic growth." It has also been shown that enucleated fragments of *Amoeba*, *Polystomella*, *Stylonychia*, and other Protista, as well as enucleated fragments of cells of Higher Plants (*Cucurbita*, etc.) are incapable of further growth and live only for a relatively short space of time.<sup>3</sup> Haberlandt<sup>4</sup> emphasised the fact that local growth in a cell-wall of a Higher Plant is always preceded by a movement of the nucleus to the point of growth. It appears that growth is associated with "an extensive interchange of materials between nucleus and cytoplasm, so that a certain proportion of nuclear and cytoplasmic substances (the 'nucleoplasmic ratio') must be maintained" if growth is to continue; and that a single spherical nucleus, through the surface of which the interchanges occur, has a strictly limited 'sphere of influence' so far as the cytoplasm is concerned.<sup>5</sup>

From our general knowledge of the effect of the nucleus on growth, in part based on the facts just set forth, we should be justified in assuming that, in the Hymenomycetes generally, just as in the Higher Plants and in Animals, the nucleus interacts with the cytosome and thus affects the growth of the hyphae which make up the thallus. In one particular Hymenomycete, *Pholiota mutabilis*,

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be seen as just having been formed across the main hypha. A minute later, the second septum—that across the base of the hook—came into view. Six minutes later a peg was pushed out from the main hypha opposite the end of the hook, and after about two minutes more the hook and peg fused completely. The hook-septum is always formed a little later than the septum across the main hypha.

<sup>1</sup> J. J. Gerassimoff, "Ueber den Einfluss des Kerns auf das Wachstum der Zelle," *Bull. Soc. Imp. Natur.*, Moscow, 1901, pp. 185-220. For Gerassimoff's other papers, *vide* E. B. Wilson, *The Cell*, p. 1164.

<sup>2</sup> E. B. Wilson, *The Cell in Development and Heredity*, New York, third edition, 1925, p. 655.

<sup>3</sup> For the literature on this subject *vide* E. B. Wilson, *loc. cit.*, pp. 657-662.

<sup>4</sup> G. Haberlandt, *Physiologische Pflanzenanatomie*, Aufl. 6, 1924, Leipzig, pp. 24-25.

<sup>5</sup> L. W. Sharp, *An Introduction to Cytology*, New York, 1926, pp. 53-54.

as a result of observations and experiments made by Harder, the effect of the nucleus on growth has been placed beyond doubt.

Harder<sup>1</sup> observed that, in *Pholiota mutabilis*, the rate of growth of a diploid mycelium (two nuclei in each cell) is about twice as great as that of either of the two haploid mycelia (one nucleus in each cell) from which it has been derived. By micro-dissection of hyphae in the state shown in Fig. 22, A, he isolated uninucleate penultimate cells and afterwards succeeded in getting them to grow and to develop into haploid mycelia. He then found that these artificially produced haploid mycelia had a rate of growth of only about one-half that of the diploid mycelia from which they had come. This difference in growth-rate can readily be explained on the supposition that the amount of nuclear material in a cell of *Pholiota mutabilis* affects the rate of growth, a conjugate pair of nuclei causing the cytosome to grow in length about twice as fast as a single nucleus.

The solution of the problem of the biological advantage accruing to a diploid mycelium from the arrangement that the hooks of the clamp-connexions grow backwards instead of forwards seems to follow naturally from Harder's work and may be stated thus. If the hook of each clamp-connexion were to grow forward, the terminal cell of each hypha, in which alone growth in length takes place, would periodically (during the formation of each clamp-connexion) have its nucleoplasmic ratio upset to the extent of being reduced to one-half, thus affecting growth adversely; whereas, when the hook grows backwards, as is actually the rule, there are always two nuclei in the terminal cell and the nucleoplasmic ratio is never greatly disturbed and remains relatively constant.

**Mlle Bensaude's Second Mode of Formation of a Clamp-connexion unconfirmed.**—Mlle Bensaude,<sup>2</sup> as a result of her cytological investigations on *Coprinus lagopus* (her *C. fimetarius*), concluded that clamp-connexions in this species are formed in two ways: (1) as shown in Fig. 21 (p. 45), and (2) as shown in Fig. 23.

<sup>1</sup> R. Harder, "Zur Frage nach der Rolle von Kern und Protoplasma im Zellgeschehen und bei der Übertragung von Eigenschaften," *Zeitschrift für Botanik*, Bd. XIX, 1927, pp. 350-351.

<sup>2</sup> Mathilde Bensaude, *loc. cit.*, pp. 64-66.

In Mlle Bensaude's first mode of clamp-connexion formation the two septa are formed *before* the hook fuses with the main hypha, whereas in her second mode (Fig. 23) the two septa are formed *after* the hook has fused with the main hypha.

My own direct observations on the development of individual clamp-connexions of *Coprinus lagopus* and *C. sterquilinus* have convinced me that the two septa of a clamp-connexion in these species are always formed *before* the hook fuses with the main hypha (Figs. 18 and 19, pp. 35 and 37) and never afterwards. This conclusion is supported by Harder's<sup>1</sup> experimental work on *Pholiota mutabilis*: the temporary uninucleate condition of the penultimate cell of the hyphae upon which he operated was a *sine qua non* for producing haploid mycelia artificially. I am therefore of the opinion that Mlle Bensaude's first mode of clamp-connexion formation was correctly conceived but that her second mode was based on some artifact or a misinterpretation of her cytological preparations.

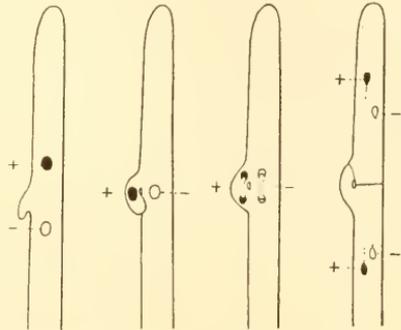


FIG. 23.—*Coprinus lagopus* (= *C. fimetarius* of Mlle Bensaude). Mlle Bensaude's diagram showing, from left to right, four stages in her supposed second mode of formation of a clamp-connexion. The hook is represented as fusing with the main hypha *before* the two septa are formed instead of afterwards (cf. Fig. 21). Copied for the author by Dr. Nellie Carter.

An account of particular observations on the formation of hypha-to-hypha, hypha-to-peg and peg-to-peg fusions in the species of fungi in which these fusions have been investigated by the author will now be given.

**Pleurae curvicolla.**—*Pleurae curvicolla*, a coprophilous Pyrenomycete having about 124 spores in each ascus, was the first species investigated. Spores were sown in hanging drops of cleared dung-agar on one day, and on the next day the mycelium thus originating formed many hyphal fusions (Fig. 24). Hypha-to-hypha,

<sup>1</sup> R. Harder, *loc. cit.*

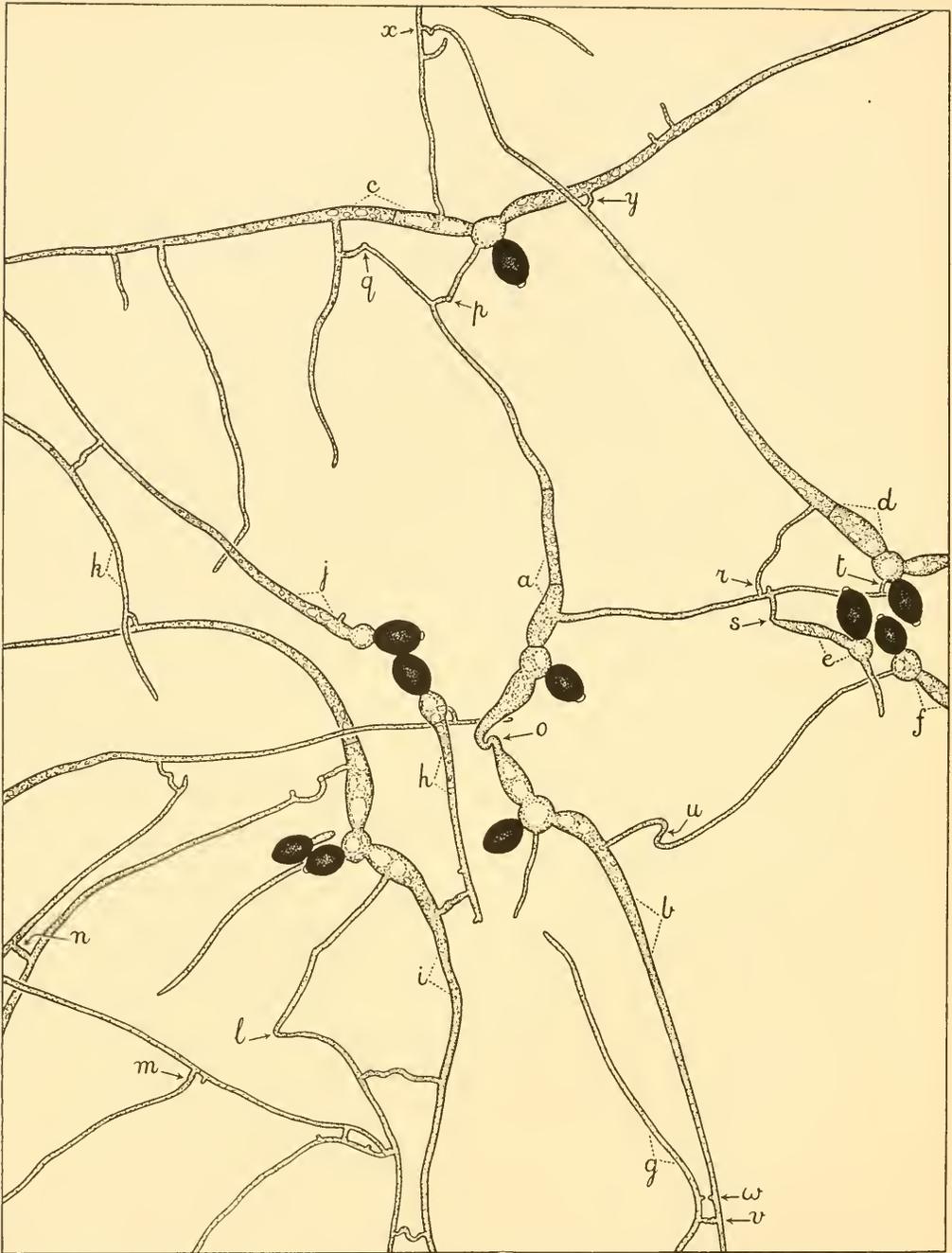


FIG. 24.—*Pleurage curvicolla*. Hyphal fusions in young mycelia. Spores sown in a hanging drop of dung-agar, the mycelia drawn with the *camera lucida* 31 hours later. The mycelium *a* is united: with the mycelium *b* by a hypha-to-hypha fusion at *o*; with the mycelium *c* by two hypha-to-peg fusions at *p* and *q*; and with the mycelia *d* and *e* by other fusions which took place at *r*, *s*, and *t*. The mycelium *b* is united with the mycelium *f* by a hypha-to-hypha fusion at *u* and with the mycelium *g* by a peg-to-peg fusion at *v*. At *w* can be seen two pegs which ceased growth before they met, the arrest of their growth probably having been caused by the near-by fusion at *v*. The main hypha of the mycelium *d* was observed growing toward the uppermost hypha of the mycelium *c* and fusing with a peg which this uppermost hypha sent out at *x*. A peg-to-peg fusion at *y* serves as another connexion between the mycelia *d* and *c*. Peg-to-peg fusions can be observed connecting the mycelium *h* with the mycelium *i*, and the mycelium *j* with the mycelium *k*. Among the remaining hyphal fusions, we may interpret that at *l* as a hypha-to-hypha fusion, that at *m* as a hypha-to-peg fusion, and that at *n* as a peg-to-peg fusion. Magnification, 404.

hypha-to-peg, and peg-to-peg fusions were all observed coming into existence.

Two stages in a hypha-to-hypha fusion, drawn with the *camera*

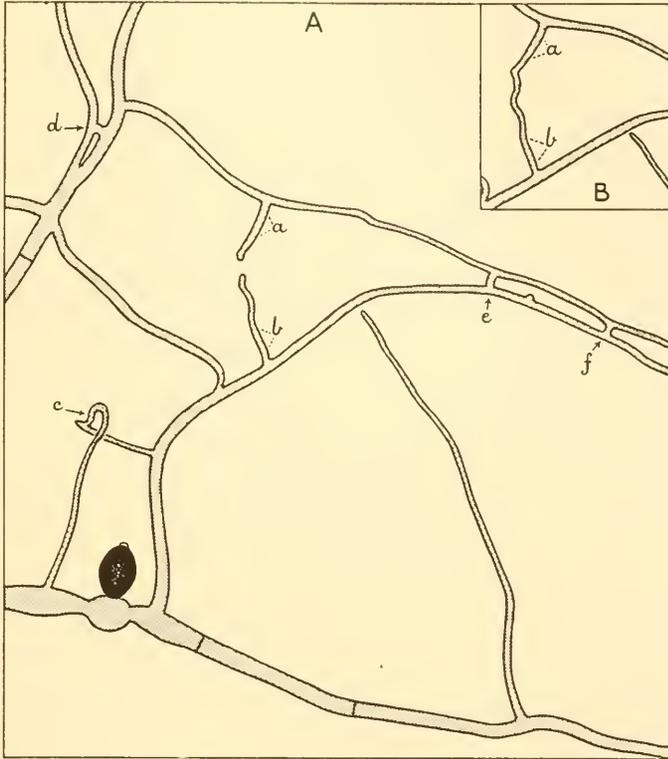


FIG. 25.—*Pleurage curvicolla*. The formation of a hypha-to-hypha fusion. Spores, of which one is shown, were sown in cleared dung-agar about 20 hours before the drawing A was made. In A are seen two branch-hyphae, *a* and *b*, which at first grew independently of one another in the culture medium, but by chance came near to one another. When about  $12\ \mu$  apart, they stimulated one another, with the result that their ends are now growing toward one another. Half an hour later their ends had met and fused, as shown in B. In A, *c* is probably a hypha-to-peg fusion, while *d*, *e* and *f* are all peg-to-peg fusions. Magnification, 434.

*lucida*, are shown in Fig. 25. In A the two branch-hyphae *a* and *b* which at first were growing independently of one another in the culture medium have now become stimulated by one another and their ends are growing toward one another. They began to react

to the tropic stimulus when about  $12\ \mu$  apart. In B the two hyphae have completely fused. The time which elapsed between stage A and stage B was 10 minutes.

Stages in two hypha-to-peg fusions are shown in Fig. 26. In A the hypha *a* has induced in the hypha *b* the formation of the peg *c* and the ends of both hypha and peg are growing toward one another.

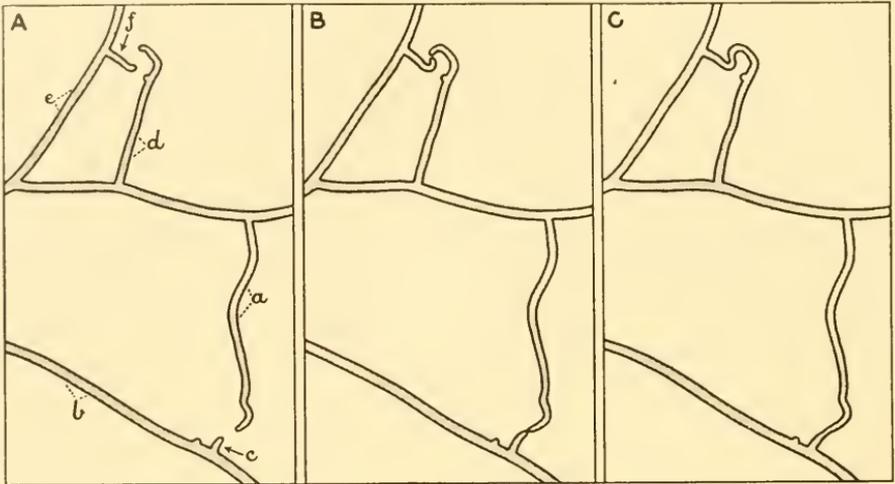


FIG. 26.—*Pleurage curvicolla*. Stages in the formation of two hypha-to-peg fusions. A: the hypha *a* by chance approached the hypha *b* obliquely; when about  $15\ \mu$  away from *b*, it turned toward *b* and caused *b* to send out the peg *c*; the ends of the hypha *a* and the peg *c* are now growing toward one another. Similarly, the hypha *d* grew toward the hypha *e* and caused *e* to send out the peg *f*; the ends of the hypha *d* and of the peg *f* are now growing toward one another. B, 10 minutes after A: the hypha *a* and the peg *c* are now touching at their ends; the end of the hypha *d* is almost touching the end of the peg *f*. C, 5 minutes after B: both of the hypha-to-peg fusions are now complete. Magnification, 434.

In B, 10 minutes after A, the hypha and peg have just met; while in C, 5 minutes after B, the hypha and peg have fused. In A the hypha *d* is curved toward the peg *f* which has grown out from the hypha *e*. In B, 10 minutes later than A, the hypha and peg are turned toward one another and have almost touched. In C, 5 minutes after B, the hypha *d* and the peg *f* have fused with one another.

The later stages in the hypha-to-peg fusion *d-x* at the top of Fig. 24 were observed. The end of the hypha *d* grew toward the side of a thin branch-hypha of the mycelium *c* and, apparently, so

stimulated this branch-hypha that the latter sent out two opposing pegs. Both of these pegs grew toward the end of the hypha *d*; but, eventually, as was actually observed, the peg *x* alone fused with the hypha *d* and then the other peg ceased to grow.

Several peg-to-peg fusions were observed being formed. Among them two are illustrated in Fig. 27. In A, the two pegs *a* and *b* have originated opposite to one another on two older more or less parallel hyphae and they are already bending their ends toward one another. In B, a few minutes later than A, the two pegs are shown after their fusion. The distance apart of the two older hyphae where the pegs were produced was about 15  $\mu$ .

In Fig. 27 there are also illustrated two stages in the formation of a fusion associated with three pegs. In A, the peg *c* produced on the older hypha *q* is faced right and left by two pegs *d* and *e* produced

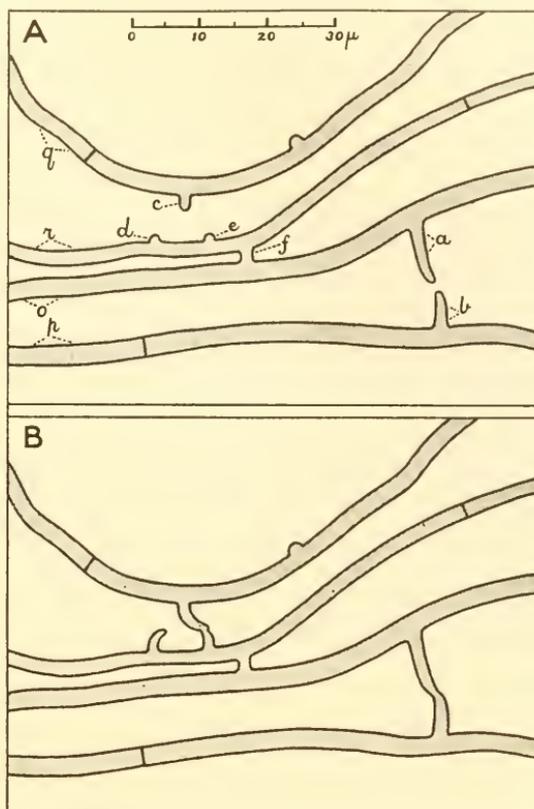


FIG. 27.—*Pleurage curvicolla*. The formation of two peg-to-peg fusions. (1) As shown in A, the two more or less parallel hyphae, *o* and *p*, have sent out opposing pegs, *a* and *b*, the ends of which are growing toward one another. Within a few minutes these pegs met and fused, and thus formed a bridging hypha, as shown in B. (2) As shown in A, the hypha *q* sent out a single peg *c* and the hypha *r* two opposing pegs, *d* and *e*. All three pegs at first grew straight forward. Then the pair of pegs *d* and *e* grew toward the single peg *c*, but the latter turned toward *e* alone and eventually fused with it, as shown in B. After the fusion had taken place, the peg *d* ceased to grow. A narrow bridging hypha *f* joining the hyphae *o* and *r* had been formed, presumably from two pegs, before the drawing A was made. A scale in micromillimeters is given in A. Magnification, 847.

by the older hypha *r*. At first all three pegs grew straight forward. Then both of the pair of pegs *d* and *e* bent toward the single peg *c*. However, the peg *c* bent toward *e* as if *d* were not in existence. Finally, as shown in B, *c* fused with *e* and then *d* ceased to grow. Probably *e* stimulated *c* slightly more than did *d* and thus won the competition by superior attractive power.

Why an older hypha should produce two pegs right and left of an opposing peg instead of one opposite peg is at present a mystery.

After one has studied all the types of fusions in actual formation, it is not difficult to interpret the nature of the fusions that one finds already in existence in an older mycelium. Thus in Fig. 24 we can interpret some of the fusions there seen as follows: those at *o* and *u*, hypha-to-hypha fusions; those at *p*, *q*, *m*, and *x*, hypha-to-

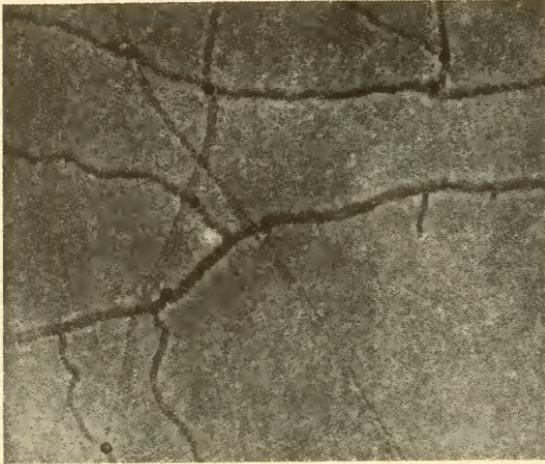


FIG. 28.—*Pleuroge curvicolla*. Photomicrograph of a mycelium produced from spores sown in a hanging drop of cleared dung-agar on the previous day. To show the very short peg-to-peg fusions which have been formed where two older hyphae have crossed one another more or less at right angles. The bridging hyphae are perpendicular to the plane of the photograph and hence appear as dark circles. Magnification, 600.

peg fusions; and those at *n* and *v*, peg-to-peg fusions.

A photomicrograph showing several very short bridging hyphae uniting long hyphae which have crossed one another more or less at right angles is reproduced in Fig. 28. These bridging hyphae, doubtless, were formed by peg-to-peg fusions. As they are directed more or less perpendicularly to the plane in which the long hyphae lie, they are seen in end view and therefore appear as dark disc-shaped structures. They were formed not immediately after the long hyphae crossed one another but much later when the culture medium was becoming exhausted. It is under such conditions that

the mycelium displays the greatest tendency to convert itself into a close-meshed network.

**Pleurage anserina.**—Spores of this fungus were sown in a hanging drop of cleared dung-agar, and next day hyphal fusions were observed forming in the mycelium.

Three hypha-to-peg fusions were observed. In two of them the pegs were prominent and distinctly peg-like, but in the third the peg never became as long as it was broad and had the form of a blunt convex process scarcely protruding from the older hypha of which it was an outgrowth. Six stages in the formation of a hypha-

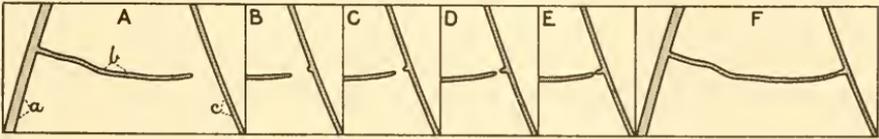


FIG. 29.—*Pleurage anserina*. The formation of a hypha-to-peg fusion. That part of a mycelium shown in A was watched continuously. At first there were present only the hyphae *a* and *c*. Then the branch-hypha *b* came into existence and, in the course of about an hour, attained the length shown in A. The end of *b* is now about  $10\ \mu$  from the side of *c* and is stimulating *c* morphogenically. B, two minutes after A; *c* has produced a peg opposite to the end of *b*. C, two minutes after B; the peg and the branch-hypha *b* are growing toward one another. D, one minute after C; the peg and the branch-hypha are rapidly approaching one another. E, one minute after D; the ends of the peg and the branch-hypha have met. F, three minutes after E; the peg and the branch-hypha have completely fused with one another. Magnification, 434.

to-peg fusion, passed through in succession in the course of about ten minutes, are shown in Fig. 29.

Two peg-to-peg fusions were witnessed. In the one illustrated in Fig. 30, A and B, the more or less parallel older hyphae which stimulated one another were  $26\ \mu$  apart at the place where they sent out their pegs.

Hypha-to-hypha fusions doubtless occur in the mycelium of *P. anserina*, but they were not actually observed taking place.

In places in the mycelium were found instances of: (1) one peg growing toward one of two opposing pegs (Fig. 30, C); (2) one peg which had fused with *one* only of two opposing pegs (Fig. 31, A); (3) one peg which had fused with *both* of two opposing pegs (Figs. 30, D, and 31, B), and (4) a hypha-to-peg fusion in which evidently the approaching hypha had induced the formation of two opposing pegs

both of which had turned toward it and with one of which it had fused (Fig. 30, E).

The monosporous mycelia of *Pleuroge anserina*, as they grow

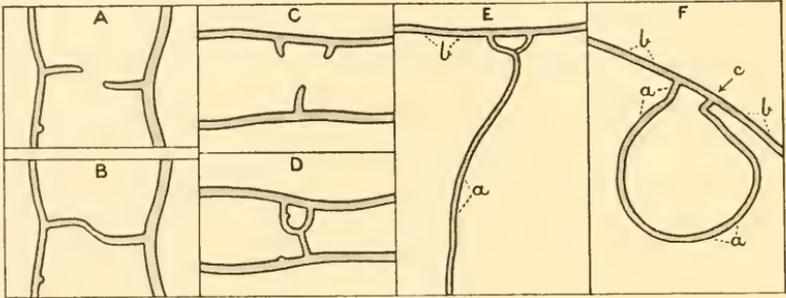


FIG. 30.—*Pleuroge anserina*. Various hyphal fusions. A and B: two stages in a peg-to-peg fusion. C: one peg and two opposing pegs: the single peg is growing toward one only of the opposing pegs but both of the pair of pegs are growing toward the single peg. D: one peg has fused with both of two opposing pegs. E: a hypha-to-peg fusion in which the hypha *a* caused the hypha *b* to send out two opposing pegs instead of the usual one; the hypha *a* has fused with one of the pegs and possibly with the other. F: a hyphal fusion in a fruit-body primordium; apparently a loop-hypha *a* has fused with a peg *c* produced by the hypha *b*. Magnification, 487.

older, develop primordia of fruit-bodies. Hyphal fusions were observed in all the primordia which were examined. One of these

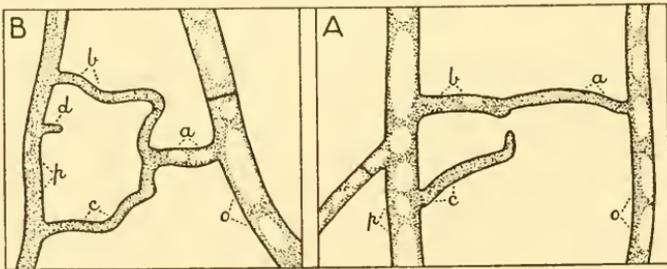


FIG. 31.—*Pleuroge anserina*. Fusions found associated with three pegs. A: the hypha *o* sent out the peg *a*, and the hypha *p* the two pegs *b* and *c*; fusion took place between *a* and *b*, and then *c* ceased to develop further. B: the hypha *o* sent out the peg *a*, and the hypha *p* the three pegs *b*, *c* and *d*; fusion took place between the pegs *a*, *b*, and *c*, apparently at their ends. The peg *d* grew no further. Magnification, 868.

fusions—probably a hypha-to-peg fusion—resulting in the formation of a loop is shown in Fig. 30, F.

At *j* in Fig. 102, A, of Volume IV (p. 178) of this work are shown two peg-to-peg fusions of *P. anserina* and to the right of them two pegs facing one another but not yet fused. The two peg-to-peg fusions were correctly called *bridging hyphae*; but, at the time the drawing was made, I was unaware that a bridging hypha is a product of the fusion of two pegs.

**Fimetaria fimicola.**—The same types of hyphal fusions can be observed in the mycelium of this fungus as in the species of Pleurage already treated of. A hanging drop of cleared dung-agar was inoculated with some mycelium from a stock culture, and next day hyphal fusions were produced in the mycelium in considerable

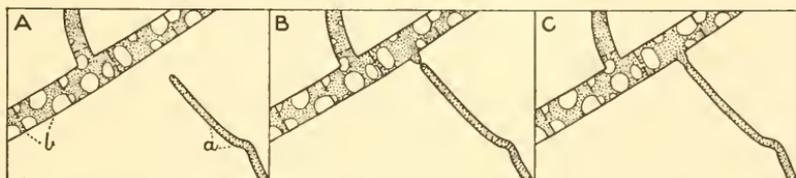


FIG. 32.—*Fimetaria fimicola*.—Three stages in the formation of a hypha-to-peg fusion. A: the hypha *a*, which is slender, happens to be approaching perpendicularly the hypha *b*, which is stout, septate and vacuolate. B: in response to a stimulus given out by *a*, *b* has put out an opposing peg: and now the hypha *a* and the peg are just coming into contact with one another. C: the hypha *a* and the peg produced by the hypha *b* have completely fused. Magnification, 434.

numbers. Some peg-to-peg fusions are illustrated at *o*, *p*, *q*, and between the hyphae *d* and *e* in the next Chapter in Fig. 59 (p. 105).

Only in one instance, a hypha-to-peg fusion, were stages in the process of fusion observed: a slender hypha, full of protoplasm, grew directly toward the side of a thick hypha which was septate and vacuolated (Fig. 32, A). As soon as the slender hypha had approached within about  $10\mu$  of the thick hypha, the latter put out a small opposing peg. The slender hypha and the peg then met and fused (B and C).

All stages in peg-to-peg fusions were found in various parts of the mycelium, but lack of time prevented my observing the actual fusion of any particular pair of pegs.

**Pyronema confluens.**—This well-known Discomycete can readily be cultivated from spores which, after having been shot on to cleared dung-agar, begin to germinate in about 4.5 hours. The

mycelium grows very rapidly. Hanging drops of cleared dung-agar inoculated with mycelium one day are ready for the study of hyphal fusions on the next day.

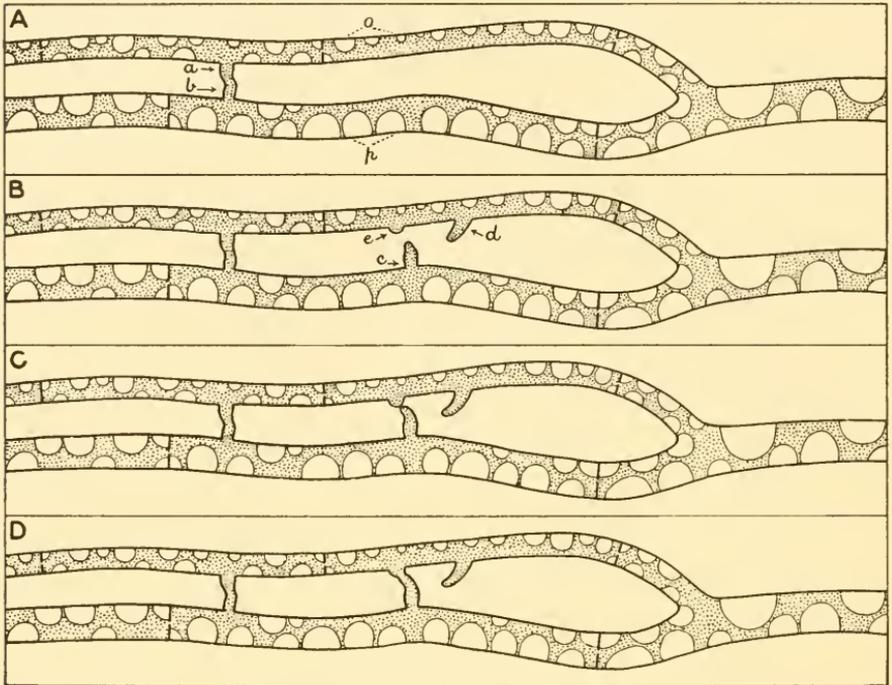


FIG. 33.—*Pyronema confluens*. Four stages in the formation of a peg-to-peg fusion. The branch-hypha *o* came off from the hypha *p* at an angle of about 45° but, with the help of a beading needle, it was brought parallel to *p*, as shown in A–D. Within about four hours after *o* had taken up its new position, four peg-to-peg fusions took place successively between *o* and *p*. The first two fusions (to the left) are not shown. The third, shown in A, was formed by the fusion of the two pegs *a* and *b*. The stages in the fourth peg-to-peg fusion are shown in A–D. A: in the region of the letters *o* and *p* the hyphae *o* and *p* are stimulating one another morphogenically. B, about 40 minutes after A; as a result of mutual stimulation, the hypha *p* has sent out the peg *c* and the hypha *o* the two opposing pegs *d* and *e*; *d* has already turned toward *c*, but *c* is turning not toward *d* but to the nearer and shorter peg *e*. C, 21 minutes after B; the ends of the pegs *c* and *e* have grown toward one another and have met. D, 8 minutes after C; the pegs *c* and *e* have completely fused with one another, thus forming the fourth bridging hypha between the hyphae *o* and *p*; the peg *d*, after the fusion was complete, ceased to elongate. The hyphae, represented in optical section, are highly vacuolated and have centrally perforate septa. Magnification, 447.

Hypha-to-hypha, hypha-to-peg, and peg-to-peg fusions are all formed in the mycelium of *Pyronema confluens* and can readily be found and identified as such in older cultures.

I observed a peg-to-peg fusion in which three pegs were involved. By a mechanical operation with a needle a branch-hypha was brought more or less parallel to the larger hypha from which it had developed. Four peg-to-peg fusions were then formed one after another between the two hyphae. The third peg-to-peg fusion is shown in Fig. 33, A. The fourth peg-to-peg fusion was initiated by the outgrowth of a single peg from the larger hypha and two pegs from the opposing smaller hypha (Fig. 33, B). The single peg grew toward the smaller of the pair of pegs, touched it (C), and fused with it (D). The remaining peg became bent toward the single peg but ceased to grow after the fusion of the two other pegs had been accomplished. The time which elapsed between stage B and stage D was 29 minutes. The distance between the hyphae at the point where these gave rise to opposing pegs was about 14  $\mu$ .

Bridging hyphae in *Pyronema confluens*, each formed by the union of two short pegs, not infrequently come into existence where two hyphae cross one another. One such bridging hypha which connected two monosporous mycelia is seen in the next Chapter in Fig. 65 at *c* (p. 120).

Hypha-to-hypha fusions in *Pyronema confluens* are often formed intrahyphally after a single cell, or two or more adjacent cells, in a hypha have been killed or have died. The formation of such fusions will be described in the next Chapter.

The observations made on the living mycelium of *Pyronema confluens* warrant the conclusion that the hyphal fusions in this Discomycete resemble in their mode of formation those of the Pyrenomycetes.

**Coprinus sterquilinus.**—*Coprinus sterquilinus* is a Hymenomycete with large fruit-bodies.<sup>1</sup> Its mycelium forms numerous hyphal fusions (*cf.* Vol. IV, Fig. 89, p. 159). In Volume IV their function was discussed in detail.

Hanging drops of cleared dung-agar were inoculated with mycelium one day, and hyphal fusions were observed forming in the drop the next day.

By continuous watching of particular parts of a mycelium I

<sup>1</sup> For illustrations, *vide* these *Researches*, Vol. III, 1924, pp. 180-236, and Vol. IV, 1931, pp. 95-98.

succeeded in observing the formation of three hypha-to-peg fusions and one peg-to-peg fusion.

In two of the hypha-to-peg fusions, a younger lateral hypha at first by chance approached the side of an older hypha at an oblique angle (Fig. 34, A); as soon as it had arrived within a distance of about  $15\ \mu$  from the older hypha, it changed its direction of growth and began to grow directly toward the older hypha (B); as soon as its apex had arrived within about  $3\text{--}4\ \mu$  of the older hypha, the latter emitted a small opposing peg (C); the lateral hypha and peg then

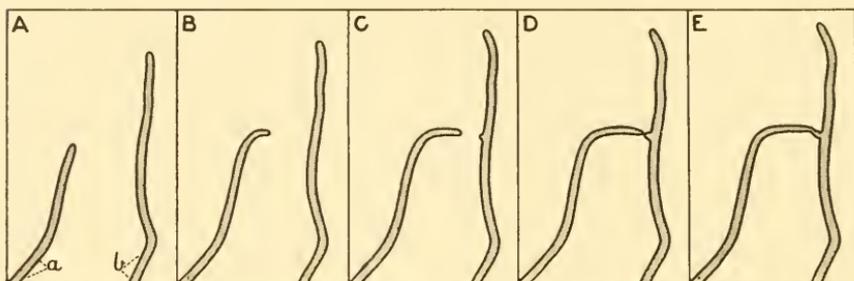


FIG. 34.—*Coprinus sterquilinus*. Five stages in the formation of a hypha-to-peg fusion. A: the hypha *a*, by chance, is growing very obliquely toward the hypha *b*. B: *a*, having come sufficiently near to the side of *b*, has become tropically stimulated by *b* and is now growing directly toward *b*. C: the hypha *a*, now much nearer to the hypha *b*, has stimulated *b* morphogenically so that *b* has formed a peg opposite to the end of *a*. D: the ends of the hypha *a* and of the peg have grown toward one another and have met. E: the hypha *a* and the peg produced by *b* have now completely fused. About 15 minutes elapsed between the stages B and E. Magnification, 434.

grew slightly in length until their tips met (D); and, finally, complete fusion took place (E). All these stages in the process of hyphal fusion were completed within half an hour. In a third hypha-to-peg fusion, the peg was longer and had already appeared when a lateral hypha was seen approaching it. Complete fusion in this instance took place forty minutes after the beginning of the observations.

In the peg-to-peg fusion (Fig. 35), two older hyphae running more or less parallel to one another emitted a pair of pegs opposite to one another. The pegs grew toward one another and fused in the usual manner.

No hypha-to-hypha fusion was seen becoming established, but an examination of an older mycelium enabled me to conclude that

a number of hypha-to-hypha fusions had taken place within it. Among the numerous hyphal fusions which an older mycelium displays, it is not difficult to find plenty of examples of all three kinds: hypha-to-hypha, hypha-to-peg, and peg-to-peg.

It is clear that in *Coprinus sterquilinus*, a typical Hymenomycete, just as in the Pyrenomycetes and the Discomycetes, all the hyphal fusions are essentially end-to-end ones.

***Coprinus lagopus*.**—*Coprinus lagopus*, often called *C. fimetarius*, is a common horse-dung Hymenomycete. Its fruit-bodies were described in Volume III and its sexual processes in Volume IV. The mycelium produces

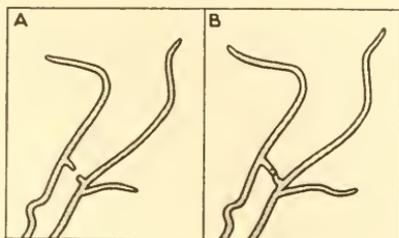


FIG. 35.—*Coprinus sterquilinus*. Two stages in the formation of a peg-to-peg fusion. A, two opposing pegs are growing toward one another. B, a few minutes later; the pegs have met and fused; the end of one peg lies over the other and the fusion plane is parallel to the plane of the paper. Magnification, 434.

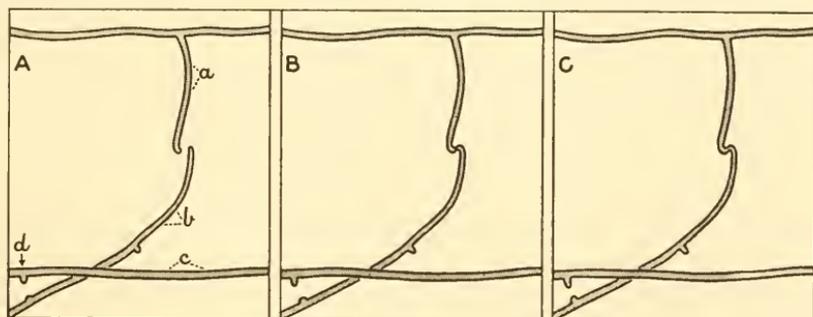


FIG. 36.—*Coprinus lagopus*. Three stages in the formation of a hypha-to-hypha fusion. The mycelium, derived from spores sown two days previously, is haploid and so does not bear clamp-connexions. A: two hyphae, *a* and *b*, which came into existence and grew for some time independently of one another, happen to have approached one another, and now their ends are stimulating and growing toward one another. B, a few minutes later than A: the ends of the two hyphae have met. C, a few minutes later than B: the hyphae *a* and *b* have fused end-to-end. At *d* in A can be seen two opposing pegs put out by the hyphae *b* and *c*. These pegs may have fused later than at the stage C, but were not observed to do so. Magnification, about 434.

many hyphal fusions, especially under starvation conditions. A mycelial network formed by fusions between the hyphae of

three monosporous mycelia is shown in Volume IV, Fig. 96, p. 170.

Some spores of *Coprinus lagopus* were sown in a hanging drop of cleared dung-agar one day, and hyphal fusions were observed being formed in the mycelium on the next day. The mycelium exhibits fusions of all three kinds: hypha-to-hypha, hypha-to-peg, and peg-to-peg.

The stages in one hypha-to-hypha and one hypha-to-peg fusion were followed.

In the hypha-to-hypha fusion (Fig. 36), two long lateral hyphae approached one another by chance. As soon as their ends arrived

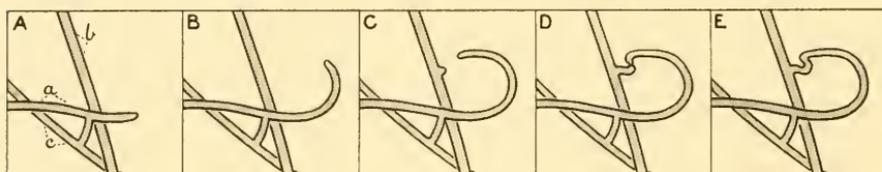


FIG. 37.—*Coprinus lagopus*. Five stages in the formation of a hypha-to-peg fusion. The mycelium, derived from spores sown about 30 hours previously, is haploid and so does not bear clamp-connexions. A: the hypha *a*, already united with the hypha *c* by a peg-to-peg fusion, is crossing over the hypha *b*. B: the hypha *a* is growing back toward *b*. C: the hypha *a* has now approached near to *b* and has caused *b* to send out an opposing peg. D: the hypha *a* and the peg have grown toward one another and have met at their ends. E: the hypha *a* and the peg produced by *b* have now completely fused. About three hours elapsed between the stage A and the stage E. Magnification, about 860.

within a certain distance of one another (10–15  $\mu$ ), the usual tropism took place: the ends of the hyphae grew almost directly toward one another. Eventually the tips turned very sharply toward one another, met, and fused (Fig. 36, A, B, and C). About the point of fusion of the two hyphae, the compound hypha displayed a very decided S-shaped twist.

In the hypha-to-peg fusion, one younger hypha grew across an older hypha almost at right angles (Fig. 37, A). Then, after growing about 7–8  $\mu$  past the older hypha, the younger hypha made a growth curvature back to the older hypha and induced there the formation of a peg (B and C). The tips of the younger hypha and of the peg eventually met and fused (D and E). This fusion took place in an older part of the mycelium where the medium was much exhausted and in consequence the growth in length was very slow. About

three hours elapsed between the first and last stages represented in Fig. 37.

From the observations just recorded, it is obvious that the hyphal fusion phenomena of *Coprinus lagopus* resemble those of *C. sterquilinus*, *Pleuroge curvicolla*, *P. anserina*, and *Pyronema confluens*.

**Sphaerobolus stellatus.**—*Sphaerobolus stellatus*, whose fruit-bodies are described in a later chapter of this volume, is a typical Gastromycete. Its mycelium was obtained by sowing a gleba, shot on to a sterilised slide, in sterilised horse dung. The mycelium exhibits

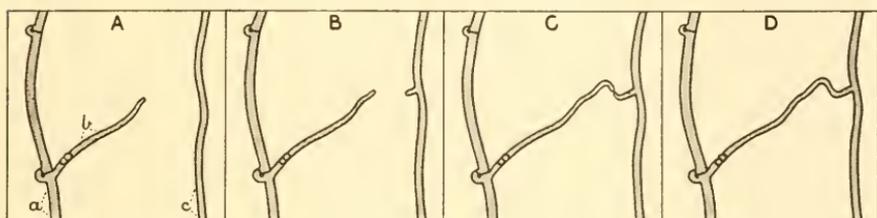


FIG. 38.—*Sphaerobolus stellatus*. Four stages in the formation of a hypha-to-peg fusion. A: the hypha *a*, which bears two clamp-connexions seen in lateral view, has given rise to a branch-hypha *b* which bears a clamp-connexion seen in face view. The hypha *b* by chance is approaching *c* obliquely. B: the hypha *b* has approached nearer to *c* and has stimulated *c* morphogenically so that *c* has sent out a peg opposite to the end of *b*. C: the hypha and the peg grew as though they would pass one another and then, as a result of mutual tropic stimulation, made growth-curvatures through a right angle, so that their ends have now met. D: the hypha *b* and the peg have now completely fused with one another. Owing to the exhaustion of the medium, the hypha *b* grew very slowly in length and three hours elapsed between stage A and stage D. Magnification, 430.

numerous hyphal fusions of all types, and three fusions are shown incidentally in the mycelium illustrated in Fig. 144, p. 291.

A hanging drop of sterilised dung-agar was inoculated with a tiny piece of mycelium taken from a stock culture and, on the next day, hyphal fusions were being formed in considerable numbers.

By continuous watching of particular parts of a mycelium, with the help of Dr. Dowding I succeeded in observing successive stages in the production of an undoubted hypha-to-peg fusion and of another fusion which I prefer to interpret as a hypha-to-peg fusion, although it may possibly have been a peg-to-peg fusion.

In the hypha-to-peg fusion, a lateral hypha bearing a clamp-connexion was seen approaching the side of an older hypha (Fig. 38, A). The older hypha sent out a peg (B); the lateral hypha

grew for a while as if it would pass the peg; the hypha and the peg then made growth curvatures which resulted in their meeting and fusing (C and D). Thus the compound hypha resulting from the fusion came to display a very decided S-shaped twist.

In the second fusion, two older hyphae happened to be more or less parallel to one another (Fig. 39, A). One of these hyphae then emitted a short branch-hypha which grew somewhat obliquely toward the other older hypha (B) which, in response, sent out a short opposing peg (C). The short branch-hypha and the peg eventually fused (D). The two

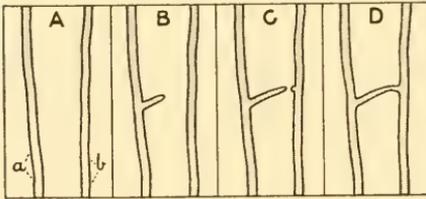


FIG. 39.—*Sphaerobotus stellatus*. Four stages in a hypha-to-peg fusion where the hypha was very short. A: *a* and *b*, two hyphae growing almost parallel to one another. B: *a* has sent out a short branch which happens to be growing obliquely toward *b*. C: in response to a stimulus from the branch-hypha, *b* has put out an opposing peg. D: the short hypha and the peg have fused. Fifteen minutes elapsed between stages B and D. Magnification, 434.

older hyphae were about  $12\mu$  apart at the place where the branch-hypha and the peg were sent out, and the time which elapsed between stage B and stage D represented in Fig. 39 was 15 minutes. Against regarding the fusion as a peg-to-peg one, we may adduce two facts: (1) the oblique growth of the longer fusing element, and (2) the fact that the fusing elements arose not simultaneously or almost so, as is usual in peg-to-peg fusions, but in

succession at different times. I have therefore interpreted the longer fusing element, not as a peg which has arisen on its parent hypha as a result of this parent hypha having been stimulated by the other older hypha, but as a very short branch-hypha which the parent hypha would have produced even if the other older hypha had not been near.

**Microsporon audouini.**—An examination of photographs of the mycelium of this fungus made by Davidson, Dowding, and Buller<sup>1</sup> caused these authors to infer that certain short bridging hyphae, connecting two parallel hyphae in a mycelium of *Microsporon*

<sup>1</sup> A. M. Davidson, E. S. Dowding, and A. H. R. Buller, "Hyphal Fusions in Dermatophytes," *Canadian Journal of Research*, Vol. VI, 1932, p. 5.

*audouini*, a ring-worm fungus, must have been formed by the simultaneous outgrowth and subsequent fusing of a pair of pegs. One of these photographs is shown in Fig. 40. The evidence from actual observations of peg-to-peg fusions in Ascomycetes and Basidiomycetes generally goes to show that the inference as to the nature of bridging hyphae in *M. audouini* was a correct one.

*Microsporon audouini* may possibly be an Ascomycete, but at present it is included in the Fungi Imperfecti. It is very probable that the mode of formation of hyphal fusions in Fungi Imperfecti resembles that exhibited by the Higher Fungi as described in this Chapter.

**Critical Remarks on Supposed End-to-Side and Side-to-Side Fusions.**—In their paper on *Microsporon audouini* Davidson, Dowding, and Buller,<sup>1</sup> in respect to the mycelium, say: "From an examination of Text-figs. 10, 11, and 12 it appears that all the fusions there illustrated were formed between the end of one hypha and the lateral wall of another hypha. This type of hyphal fusion has been described and illustrated by Buller."

In view of the evidence given in this Chapter that all hyphal fusions in the mycelium of typical Pyrenomycetes, Discomycetes, Hymenomycetes, and Gastromycetes are essentially end-to-end ones, I now feel that there is no support for the view, expressed in Volume IV,<sup>2</sup> that one younger hypha fuses directly with the lateral wall of an older hypha. Rather I am inclined to think that, on the approach of a younger hypha, the older hypha always sends out a longer or shorter peg to meet the younger

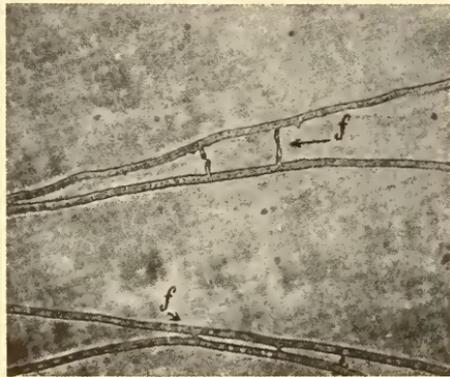


FIG. 40.—*Microsporon audouini*. Mycelium grown in a hanging drop of Sabouraud's medium, ten days after inoculation, showing peg-to-peg fusions between older hyphae. From the paper of Davidson, Dowding, and Buller on *Hyphal Fusions in Dermatophytes*. Magnification, 250.

<sup>1</sup> A. M. Davidson, E. S. Dowding, and A. H. R. Buller, "Hyphal Fusions in Dermatophytes," *Canadian Journal of Research*, Vol. VI, 1932, p. 5.

<sup>2</sup> These *Researches*, Vol. IV, 1931, pp. 152-154.

hypha, so that the fusion in question is not an end-to-side but, in reality, an end-to-end one.

Mlle Bensaude,<sup>1</sup> in 1900, in her well-known paper on the sexual process in *Coprinus fimetarius* (the *C. lagopus* of these volumes), by studying the position of clamp-connexions in mature mycelia

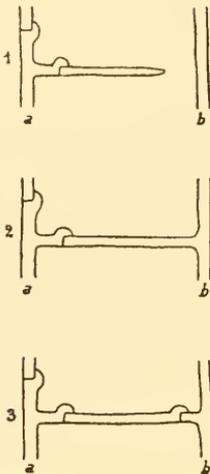


FIG. 41.—*Coprinus fimetarius*. Diagrams showing stages in the formation of an anastomosing branch between two parallel hyphae. The clamp-connexion formed near *b* after the fusion belongs morphologically to the branch sent out from *a*. Drawn and described by Mlle Bensaude.

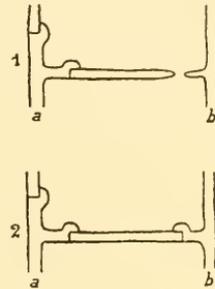


FIG. 42.—*Coprinus fimetarius*. Diagrams showing stages in the formation of an anastomosing branch between two parallel hyphae. The clamp-connexion formed after the fusion belongs morphologically to the little branch sent out by *b*. Drawn and described by Mlle Bensaude.

exhibiting completed fusions, came to the conclusion that hyphal fusions are formed in three ways which she illustrated with diagrams : (1) end of one hypha to side of another hypha (Fig. 41) ; (2) end of one longer hypha to end of a short hypha emitted from the side of another hypha (Fig. 42) ; and (3) the end of one longer hypha to the end of another longer hypha (Fig. 43). Her second and third types of fusions correspond to my hypha-to-peg and hypha-to-hypha types

<sup>1</sup> Mathilde Bensaude, *Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes*, Nemours, 1918, pp. 53-54.

respectively and were correctly conceived, as shown by my observations on the actual formation of fusions in developing mycelia. However, her first type of fusion, an end-to-side one, I am unable to admit and have no doubt that the fusion which she had in mind and illustrated in her diagram was in reality a hypha-to-peg fusion, *i.e.* an end-to-end fusion. Finally, it may be remarked that Mlle Bensaude did not include in her classification the peg-to-peg fusions described in this Chapter.

Hein,<sup>1</sup> in 1930, as a result of studies made "on material prepared by the usual fixing and staining methods as well as on fresh material" came to the conclusion that in *Psalliota campestris* hyphae frequently anastomose with one another *laterally*. Thus he<sup>2</sup> says: "Anastomoses occur apically and laterally between hyphal branches from the same spore and between hyphae produced from different spores" and, further, in respect to the formation of the large hyphae of a mycelial cord, he says: "Where two or more hyphae fuse by the dissolution of their lateral adjoining walls as in Figs. 12 and 13 a cell of large diameter results. In this manner the vascular

elements of large diameter originate. The large lumen in the vascular hyphae of *Psalliota campestris* does not appear to be entirely the result of swelling as Falck described for species of *Merulius* (1912), but is at least partly the result of lateral fusion of hyphae of smaller diameters." In his Fig. 14 he<sup>2</sup> represents the tip of an older strand and concerning it remarks: "The hyphae are all of uniform diameter, filled with protoplasm and actively growing. These are the 'Bildungshyphen' of Falck (1912). The latter hyphae will become differentiated by lateral fusion into vascular hyphae of large diameters."

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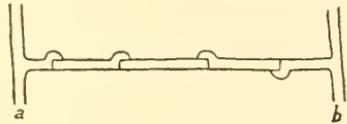


FIG. 43.—*Coprinus fimetarius*. Diagram showing the formation of an anastomosing branch between two parallel hyphae by means of an end-to-end fusion of two lateral branches. As indicated by the position of their secondary septa, two clamp-connections belong morphologically to the hypha *a* and two to the hypha *b*. Drawn and described by Mlle Bensaude.

<sup>1</sup> I. Hein, "Studies on the Mycelium of *Psalliota campestris*," *American Journal of Botany*, Vol. XVII, 1930, pp. 197-211.

<sup>2</sup> *Ibid.*, p. 203.

Hein's supposed lateral fusions we may call *side-to-side* fusions and in this way clearly distinguish them from the other types of fusion already described in this Chapter.

It seems to me that Hein has misinterpreted his preparations and that we are not justified in accepting the view that side-to-side fusions take place in *Psalliota campestris* either in the ordinary vegetative mycelium or in the mycelial cords. The supposed side-to-side fusions which Hein shows in his Figs. 12 and 13 are evidently very short local peg-to-peg fusions of the kind illustrated in this Chapter in Fig. 16 (p. 32); and the evidence which Hein brings forward to support his contention that the large vascular elements in the strands of *Psalliota campestris* develop not as in *Merulius lacrymans* by the swelling of individual hyphae, but by the side-to-side longitudinal fusion of a number of smaller hyphae which are in contact with one another, seems to me to be entirely unconvincing.

**Action at a Distance in Vegetative Hyphal Fusions and its Theoretical Explanation.**—As a result of precise experiments made by Pfeffer and others we have become acquainted with the phenomena of chemotaxis and chemotropism; so that we attribute: (1) to *chemotactic stimuli* the movements of the spermatozoids of Ferns, Mosses, and their allies toward the mouths of archegonia, the movements of bacteria toward or away from oxygen, hydrogen bisulphide, meat extract, and other chemical substances, and the movements of white blood corpuscles toward the surfaces of wounds; and (2) to *chemotropic stimuli* the growth-movements of pollen-tubes toward stigmas and the mouths of ovules. On the basis of our knowledge of chemotactic and chemotropic phenomena in other organisms, we are justified in attempting to explain the teleomorphic and zygotropic reactions which take place in connexion with sexual and non-sexual hyphal fusions in Fungi as being due to chemical excretions.

Already, as set forth in the introduction to this Chapter, Burgeff has sought to explain the mutual teleomorphic and zygotropic reactions which take place in heterothallic Mucorineae when (+) and (−) mycelia come near to one another as being due to the excretion of two sexual substances, one excreted by (+) mycelia and (+) zygophores and the other by (−) mycelia and (−) zygophores.

In the mycelia of the Ascomycetes, Basidiomycetes, and Fungi

Imperfecti, the vegetative fusions which are formed in such large numbers are in no way connected with sex ; for they are formed between the hyphae of one and the same haploid mycelium and between the hyphae of any two haploid mycelia of the same sexual kind ; and, in the Basidiomycetes, they are also formed between the hyphae of one and the same diploid mycelium and between the hyphae of any two diploid mycelia.

While the telemorphic and zygotropic phenomena observed in vegetative hyphal fusions may be initiated by the excretion of chemical substances, it is not easy to formulate a precise theory, based on the assumption of chemical stimuli, which will account for all the observed reactions.

If on a substratum of agar or gelatine a pollen-grain is set a little distance away from a stigma, when it germinates its pollen-tube grows directly toward the stigma, *i.e.* it grows from a region where the chemical excretion of the stigma is in lesser concentration to a region where it is in greater concentration.

If in a vegetative hypha-to-hypha fusion of a fungus the tips of both the hyphae were to excrete one and the same chemical substance, then if we may judge by what has been observed in the chemotropism of pollen-grains the two hyphae could not approach one another, for there would be no gradient of weaker to stronger concentration of the substance extending from each hyphal tip to the other hyphal tip. It therefore appears that the excretion of one and the same chemical substance by two hyphae bending toward one another zygotropically is inadequate to account for the growth-movements observed.

If one substance is not enough for our chemical theory, let us try two. Let us suppose that, in a hypha-to-hypha fusion, the tip of one of the hyphae excretes one chemotropic substance and the tip of the other another chemotropic substance and that each hypha, while unable to react chemotropically to the substance which it itself excretes, is yet chemotropically sensitive to the substance which the other hypha excretes. Under the conditions here assumed it is theoretically possible for the two hyphae in a hypha-to-hypha fusion to grow toward one another until they meet.

However, the assumption that in a hypha-to-hypha fusion two

substances are excreted, one by one hypha and the other by the other hypha, leads us into difficulties when we try to account for the fact that, in a single mycelium, any hypha will fuse with any other hypha that is near enough to it and that, in a single species, any mycelium produced from a spore, a conidium, or an oidium will fuse with any other mycelium. Let us consider how our theory of two chemical substances for each vegetative hypha-to-hypha fusion becomes complicated when we have to explain the formation of fusions between any two mycelia of a single species.

Let A, B, C, D, . . . . . designate a series of mycelia all belonging to a single fungus species and all sexually similar, and let us suppose that, in any combination of two of these mycelia, there is formed a single hypha-to-hypha fusion. When A fuses with B, on our theory, two substances are excreted. Let *a* be the substance excreted by A and *b* the substance excreted by B. Then *a* and *b* are different substances. When A fuses with C, the substance excreted by C must be different from that excreted by A. Let *c* be the substance excreted by C. When B fuses with C, the substance excreted by B must be different from that excreted by C, *i.e.* the substances *b* and *c* are different from one another. So far, we have been obliged to assume the existence of three distinct chemotropic substances *a*, *b*, and *c* excreted by A, B, and C respectively. With every additional mycelium, D, E, F, . . . . . that we take into consideration we must assume the existence of a new chemical excretion, *d*, *e*, *f*, . . . . . to account for the phenomena observed. Thus, if we have interfusions between any two of ten thousand mycelia, we are forced to assume the existence of ten thousand different chemical excretions to account for the tropic phenomena observable. Thus in developing the theory that two different chemical substances are excreted in each hypha-to-hypha fusion between any two like mycelia of one and the same species we are faced with a *reductio ad absurdum*. We cannot accept the view that, in any fungus species, every mycelium is able to produce an excretion different from that of every other mycelium and that the number of such excretions is indefinitely great.

How then shall we account for the fact that between any two like mycelia of one and the same species, hyphal fusions are readily

produced? It seems to me probable that, in a single species, all the vegetative fusions, whether taking place between two hyphae of a single mycelium or between a hypha of one mycelium and a hypha of another mycelium, are alike, *i.e.* that the chemical excretions taking part in the telemorphic and zygotropic phenomena of one hyphal fusion are identical in composition with those taking part in the similar phenomena in any other hyphal fusion. Granted that all the hypha-to-hypha fusions in any species of the Higher Fungi are due to a single pair of chemical substances excreted by the ends of the two hyphae concerned in each fusion, we are still confronted with theoretical difficulties; for one may ask: in any hypha-to-hypha fusion, what causes one hypha to produce one of the two substances and the other hypha the other of the two substances? To this question I can give no satisfactory answer.

Sufficient has been said to indicate the difficulty of accounting for the telemorphic and zygotropic reactions which take place in vegetative hyphal fusions on the basis of chemical excretions. So far as vegetative fusions are concerned, we have as yet no experimental evidence that the hyphae excrete any chemotropic substances whatever. It is possible that the stimuli which cause the two hyphae entering into a hypha-to-hypha fusion to become, as it were, polarised in respect to one another, may be non-chemical. If they are purely chemical, it remains for this to be demonstrated by exact experiment.

Recently and since the above was written, Vandendries and Brodie<sup>1</sup> have announced the results of some remarkable experiments which they have made on the cause of the mutual repulsion which takes place between certain haploid mycelia, between (*Ab*) and (*AB*) or between (*ab*) and (*aB*), of *Lenzites betulina*. The repulsion is more marked in aerial hyphae than in hyphae embedded in the substratum, and can be observed when the hyphae are separated from one another by a distance of 3·5 mm. The introduction of a north or south pole of a magnet between the mycelia

<sup>1</sup> R. Vandendries and H. J. Brodie, "La Tétrapolarité et l'Étude expérimentale des Barrages sexuels chez les Basidiomycètes" (Note préliminaire), *Bull. de l'Acad. roy. de Belgique*, classe de Sci., sér. 5, T. XIX, 1933, pp. 3-8.

was without effect on the phenomenon. However, when a thin plate of glass or of mica was placed between the two mycelia in such a way as to form a water-tight partition (cloison *étanche*), the repulsive action was just as strong or nearly as strong as if the mycelia had not been separated. On the other hand, a thin water-tight partition of lead suppressed the repulsive action completely. Vandendries and Brodie, as a result of these and other experiments, have come to the following conclusions: (1) the repulsive action is not of a chemical nature but is due to radiation; (2) this radiation is more or less arrested by partitions placed between the mycelia; and (3) the action of the partition is dependent on the substance of which it is composed, its density, and its thickness.

If the mutual repulsion of hyphae belonging to different haplonts of *Lenzites betulina*, as observed by Vandendries and Brodie, is due to radiation, it may well be that, in the fungi generally, the mutual attraction shown by two hyphae about to fuse with one another is also due to radiation.

**Passage of Nuclei through Hyphal Fusions.**—We now know that, in the Hymenomycetes and the Uredineae, when two haploid mycelia of opposite sex fuse together, they subsequently diploidise one another. We are therefore justified in inferring that, during the diploidisation process, one or more nuclei of each mycelium pass through the hyphal fusions into the other mycelium.<sup>1</sup>

To what extent, if any, in ordinary vegetative hyphal fusions nuclei pass through the junction-places from one hypha to another still remains to be determined by exact observation and experiment. As will be shown in the next Chapter, great quantities of cytoplasm often pass through hyphal bridges from one mycelium to another; but to what extent, if any, the streams of cytoplasm carry nuclei with them is at present unknown.

Brierley,<sup>2</sup> in discussing variation or subspecific grouping in fungi, has taken into account the possibility of nuclei passing *via* hyphal anastomoses from one strain of a fungus to another strain.

<sup>1</sup> Cf. these *Researches*, Vol. IV, 1931, Part II, Chapter II.

<sup>2</sup> W. B. Brierley, "Variation in Fungi and Bacteria," *Proc. Internat. Cong. Plant Sci. Ithaca*, New York, Vol. II, 1929, pp. 1629-1654; also "Biological Races in Fungi and their Significance in Evolution," *Ann. App. Biol.*, Vol. XVIII, 1931, pp. 420-434.

Hansen and Smith<sup>1</sup> mixed two strains of *Botrytis cinerea* (a fungus in which hyphal anastomoses between various strains are readily formed), analysed their progeny and, as a result of their work, suggested that "by the mechanism of anastomosis nuclei of one strain may migrate into the cells of other strains and thus give rise to cells and spores containing two or more kinds of genetically different nuclei"; and these authors also concluded that "variable forms of Fungi Imperfecti may owe their instability, not to mutation, but to nuclear heterogeneity (heterocaryosis), and that this condition can be brought about both *in vivo* and *in vitro* by nuclei of one strain entering the cells of another strain through anastomoses, and that the re-assortment of these diverse nuclei is accomplished by the mechanism of anastomosis and unequal cell divisions."

Whether or not the explanation given by Hansen and Smith for the results of their observations is the correct one remains to be determined by further work.

In *Ustilago zae*, although each yeast cell contains a single nucleus, discontinuous variations (saltations, mutations), as the work of Stakman<sup>2</sup> and others has shown, occur very frequently. In this fungus at least, heterocaryosis of the kind postulated by Hansen and Smith cannot possibly be the cause of the large amount of variation.

If, in *Botrytis cinerea*, one nucleus passed *via* an anastomosis into a cell of another strain containing say five nuclei, so that the cell became heterocaryotic, and from this cell a mycelium originated which produced numerous spores having six nuclei like those of our original cell, then conceivably we might obtain a new strain in which all the spores would be alike; but such an even and uniform distribution of nuclei in the spores could scarcely be brought about without the mechanism of conjugate nuclear division. So far as I know no such conjugate nuclear division has been observed in *Botrytis* or in any of its relatives.

The fact that there are a great many strains in *Botrytis cinerea*

<sup>1</sup> H. N. Hansen and R. E. Smith, "The Mechanism of Variation in Imperfect Fungi: *Botrytis cinerea*," *Phytopathology*, Vol. XXII, 1932, pp. 953-964.

<sup>2</sup> E. C. Stakman, J. J. Christensen, C. J. Eide, and B. Peturson, "Mutation and Hybridization in *Ustilago zae*," *Minn. Agr. Exp. Sta. Tech. Bull.*, No. 65, 1929.

suggests that in these strains taken altogether there are a considerable number of differently constituted nuclei. Possibly much of the variation in *Botrytis* strains is due to sudden change in nuclear structure.

Müller,<sup>1</sup> who has observed that, in *Hypochnus solani* (= *Rhizoctonia solani*), the mycelium is diploid from the first, that several nuclei divide conjugately in the terminal cell of each growing hypha, and that hyphal fusions take place frequently between the hyphae of any two monosporous mycelia, has suggested, with the help of diagrams, that sometimes a nucleus may pass from a hypha of one strain, which we may call (*A*), through the passage-way of a hyphal fusion into a hypha of another strain, which we may call (*B*), and there divide conjugately with several (*B*) nuclei, so that in the end, in some or all of the young basidia of a fruit-body produced on the (*B*) mycelium, fusions may take place between (*A*) and (*B*) nuclei, thus making the production of a hybrid strain possible. This suggestion, to which no theoretical objection applies, so far has not been brought to the test of experiment.<sup>2</sup>

<sup>1</sup> K. O. Müller, "Untersuchungen zur Entwicklungsgeschichte und Biologie von *Hypochnus solani* P. n. D. (*Rhizoctonia solani* K.)," *Arbeiten aus der Biologischen Reichsanstalt für Land- und Forstwirtschaft*, Bd. XIII, 1924, pp. 216-221.

<sup>2</sup> In a paper which has come to hand during the reading of the proofs of this chapter, S. Dickinson ("The Nature of Saltation in *Fusarium* and *Helminthosporium*," *University of Minnesota Agric. Exp. Sta. Tech. Bull.*, No. 88, Nov., 1932, pp. 1-42, Text-figs. 1-6) records that, employing microscissors, he cut out hyphae from mycelia of species of *Helminthosporium* and *Fusarium* and mated them side by side on agar. Under these conditions, he found that, in *Fusarium*, hyphal fusions "were more easily induced between cells of the same strain, or between cells of closely related strains, than between cells with a more distant relationship." Fusion cells formed between pairs of cells of two contrasting saltant strains of *Fusarium fructigenum*, after isolation, produced hyphae which, after isolation, developed into mycelia showing the characteristics of one or other of the parent forms and not of any new form. On the basis of (1) these and other experiments and (2) a cytological investigation, Dickinson has come to the conclusion that heterocaryosis arising through hyphal fusions cannot account for the phenomenon of saltation in the mycelia of the species which he studied.

## CHAPTER II

### THE TRANSLOCATION OF PROTOPLASM THROUGH THE SEPTATE MYCELIUM OF CERTAIN PYRENOAMYCETES, DISCOMYCETES, AND HYMENAMYCETES

Historical Introduction—Investigations by the Author—*Fimctaria fimicola*—Cultures—Protoplasmic Streaming—Passage of Vacuoles through the Pores in the Septa—Rate of Flow of the Protoplasm—General Direction of the Protoplasmic Current—*Gelasinospora tetrasperma*—*Pyronema confluens*—Hanging-drop Cultures—Growth of the Mycelium and Development of Vacuoles—Protoplasmic Streaming—Streaming of Protoplasm from one Mycelium to Another—Deformation of Vacuoles by Flowing Protoplasm—Causes of Streaming—Biological Significance of Streaming—Rate of Growth of the Mycelium in a Dung-agar Plate—Observations with Dark-field Illumination—Woronin Bodies and their Movements—Pore Plugs and their Formation under Experimental Conditions—Formation of Pore Plugs in Old Mycelia when Individual Cells Die—Pore Plugs in Other Fungi—The Origin of Intrahyphal Hyphae from Septal Walls and their Growth through Older Dead Hyphae under Experimental Conditions—*Ascophanus carneus*—A Ciboria on Male Birch Catkins—The Hymenomyces—Mycetozoa and Fungi—Phycomycetes compared with Ascomycetes and Basidiomycetes—The Biological Significance of Septa in the Mycelium of the Higher Fungi—Time taken for the Formation of a Septum—Temporary Bulging of Septa toward Growing Points—The Passage of Nuclei through the Haploid Mycelium of Hymenomyces during the Diploidisation Process

**Historical Introduction.**—Streaming movements in the cells of Phanerogams were first observed by Corti<sup>1</sup> in 1774 and, during the next century, his observations were amplified and extended by Fontana, Treviranus, Amici, Meyen, Dutrochet, Schleiden, and Hassal.<sup>2</sup> At first it was thought that the seat of the movement was the cell-sap, and it was not until 1852, shortly after von Mohl

<sup>1</sup> B. Corti, "Osservazioni microscopiche sulla Tremella e sulla circolazione del fluido in una pianta acquajuola (Chara)," Lucca, 1774, p. 127. Cited from Pfeffer.

<sup>2</sup> For references to the early literature on streaming *vide*: W. Pfeffer, *The Physiology of Plants*, Oxford, Vol. III, 1906, p. 289; and A. J. Ewart, *On the Physics and Physiology of Protoplasmic Streaming in Plants*, Oxford, 1903, pp. 1-5.

had established the fact that the protoplasm is the essential living substance of all animal and vegetable cells, that Schacht<sup>1</sup> showed that it is the protoplasm which moves and not the sap and concluded that streaming is but an outward and visible sign of protoplasmic activity.

The streaming of protoplasm in the mycelium of Phycomycetes was first observed by Hugo de Vries<sup>2</sup> in 1885 in the sporangiophores of *Phycomyces nitens*, and he regarded it as an aid in the transfer of nutrient materials to points where growth is taking place.

In 1897, Arthur<sup>3</sup> published the results of his investigations on streaming in Phycomycetes. His observations and conclusions may be thus summarised. Streaming occurs in *Mucor Mucedo*, *M. racemosus*, *Rhizopus nigricans*, *R. elegans*, *Phycomyces nitens*, *Sporodinia Aspergillus*, *Thamnidium elegans*, and *Pilobolus crystallinus*. The movement (specially investigated in *Rhizopus nigricans*) is most evident under moist conditions, takes place in hyphae which have attained a certain maturity, at one and the same time is restricted to some of the main hyphae and a few of their branches, and involves the cytoplasm, microsomes, food-bodies, nuclei, and vacuoles (Fig. 44). The current may be compared with that of a somewhat viscous colourless fluid flowing through a pipe, but there is an ectoplasmic layer of protoplasm lining the cell-wall, sometimes visible but often not, which takes no part in the movement. The streaming of the protoplasm is somewhat fitful. It starts, stops without apparent cause, and then begins again either in the same direction or, more often, in the opposite direction. The rate of movement varies greatly, but at 28° C. it was found to average 3·3 mm. per minute, which is about twice as fast as the rotation in *Nitella* and four times as fast as the circulation in *Tradescantia*. In a rapidly moving current the vacuoles become more convex at the anterior end and less convex, flat, or even concave at the posterior end (Fig. 45, B). A large vacuole, on coming to a fork, is often bisected so that one part goes along one hypha and the other part

<sup>1</sup> H. Schacht, *Die Pflanzenzelle*, 1852, p. 340. Cited from Pfeffer.

<sup>2</sup> H. de Vries, "Über die Bedeutung der Circulation und der Rotation des Protoplasma für Stofftransport in der Pflanze," *Bot. Zeit.*, Bd. XLIII, pp. 1-6, 16-26.

<sup>3</sup> J. C. Arthur, "The Movement of Protoplasm in Coenocytic Hyphae," *Annals of Botany*, Vol. XI, 1897, pp. 491-507.

along the other hypha. A large vacuole may also be disrupted by protoplasm flowing into it from a side branch (Fig. 45, A). In a hypha exhibiting streaming return currents are sometimes seen.

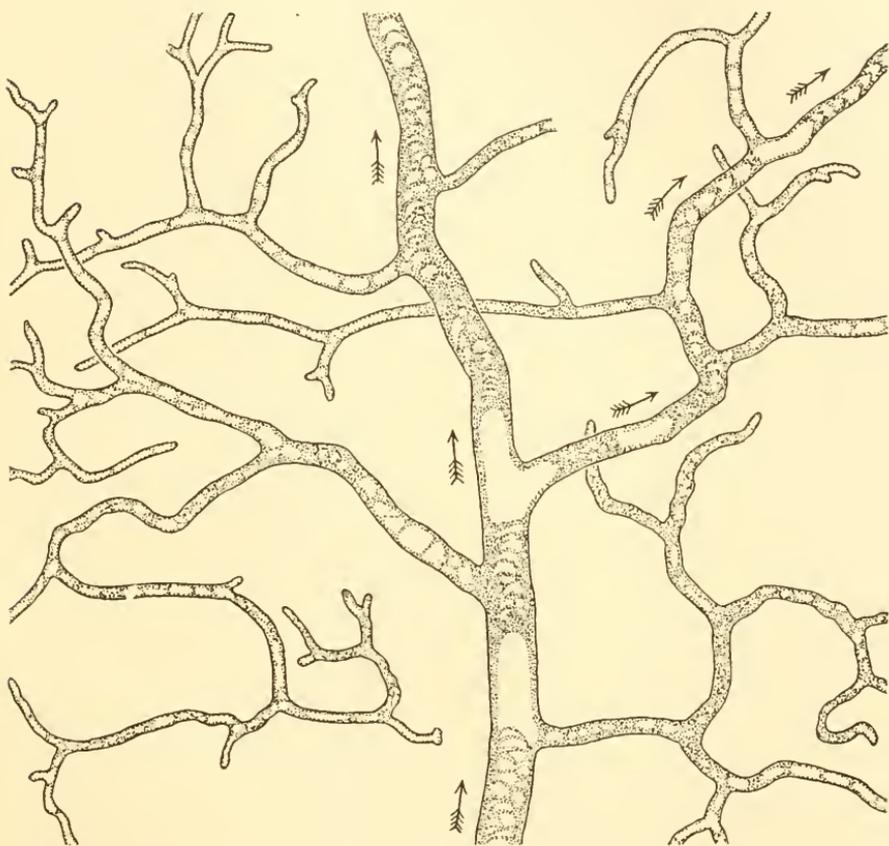


FIG. 44.—*Rhizopus nigricans*. Drawing of part of a mycelium grown upon nutrient gelatine, to show protoplasmic streaming. The protoplasm and vacuoles are flowing along a main hypha and one of its stouter branches in the direction shown by the arrows. The moving vacuoles are convex in front and plane or concave behind. In the smaller hyphae, at the moment, no streaming is perceptible. Copied by the author in black-and-white from Arthur's pencil drawing in his *The Movement of Protoplasm in Coenocytic Hyphae*.

These are situated at the periphery of the hyphal cavity and carry no vacuoles (Fig. 46, B). Streaming is due to the change in water-content at the extremities of the mycelium and the movement is more frequently seen toward aerial parts, including growing branches

and developing sporangia, than in the opposite direction. The application to the mycelium of a drop of 20 per cent. solution of

potassium nitrate causes vigorous movement for a time toward the place of application. The streaming movement aids in the transfer of nutrient materials to points where growth is taking place, but growth is not dependent on it, and full and normal development may take place without the movement coming into action.

In 1905, Schröter,<sup>1</sup> in a detailed study of protoplasmic streaming in Phycomycetes, added but little to the description of the movement as given by Arthur, but determined with some precision the rôle of the different factors which cause the stream to flow. The following is a summary of the results of his investigations. The streaming of protoplasm in *Mucor stolonifer* (= *Rhizopus nigricans*) and *Phycomyces nitens* is dependent upon osmosis and transpiration. When a mycelium grows submerged in a homogeneous substratum or in air saturated with water vapour, its protoplasm does not exhibit streaming. Streaming begins only when the medium has local differences of concentration or when

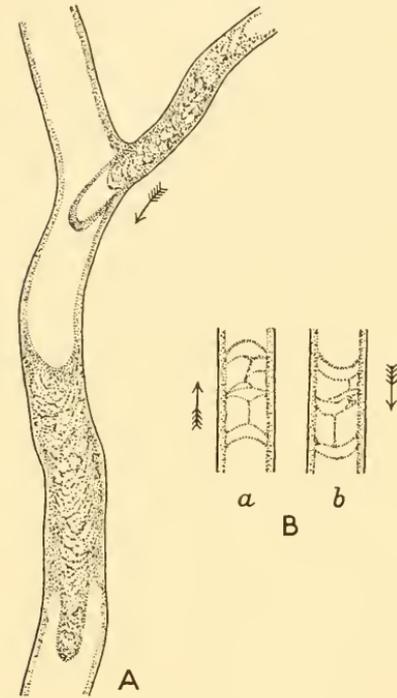


FIG. 45.—*Rhizopus nigricans*. Protoplasmic streaming in mycelial hyphae. A, the current from a branch is rushing into a long vacuole in the main hypha. Further along the main hypha, the protoplasm is pushing into a large vacuole (only partially shown). B, part of a highly vacuolated hypha with the protoplasm in motion: *a* just before, and *b* just after, a change in the direction of the current. Copied by the author in black-and-white from Arthur's pencil drawing in his *The Movement of Protoplasm in Coenocytic Hyphae*.

transpiration becomes active. Subjection to dry air causes lively streaming and hastens that already present owing to the increase

<sup>1</sup> A. Schröter, "Über Protoplasmaströmung bei Mucorineen," *Flora*, Bd. XCV, 1905, pp. 1-30.

in transpiration. If dry air is passed rapidly over the mycelium, streaming ends with the collapse of some hyphae and the bursting of a number of other hyphae. The local application of solutions of cane-sugar, potassium nitrate, etc., causes a streaming of protoplasm toward the place of application. Repeated change of direction of streaming in a hypha can be caused by appropriate local applications of a sugar solution. Protoplasmic streaming in *Mucor stolonifer* and *Phycomyces nitens* is in the main a to-and-fro flowing of the whole of the protoplasm. It should be called neither rotation nor circulation; it rather resembles the mass-translocation of protoplasm in the plasmodium of the Mycetozoa. Sometimes, while the central protoplasm, vacuoles, and cell-sap, in the form of a central cylinder, are moving forward, an outer mass of protoplasm, free from vacuoles, in the form of a cylinder-mantle, is moving basipetally (cf. Fig. 46, B). Whilst light, in general, has but little influence on the movement of the protoplasm, after the mycelium has been in the dark sudden exposure to light may initiate or hasten streaming. Changes of temperature affect streaming in the same manner as they do in Phanerogams. A rise of temperature hastens streaming or, if the protoplasm is at rest, may cause streaming to begin. Cooling may cause streaming to cease. Too high a temperature results in a backward streaming, and at about 55° C. streaming ceases and the mycelium dies. The optimum and minimum temperatures for streaming for *Mucor*

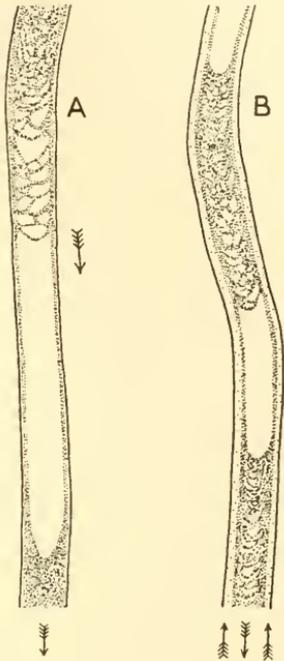


FIG. 46.—*Rhizopus nigricans*. Protoplasmic streaming in mycelial hyphae. A, a long vacuole is pushing forward into dense protoplasm, followed by highly vacuolated protoplasm. B, a hypha in which the protoplasm is flowing in opposite directions at the same time. There is a broad vacuolated axial stream of protoplasm and a sheath-like peripheral return-current of non-vacuolated protoplasm. Copied by the author in black-and-white from Arthur's pencil drawing in his *The Movement of Protoplasm in Coenocytic Hyphae*.

*stolonifer* are about 26° C. and 10°–12° C. respectively, and for *Phycomyces nitens* 28° C. and 13°–15° C. respectively. Injuries influence streaming adversely: they cause a sudden outflow of protoplasm at the point of injury and a cessation of streaming for a long time or altogether. Streaming continues to take place slowly at an air pressure of 10 mm., and therefore at a very low partial pressure of oxygen.

In a discussion of the biological significance of streaming, Schröter makes the following remarks. Streaming at first is acropetal and with short breaks continues so for some time. In this way material is transported to the ends of the hyphae. The most rapid growth takes place during acropetal streaming, less rapid growth whilst the protoplasm is at rest, and no growth during basipetal streaming. In a mature mycelium the protoplasm flows into the developing sporangiophores and, finally, into the sporangia where it is used in the formation of the spores. In *Mucor stolonifer*, streaming ceases first of all in the hyphae of the mycelium, then in the stolons and, lastly, in the sporangiophores. We thus see that Schröter, like de Vries and Arthur, regarded streaming as an important means of transferring material to points of growth.

In 1911, Raybaud,<sup>1</sup> in a long memoir, gave an account of the influence on the form and mode of growth of *Phycomyces nitens* and *Rhizopus nigricans* exerted by ordinary light, ultraviolet radiations, temperature, pressure, the hygrometric state of the air, osmosis, transpiration, and the acidity or alkalinity of the nutritive substratum, in the course of which he added the following facts, among others, to our knowledge of the movement of protoplasm within the hyphae. A germinating spore which in the dark has swollen up and is about to emit a germ-tube, if suddenly subjected to bright light, may be killed; but, if the spore has put out a germ-tube, sudden exposure to light does not kill the plant but causes the protoplasm to contract and flow toward the spore. If a mycelium, grown in the dark, is suddenly exposed to light, the protoplasm flows basipetally from the ends of the hyphae filled with homogeneous protoplasm toward the more vacuolated parts of the

<sup>1</sup> L. Raybaud, "Influence du milieu sur les Mucorinées," *Annales de la Faculté des Sciences de Marseille*, T. XX, 1911, pp. 1–248, Pl. I–V.

protoplasm. If a spore with a germ-tube, grown in the dark, is subjected not only to bright light but also to an increase of pressure and loss of water, the protoplasm in its entirety may flow out of the germ-tube into the spore, leaving the germ-tube completely empty and contracted (Fig. 47, A, *a*). If the plant is again placed in the dark, the protoplasm re-enters the germ-tube and normal growth is resumed (A, *b*). In general, if a mycelium is grown in the light and

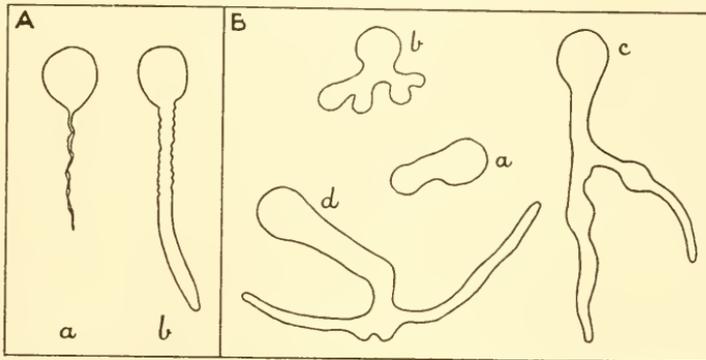


FIG. 47.—*Rhizopus nigricans*. Effect of light and darkness on germinating spores. A : *a*, the spore was germinated in a shallow hanging drop in a partially closed van-Tieghem cell in the dark and was then suddenly brought into the light, its protoplasm then flowed into the spore and left the germ-tube empty and plicate : *b*, the same spore and germ-tube after having been placed in the dark again, the protoplasm has re-entered the germ-tube and this has begun to grow again, the old plications in the wall can still be seen. B : the spores *a*, *b*, *c*, and *d* germinated in the light and were then placed in the dark ; the ends of the germ-tubes then became dilated ; in *c* and *d* the dilatations have persisted after normal growth has been resumed. Magnification, 345. The drawings copied by the author from Plate I of Raybaud's *Influence du milieu sur les Mucorinées*.

is then placed in the dark, the current of protoplasm flows in the direction the reverse of that taken when a mycelium is grown in the dark and then placed in the light. Movements induced by light take place not only during the application of the light but for some time afterwards. When spores are germinated in the light and are then placed in the dark, the ends of the germ-tubes become dilated and afterwards normal growth is resumed (Fig. 47, B). When hyphae which have been grown in the dark are placed in the light, the vacuoles and oil-drops increase in number (Fig. 48). In long hyphae, as Matruchot had pointed out earlier, intraproto-

plasmic movements can be seen which suggest that the protoplasm is permeated by a large number of minute, more or less parallel, often anastomosing tubules in the interior of which the granules move.

In 1912, Andrews<sup>1</sup> recorded the results of his studies on the effects of transpiration, osmosis, injury, light, and changes of temperature on protoplasmic streaming in *Rhizopus nigricans* (his

*Mucor stolonifer*), *Mucor Mucedo*, and *Phycomyces nitens*. For the most part his work served to corroborate the conclusions which had been arrived at by his predecessors. He remarked that the kind of nutrient medium is of great importance for growing the fungi successfully. By means of a suitable apparatus he was able to change at will the air in the small chamber in which a mycelium was growing and, as a result of using this apparatus, he found that streaming in a mycelium is strong or weak, the rate varying directly with the intensity of the transpiration. In saturated air streaming

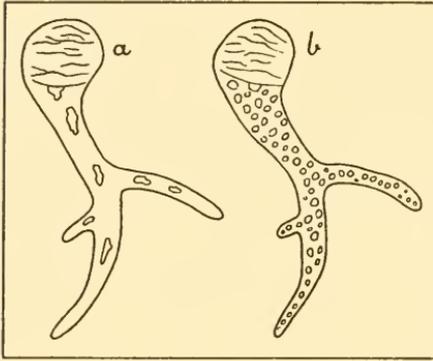


FIG. 48.—*Phycomyces nitens*. Effect of light on the vacuoles in germ-tubes: *a*, a spore which was germinated in darkness or weak light, the vacuoles in its branched germ-tube are few and irregular; *b*, the same, a few minutes after having been exposed to sunlight, the vacuoles have increased greatly in number. Magnification, 345. The drawings copied by the author from Plate I of Raybaud's *Influence du milieu sur les Mucorinées*.

ceases and, when relatively dry air is supplied, streaming begins and proceeds toward the tips of transpiring aerial hyphae. By means of a fine capillary tube a sugar solution may be applied to particular hyphae of a mycelium completely submerged in a drop of culture medium beneath a cover-glass. Under these conditions streaming takes place always toward the sugar solution and is exclusively due to osmosis. Among Andrews's other conclusions are the following. Injury caused by cutting off the tip of a hypha or by cutting a filament into two pieces may stop streaming altogether; but the

<sup>1</sup> F. M. Andrews, "Protoplasmic Streaming in Mucor," *Bull. Torrey Bot. Club*, Vol. XXXIX, 1912, pp. 455-499.

wound heals and streaming may be resumed. Light, when alternated with darkness, may cause or accelerate streaming. Streaming may be caused by a sudden change of temperature of several degrees. Streaming may occur or be caused to take place in unbranched as well as in branched filaments.

Arthur and, subsequently, Schröter had stated that in *Rhizopus nigricans*, when streaming is actively taking place in the centre of a hypha in one direction, a thin peripheral sheath-like non-vacuolated layer of protoplasm can sometimes be seen moving in the opposite direction, and both these authors had indicated the double current in a text-figure (for Arthur's illustration *vide* Fig. 46, B). However, Andrews was not only unable to observe any peripheral return current in a streaming hypha but positively denied its existence: "There is no streaming or movement in the opposite direction as stated and figured by Schröter . . . I can therefore not agree with Schröter on this point but find, as stated by Ternetz for *Ascophanus carneus*, that during streaming all of the moving protoplasm of *Mucor stolonifer* and *M. Mucedo* goes only in one direction. It is hardly possible, even if a reverse movement did take place along the wall during streaming, that it would be sufficient to account for the return of the protoplasm. The streaming occurs first in one direction, and when the factor that has caused this subsides or is overcome, it streams back in the opposite direction."

In 1932, assisted by one of my pupils, Mr. C. C. Neufeld, I examined cultures of the mycelium of *Rhizopus nigricans* with a view to finding out whether or not the return current in streaming hyphae can be observed. Spores were sown in hanging drops of 5 per cent. cane-sugar solution. Forty-one hours later the mycelia were found to be well developed. The temperature of the room was about 26° C. After the cover-glasses had been raised for a short time, streaming became very active in the thicker hyphae. In a number of these hyphae the *double flow of protoplasm in opposite directions was clearly seen*—a rapid massive streaming of protoplasm and vacuoles down the centre of a hypha in one direction, and a peripheral flow of a very thin sheath-like layer of non-vacuolated protoplasm in the opposite direction. The particles in the peripheral stream of protoplasm were watched moving quite steadily

and rapidly for long distances from one part of a hypha to another. In one preparation the main central mass of protoplasm moving in one direction and the thin peripheral layer of protoplasm moving in the opposite direction were seen to reverse their directions of movement simultaneously at intervals of from one to four minutes.

The observations on the double flow of protoplasm in certain hyphae of *Rhizopus nigricans* which have just been recorded confirm those made by Arthur and Schröter and indicate that Andrews's denial of the occurrence of the peripheral reversed flow of protoplasm was not well based.

While protoplasmic streaming in the non-septate hyphae of the Phycomycetes is now a well-known phenomenon, few mycologists seem to be aware that very active streaming also takes place in the septate hyphae of certain Ascomycetes. Hitherto, our knowledge of streaming in the Ascomycetes has been based upon brief statements made by Woronin, Rothert, Reinhardt, and Köhler and upon the results of a detailed investigation made by Ternetz.

In 1866, Woronin,<sup>1</sup> in the course of his description of the development of the apothecium of *Ascobolus pulcherrimus* Cr. (= *Lasiobolus pulcherrimus*), stated in a few words that, after an anastomosis has been formed between two hyphae, protoplasmic streaming, which is characteristic of the living cells of the mycelium, takes place from one cell to the other through the bridge; and, in a drawing, he represented the direction of streaming with arrows.

In 1892, Rothert<sup>2</sup> published the results of his investigations on *Sclerotium hydrophilum*. In cultures the sclerotia never fruited but gave rise to a mycelium which was septate and without clamp-connexions. A comparison of Rothert's illustrations with my own drawings of the hyphae of *Fimetaria fimicola* and *Pyronema confluens* suggests that *Sclerotium hydrophilum* is of ascomycetous origin with affinities with either the Pyrenomycetes or the Discomycetes. In the course of his studies upon this fungus Rothert noticed protoplasmic movement in the living cells. He<sup>3</sup> says: "In living cells

<sup>1</sup> M. Woronin, "Entwicklungsgeschichte des *Ascobolus pulcherrimus*," in de Bary and Woronin's *Beiträge zur Morphologie und Physiologie der Pilze*, II, 1866, p. 2.

<sup>2</sup> W. Rothert, "Ueber *Sclerotium hydrophilum* Sacc., einen sporenlosen Pilz," *Bot. Zeit.*, Jahrg. L, 1892, pp. 321-329, *et seq.*

<sup>3</sup> *Ibid.*, p. 361.

the protoplasm is engaged in continuous gliding (gleitender) movement, which does not strike one at once but is very noticeable when one watches continuously. One sees the vacuoles change their form, move from their positions, divide, and melt together. The movements of the protoplasm, which are the cause of all that, are nevertheless so rapid that they make it quite impossible to prepare an exact drawing of a vacuolated cell: in the time required the protoplasm completely changes its configuration." Apparently, Rothert did not observe the passage of the protoplasm from cell to cell. However, after recording that some hyphae lose protoplasm while others which are growing rapidly are full of it, he<sup>1</sup> suggested that the septa are perforated and that the protoplasm in consequence is able to pass through the septa and to move through the mycelium.

In 1892, Reinhardt,<sup>2</sup> in the course of his studies of the growth of hyphae of *Sclerotinia sclerotiorum* (his *Peziza Sclerotiorum*), observed the passage of protoplasm from one cell to another and described the phenomenon in a few words as follows: "Often one sees that the streaming plasm of a cell of a hypha, on coming to a young septum, does not turn back in its entirety, but certain portions of it, which can be clearly seen, pass through the cell-wall into the next cell; the passage is by jerks, somewhat like the passage of the gonoplasm of the antheridium of *Phytophthora* and *Pythium* through the narrow opening into the egg. This jerky clearly visible passage of protoplasm into a neighbouring cell always takes place in the very middle of the cross-wall." Doubtless moving protoplasm was streaming from one cell to another through a small pore situated at the centre of each septum.<sup>3</sup>

In 1900, Charlotte Ternetz<sup>4</sup> discovered and investigated protoplasmic streaming in another Discomycete, *Ascophanus carneus*. She stated that: the movement is neither a rotation nor a circulation and should be called simply "streaming"; for the protoplasm

<sup>1</sup> W. Rothert, "Ueber *Sclerotium hydrophilum* Sacc., einen sporenlosen Pilz," *Bot. Zeit.*, Jahrg. L, 1892, p. 383.

<sup>2</sup> M. O. Reinhardt, "Das Wachsthum der Pilzhyphe," *Jahrb. f. wiss. Bot.*, Bd. XXIII, 1892, p. 562.

<sup>3</sup> In three species of *Sclerotinia* a small central pore in each septum has actually been observed by Wahrlich and others (*vide infra*).

<sup>4</sup> Charlotte Ternetz, "Protoplasmabewegung und Fruchtkörperbildung bei *Ascophanus carneus*," *Jahrb. f. wiss. Bot.*, Bd. XXXV, 1900, pp. 273-312.

flows unhindered from one cell to another through the often very numerous cross-walls and from one system of hyphae to another *via* anastomoses, taking here an acropetal and there a basipetal direction and, under favourable conditions, passing through twenty or more hyphae. She pointed out that, whereas in the hyphae of Mucorineae streaming often takes place in both directions at the same time, in the hyphae of *A. carneus* streaming is always in one direction only. She was able to induce protoplasmic streaming in hyphae with the help of local applications of 10 per cent. cane-sugar solution, and she came to the conclusion that the movement of the protoplasm is due to differences of osmotic pressure in the cells of the hyphae. She sometimes observed the passage of vacuoles through the cross-walls, but was not able to form a clear conception of the manner in which these walls are perforated.

By a series of investigations which were made in part before Ternetz's paper appeared and in part afterwards, it has been established in respect to the mycelium of Ascomycetes, Basidiomycetes, and Fungi Imperfecti: (1) that each septum is provided with a *small central open pore*; (2) that through each pore *protoplasm extends from one cell to the next*; and (3) that, consequently, there is *protoplasmic continuity* between the successive cells of every living hypha or mycelium. These facts have an important bearing on the phenomenon of streaming in septate mycelia and, on this account, the history of their discovery will now be reviewed.

Between the years 1879 and 1901, through the work of Tangl,<sup>1</sup> Russow,<sup>2</sup> Gardiner,<sup>3</sup> Kienitz-Gerloff,<sup>4</sup> Arthur Meyer,<sup>5</sup>

<sup>1</sup> E. Tangl, "Ueber offene Communicationen zwischen den Zellen des Endosperms einiger Samen," *Jahrb. f. wiss. Bot.*, Bd. XII, 1879-1881, pp. 170-189.

<sup>2</sup> E. Russow, "Ueber den Zusammenhang der Protoplasmakörper benachbarter Zellen," *Sitzungsber. d. Dorpater Naturf.-Gesell.*, 1882, pp. 350-389. Cited from Just's *Bot. Jahresber.* for 1883.

<sup>3</sup> W. Gardiner, "On the Continuity of the Protoplasm through the Walls of Vegetable Cells," *Arbeiten des botanischen Instituts in Würzburg*, herausgegeben von J. Sachs, Leipzig, Bd. III, 1888, pp. 52-87.

<sup>4</sup> F. Kienitz-Gerloff, "Die Protoplasmaverbindungen zwischen benachbarten Gewebeelementen in der Pflanze," *Bot. Zeit.*, Jahrg. XLIX, 1891, pp. 1-10, 17-26, 33-46, 49-60, 65-74.

<sup>5</sup> A. Meyer, "Das Irrthümliche der Angaben über das Vorkommen dicker Plasmaverbindungen zwischen den Parenchymzellen einiger Filicinen und Angiospermen," *Ber. d. D. bot. Gesell.*, Bd. XIV, 1896, pp. 154-158.

Kuhla,<sup>1</sup> Kohl,<sup>2</sup> A. W. Hill,<sup>3</sup> Strasburger,<sup>4</sup> and others, it was established that, in the Phanerogamia, Pteridophyta, and Muscineae, living cells are connected with one another by extremely delicate protoplasmic filaments which Strasburger<sup>5</sup> called *plasmodesmae*. These filaments traverse the closing membranes of pits in large numbers or pass singly through the more or less thickened unpitted regions of the cell-wall.<sup>6</sup> As was shown by

<sup>1</sup> F. Kuhla, "Die Plasmaverbindungen bei *Viscum album*," *Bot. Zeit.*, Jahrg. LVIII, 1900, pp. 29-58.

<sup>2</sup> F. G. Kohl, "Dimorphismus der Plasmaverbindungen," *Ber. d. D. bot. Gesell.*, Bd. XVIII, 1900, pp. 364-372.

<sup>3</sup> A. W. Hill, "The Distribution and Character of 'Connecting Threads' in the Tissues of *Pinus sylvestris* and other Allied Species," *Proc. Roy. Soc.*, Vol. LXVII, 1901, pp. 437-439; also *Phil. Trans. Roy. Soc.*, Series B, Vol. CXCIV, 1901, pp. 83-125.

<sup>4</sup> E. Strasburger, "Über Plasmaverbindungen pflanzlicher Zellen," *Jahrb. f. wiss. Bot.*, Bd. XXXVI, 1901, p. 503.

<sup>5</sup> E. Strasburger, *loc. cit.*, p. 503.

<sup>6</sup> Theodor Hartig (*Bot. Zeit.*) discovered sieve-tubes in 1854. Sachs (*Flora*) in 1863 and Hanstein (*Die Milchsaftgefäße*, Berlin) in 1864 then demonstrated that the cells of sieve-tubes are connected by protoplasmic threads passing through the sieve-plates. One of the more important later papers treating of the details of structure of sieve-tubes and the mode of formation of sieve-plates was that of Hill ("The Histology of the Sieve-tubes of Angiosperms," *Annals of Botany*, Vol. XXII) published in 1908.

Tangl (1879-1881) first proved the correctness of suggestions made by Hofmeister, Sachs, and Strasburger that ordinary living cells in a higher plant are intimately connected with one another by demonstrating the existence of plasmodesmae in the ripe endosperm cells of *Strychnos*, *Phoenix*, and *Areca*. Russow found plasmodesmae in the secondary phloem of certain Dicotyledons. Gardiner confirmed Tangl's observations by observing protoplasmic connexions in the endosperm of fifty species of Palms and of many other dicotyledonous and monocotyledonous plants. He also found the connexions in the pulvinus of *Mimosa pudica*, *Robinia pseud-acacia*, etc. Kienitz-Gerloff found connexions in a great variety of plants from Liverworts to Phanerogams; but, as pointed out by Meyer, the worth of his work was unfortunately diminished by a faulty technique. At Meyer's suggestion, Kuhla, as a result of a very careful and laborious investigation, showed that, in *Viscum album*, the protoplasm of all the living cells of an individual plant is connected together by bridges into a single symplastic unit. In an interesting schematic diagram of a cross-section of a one-year-old stem of *Viscum* showing cells of all the tissues from the epidermis to the pith, he gives for every cell-wall the area occupied by pits and the number of plasmodesmae per 100 square  $\mu$  of wall surface. Kohl investigated the occurrence of *solitary* plasmodesmae, *i.e.* those that go through the whole thickness of the cell-wall, and of *aggregated* plasmodesmae, *i.e.* those that occur in groups and are confined to the closing membranes of pits, and he found that, usually, the two types of plasmodesmae do not occur in one and the same cell although they may occur in different tissues of the same plant. Hill showed that plasmodesmae are very generally present throughout the tissues of the young seedling of *Pinus pinea*

Strasburger<sup>1</sup> they are not persistent nuclear spindle-fibres, but are secondary formations which grow through the primary cell-wall after this has become completely formed but before the deposition upon it of thickening layers. This mode of origin, which is not dependent upon nuclear division, permits of plasmodesmae being formed between such tissues as the epidermis and the outer cells of the cortex<sup>2</sup> and between the living cells of a scion and its stock.<sup>3</sup> In the Fungi, as we shall see, the protoplasmic bridges between the cells, except in so far as fusions between different hyphae are concerned, are not secondary but primary.

In the Red Algae, the Brown Algae, and certain Green and Blue-green Algae, there are pits between adjacent cells; and, where such pits occur, it is probable that there is protoplasmic continuity between neighbouring cells.<sup>4</sup> Arthur Meyer<sup>5</sup> demonstrated protoplasmic continuity in three species of *Volvox* in 1896. According to Falkenberg<sup>6</sup> the pits of the Florideae have closing membranes which, when isolated, appear punctate and bear minute protoplasmic fibrillae, thus suggesting that, under normal conditions, they are threaded by very fine plasmodesmae. In the Laminariaceae, sieve-tubes were discovered by Reinke<sup>7</sup> in 1876 and, subsequently, they were studied by Wille, Will. Rosenthal, Oliver, and others.<sup>8</sup> Sykes,<sup>9</sup> in 1908, as a result of investigations made with the most modern technique, came to the conclusion that, in *Macrocystis pyrifera*

and of the adult stem, leaf, and root of *P. sylvestris*, including the root-cap and the junction of the endodermis with the cortex in a leaf. Strasburger, in his masterly paper, solved problems connected with the origin of plasmodesmae.

<sup>1</sup> E. Strasburger, *loc. cit.*, pp. 493-503.

<sup>2</sup> *Ibid.*, pp. 495-500.

<sup>3</sup> *Ibid.*, pp. 582-585.

<sup>4</sup> For references to the literature on plasmodesmae in Algae, *vide* F. Oltmanns, *Morphologie und Biologie der Algen*, Aufl. 2, Jena, Bd. III, 1932, pp. 5-6.

<sup>5</sup> A. Meyer, "Die Plasmaverbindungen und die Membranen von *Volvox globator*, *aurcus* und *tertius*, mit Rücksicht auf die thierischen Zellen," *Bot. Zeit.*, Bd. LIV, 1896, pp. 187-217.

<sup>6</sup> P. Falkenberg, "Rhodomelaceen des Golfes von Neapel," *Fauna und Flora des Golfes von Neapel*, Berlin, 1901, pp. 16-27.

<sup>7</sup> J. Reinke, "Beiträge zur Kenntniss der Tange," *Jahrb. f. wiss. Bot.* Bd. X, 1876, p. 373.

<sup>8</sup> For references to this series of papers, *vide* Sykes.

<sup>9</sup> M. G. Sykes, "Anatomy and Histology of *Macrocystis pyrifera* and *Laminaria saccharina*," *Annals of Botany*, Vol. XXII, 1908, pp. 291-325.

and *Laminaria saccharina*, the "trumpet hyphae" are true sieve-tubes and the structure of these sieve-tubes resembles that of Phanerogams. In these species she also demonstrated the existence of protoplasmic connecting-threads passing through the closing membranes of pits throughout the tissues.

It was natural that, after protoplasmic connexions had been found to exist between adjacent cells in Phanerogamia, Pteridophyta, Muscineae, and Algae, they should be looked for in Fungi. The first to notice pits in fungus walls was de Bary,<sup>1</sup> in his well-known Text-book published in 1884, but he gave no indication whether the pits were closed or open. He says: "The thick transverse walls of *Dactylium macrosporum* Fr. which are formed of two semi-lenticular lamellae have a large pit in their centre, just in the same way as it occurs in the transverse walls of filiform Florideae like *Callithamnion*. I have never seen pits in other Hyphomycetes; their transverse walls are usually delicate but often, as in *Botrytis cinerea*, they appear to be thinner in the middle than at the margin."

Strasburger,<sup>2</sup> in 1884, in his *Practical Botany*, described and illustrated pits in the septa of the stipes of *Agaricus* (= *Psalliota*) *campestris* and *A. pratensis*.

Chmielewski,<sup>3</sup> in 1886, discovered and described perforations in the septa and protoplasmic connexions between successive cells in the hyphae of *Haplotrichum roseum* Corda.

In 1893, Wahrlich,<sup>4</sup> in an important paper, published in both Russian and German, demonstrated that septal pores and protoplasmic bridges between cells are characteristic of Fungi in general (Figs. 49-52). In making his preparations he used fresh material and fixed it with a watery solution of iodine in potassium iodide, with more iodine than usual, so as to obtain a darker colour in the

<sup>1</sup> A. de Bary, *Vergleichende Morphologie und Biologie der Pilze*, Leipzig, 1884, p. 14.

<sup>2</sup> E. Strasburger, *Das Botanische Practicum*, Jena, 1884, pp. 324-325.

<sup>3</sup> Chmielewski, "Zur Morphologie von *Haplotrichum roseum* (Corda)," *Ber. der neuruss. Naturf.-Gesell.*, Bd. VI, Odessa, 1886 (in Russian). Cited from Wahrlich.

<sup>4</sup> W. Wahrlich, "Zur Anatomie der Zelle bei Pilzen und Fadenalgen," *Scripta Botanica Horti Universitatis Imperialis Petropolitanae*, T. IV, 1893, pp. 101-155, Tab. II-IV.

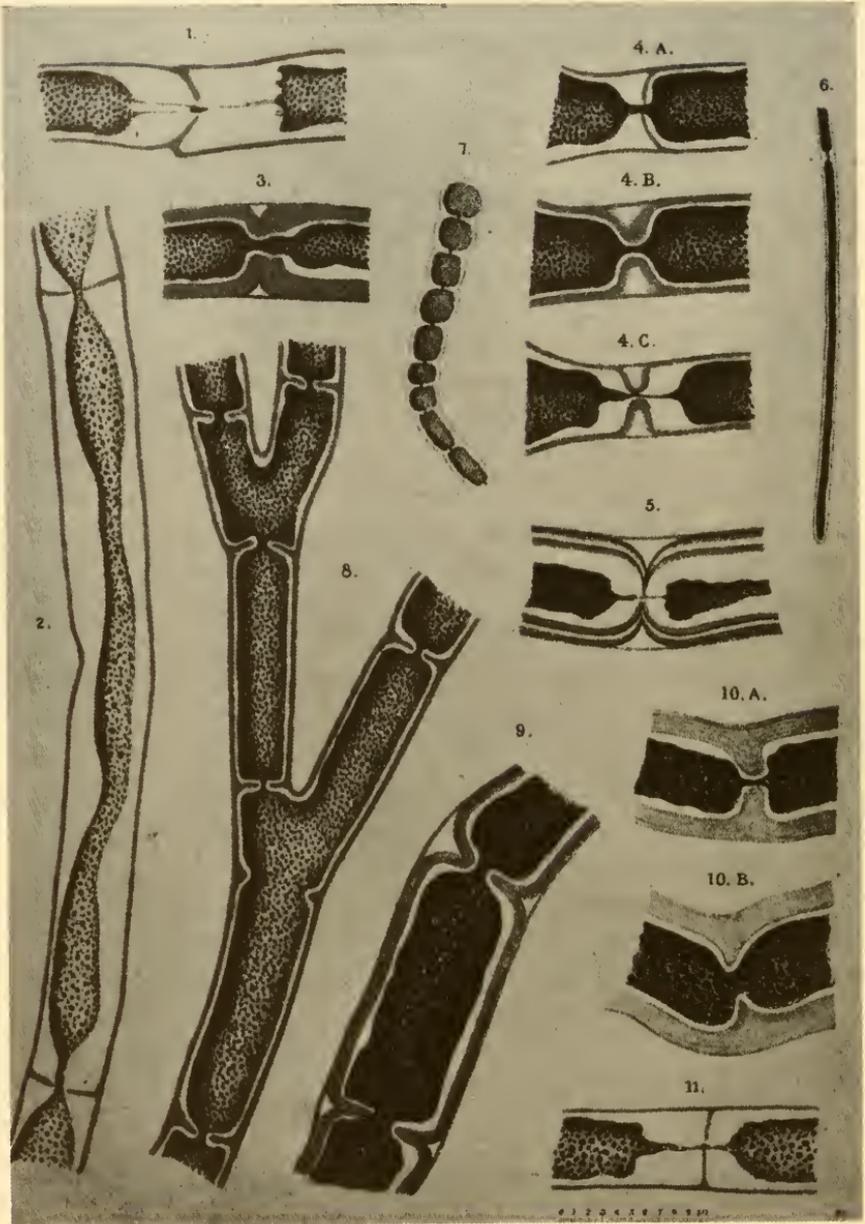


FIG. 49.—Septal pores and protoplasmic bridges between adjacent cells in the hyphae of various fungi. Nos. 1-6, *Eurotium herbariorum*. No. 1, hypha of a young mycelium after treatment with chlor-zinc iodine. No. 2, hypha of a young mycelium; plasmolysis has been effected by means of a sodium-chloride solution. No. 3, hypha of an old mycelium treated with chlor-zinc

protoplasm<sup>1</sup>; and then, to cause the septa to swell up, he employed, instead of sulphuric acid, chlor-zinc iodine. For certain fungi he found it necessary to increase the action of the chlor-zinc iodine by warming cautiously "until the production of the first bubbles." An examination of preparations thus treated allowed Wahrlich to observe protoplasmic connexions between adjacent cells in all the fungi which he investigated, with the exception of *Oidium lactis*. The species in which the connexions were found, as named by Wahrlich, were as follows<sup>2</sup>:

- I. *Mucorini*: *Mucor stolonifer* (sometimes in old hyphae which have become septate).
- II. *Ustilagineae*: *Urocystis Anemones* (mycelium); *Graphiola Phoenicis* (between young incompletely developed spores).
- III. *Uredineae*: *Puccinia fusca* (mycelium); *P. graminis* and *P. caricis* (mycelium and unripe aecidiospores).
- IV. *Agaricineae*: *Lactarius theiogalus* (+), *Russula alutacea* (+), *Omphalia campanella* (connexions everywhere splendidly visible), *Marasmius rotula* (+), *M.*

FIG. 49—cont.

iodine, washed, and then stained with haematoxylin; the cross-wall appears as a distinct fold of the second layer; the walls of the hyphae are unequally thickened. No. 4, hypha from a young mycelium: A, fixed with iodine in potassium iodide; B, the same hypha treated with chlor-zinc iodine, the cross-wall is much swollen and in its inner part a clear space can be seen; C, the same hypha stained with haematoxylin after being washed with water, the cross-wall appears as a ring-like fold of the inner (second) membrane-layer. No. 5, hypha from an old mycelium, treated with chlor-zinc iodine; the cell-walls clearly have a layered structure, and the layers have separated from one another. No. 6, an extremely thin young branch-hypha, after treatment with chlor-zinc iodine. No. 7, *Graphiola Phoenicis* (? an imperfect fungus); a chain of unripe spores, after treatment with chlor-zinc iodine; between the spores thin protoplasmic connexions can be seen. No. 8, *Achorion Schönleini* (a dermatophyte causing favus); a branched hypha from a young bouillon culture; treated with chlor-zinc iodine. No. 9, *Botrytis cinerea*; an old mycelial hypha, after treatment with chlor-zinc iodine. No. 10, *Sclerotinia Libertiana*; hyphae from the pith of a sclerotium; A, from a fully developed dry sclerotium; B, from a young sclerotium; treated with chlor-zinc iodine. No. 11, *Marasmius oreades*; hypha from the pith of the stipe where this passes into the pileus, after treatment with chlor-zinc iodine. From Wahrlich's *Zur Anatomie der Zelle bei Pilzen und Fadenalgen*. Magnification, 1800.

<sup>1</sup> A. Meyer (*vide infra*) gives the proportions of iodine, potassium iodide, and water as 3 : 3 : 20.

<sup>2</sup> A (+) sign after a name indicates that the protoplasmic connexions were somewhat difficult to observe, so that the preparation had to be warmed.

## RESEARCHES ON FUNGI

scorodonius (+), *M. oreades* (+), *Agaricus ostreatus* (+), *A. campestris*, *Hypholoma fasciculare* (+), *Coprinus atramentarius* (connexions everywhere splendidly visible). In all these fungi all the tissues of the fruit-bodies, chiefly young ones, were investigated and the connexions were found everywhere.

- V. *Polyporaceae* : *Boletus versipellis* (connexions everywhere can be easily seen), *Polyporus fuscidulus* (+), *Polyporus perennis* (+), and *Daedalea Poetschii* (+), (hymenial layer not investigated, in other parts the connexions were rather difficult to observe), *Merulius lacrymans* (mycelium only investigated).
- VI. *Hydnaceae* : *Hydnum compactum* (+).
- VII. *Clavariaceae* : *Corallium pratense* (+).
- VIII. *Tremellineae* : *Calocera viscosa* (+), *Tremella undulata* (connexions everywhere can easily be seen).
- IX. *Hymenogastreae* : *Hydnangium carneum* (connexions everywhere present).
- X. *Lycoperdaceae* : *Bovista nuciformis* (+), *Scleroderma vulgare* (+).
- XI. *Nidularieae* : *Crucibulum vulgare* (+).
- XII. *Saccharomycetes* : *Saccharomyces cerevisiae* (the young cells remain for some time connected with the mother-cells by a thread of protoplasm).
- XIII. *Perisporiaceae* : *Eurotium herbariorum*, *Aspergillus niger*, *Penicillium glaucum*. In all these three fungi there are connexions between all the cells of the mycelium from the finest branches right up to the conidia.
- XIV. *Hypocreaceae* : *Claviceps purpurea*. Only sclerotia investigated. Connexions somewhat difficult to find, best seen in preparations kept in an alcoholic sublimate solution for 24 hours, then washed, and then treated with chlor-zinc iodine.

- XV. *Pezizeae* : *Sclerotinia Fuckeliana* (the conidia-forming mycelium, *Botrytis cinerea*, and mycelial branches with spermatia only investigated, in both the connexions could be seen splendidly), *Sclerotinia Libertiana* (sclerotia), *Sclerotinia Rhododendri* (sclerotia), *Humaria scutellata* (connexions found in all parts of the fruit-body, they can be especially well seen where they go into asci and paraphyses ; in the small bristles at the margin of the apothecium large open pores can be seen in the septa), *Peziza acetabulum* (connexions found in all parts).
- XVI. *Helvellaceae* : *Helvella esculenta* (young fruit-bodies in which the hymenium had not yet developed were investigated and everywhere connexions could be seen splendidly).
- XVII. *Tuberaceae* : *Tuber melanosporum* (connexions found everywhere).
- XVIII. *Fungi with an imperfectly investigated life-history* : *Achorion Schönleinii* (connexions everywhere present, the thickest  $1\ \mu$  in diameter), *Oidium subtile* (or *Microsporon furfur*, the connexions are extremely fine), *Oidium albicans* (the mycelium-forming form investigated, the connexions are extremely fine), *Oidium lactis* (this fungus has no connexions).
- XIX. *Fungi not identified*. In one, obtained from a pine-board, the connexions between the cells in the multicellular spores could be seen even with a low magnification.

Wahrlich observed protoplasmic connexions between : ordinary mycelial cells (Fig. 49, Nos. 1-6, 8, and 9) ; the cells of sclerotia (Fig. 49, No. 10) ; the cells of fruit-bodies (Fig. 49, No. 11, and Fig. 52, No. 15) ; subhymenial cells and asci (Fig. 50, No. 33, B) ; subhymenial cells and paraphyses (Fig. 50, No. 33, A) ; subhymenial cells and basidia (Fig. 51) ; conidiophores and conidia ; and between the cells of multicellular spores (Fig. 50, No. 34, A). Everywhere the

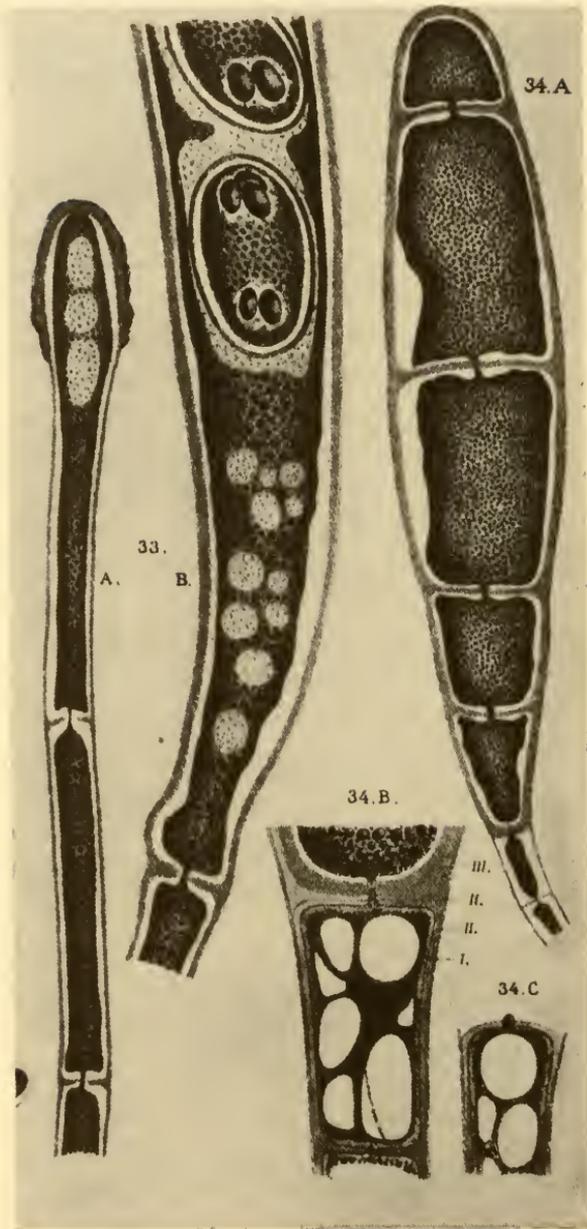


FIG. 50.—Septal pores and protoplasmic bridges between adjacent cells in certain fungi. No. 33, *Humaria scutellata*; A, paraphysis; B, ascus; treated with chlor-zinc iodine. No. 34, an unidentified fungus growing on a pine-board; A, a five-celled spore seated on its pedicel; B, the upper part of

septum was found to have a simple central open pore, devoid of a closing membrane, through which passed a single thread of protoplasm; only in one instance (an unidentified fungus) was a more complicated arrangement met with. In a clamp-connexion each of the two septa has a pore (Fig. 52, No. 16). The thread of protoplasm passing through a pore is about  $1.0-1.5\mu$  thick and consists, externally, of a thin layer of homogeneous protoplasm and, internally, as may be seen in favourable preparations, of granular protoplasm. A pore in a septum arises not through subsequent resorption of a piece of membrane, but is there from the first owing to the septum never having become fully formed at its centre. Cell-division in septate hyphae is therefore never complete. Of this Wahrlich obtained convincing evidence from a young culture of *Achorion Schönleinii* (Fig. 49, No. 8). A septum begins its formation as a ring-like projection from the inside of the wall of the mother-cell and gradually pushes its way toward the centre of the cell. However, it never extends completely to the centre of the cell so that, when it ceases to develop, it is already provided with a median pore.<sup>1</sup>

So far as the function of the protoplasmic bridges is concerned, Wahrlich expressed the view that they serve as ways for the transportation of material and, in particular, of granular protoplasm. In support of this he pointed to the fact that

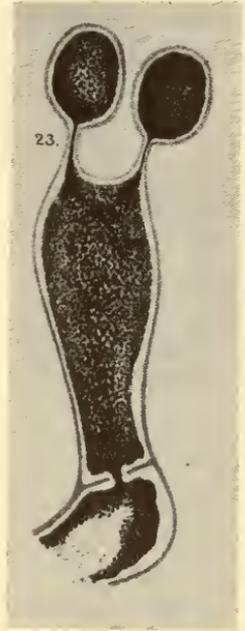


FIG. 51.—*Coprinus atramentarius*, a basidium. After Wahrlich. Mag., 1800.

FIG. 50—cont.

the pedicel with the base of the spore, the Roman numerals indicate the different membrane-layers; C, the top of the pedicel torn away from the spore, a granule of protoplasm is left sticking in the pore; treated with chlorzinc iodine. From Wahrlich's *Zur Anatomie der Zelle bei Pilzen und Fadenalgen*. Magnification: No. 34, A, 600; the others 1800.

<sup>1</sup> Wahrlich believed that, in *Spirogyra*, and probably also in *Fungi*, the primary wall of the septum begins its development as a true ring-fold formed within a constriction of the protoplast.

cells and parts of mycelia often become empty of protoplasm and stated that, through careful one-sided removal of water from a living mycelium of *Eurotium herbariorum*, he had succeeded in setting the cell-contents in slow motion, as a result of which he had been able directly to observe the passage of granular protoplasm out of one cell into another.

In 1896, in Part I of his great monograph on the Laboulbeniales, Thaxter<sup>1</sup> stated that in these fungi: "The protoplasm of adjacent

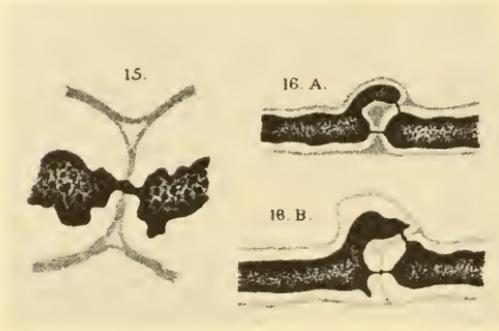


FIG. 52.—Septal pores and protoplasmic connexions. No. 15, *Coprinus atramentarius*; a hypha from the stipe. No. 16, A and B, two clamp-connexions from a mycelium, presumably of *Merulius lacrymans*. Treated with chlor-zinc iodine. After Wahrlich. Magnification, 1800.

cells, the origin of which is the same, is connected by a conspicuous strand of the same substance, which passes from one cell to the other through a well-marked perforation of the cell wall, the connection being demonstrated with great ease by potash and subsequent staining"; and he illustrated the pits and the supposed simple protoplasmic strands very beautifully. In 1912, Faull<sup>2</sup> attempted to

verify Thaxter's observations by means of investigations on *Laboulbenia chaetophora*. With gross material treated with potash he obtained results like those of Thaxter; but, when he came to examine microtome sections prepared in the usual manner, he found that the pits, as a rule, have a closing membrane. Of this membrane, which apparently Thaxter had overlooked, Faull says that, in favourable preparations, it "can be seen to be perforated by a very fine pore and, in some instances, there is the appearance of several minute perforations."

<sup>1</sup> R. Thaxter, "Contributions toward a Monograph of the Laboulbeniaceae; Part I," *Mem. Americ. Acad. of Arts and Sci.*, Vol. XII, 1896, p. 236, Plate II, Figs. 16-18, and Plate III, Figs. 11-12.

<sup>2</sup> J. H. Faull, "The Cytology of *Laboulbenia chaetophora* and *L. Gyridarum*," *Annals of Botany*, Vol. XXVI, 1912, pp. 330-333.

Wahrlich's work on fungi in general was subsequently confirmed by other investigators; for a single protoplasmic strand passing through a median pore in each septum was observed: by A. Meyer<sup>1</sup> (1896-1902) in *Hypomyces rosellus* and *Pleurotus ostreatus*; by Dangeard<sup>2</sup> in *Sphaerotheca Castagnei* (1897), and *Bactridium flavum* (1900); by Woronin<sup>3</sup> (1900), in *Sclerotinia cinerea* and *S. fructigena*; and by Kienitz-Gerloff<sup>4</sup> (1902), in *Sclerotinia cinerea*, *S. fructigena*, *Peziza aurantiaca*, the sclerotium of *Claviceps purpurea*, a *Verticillium*, and a *Cephalothecium*.

In 1894, Poirault<sup>5</sup> published a brief note in which he stated that, in *Cladonia rangiferina*, *Peltigera canina*, *Calicium chrysocephalum*, and other Lichens, the septa in the hyphae of the thallus are traversed by several protoplasmic threads, whilst those in the paraphyses are traversed by a single thread. In 1902, this was confirmed by A. Meyer<sup>6</sup> who found that in *Peltigera canina* the normal cross-walls of the hyphae, when sufficiently large, have several perforations and, when small, have only one perforation. Septa with two perforations were observed by Strasburger<sup>7</sup> (1901) in *Cora pavonia*, and septa having 2-6 perforations and looking like the sieve-plates of Phanerogamia were observed by Kienitz-Gerloff<sup>8</sup> (1902) in *Peltigera canina* and *P. polydactyla* after he had destroyed the cell-contents with eau de Javelle. Protoplasmic continuity

<sup>1</sup> A. Meyer, "Das Vorkommen von Plasmaverbindungen bei den Pilzen," *Ber. d. D. bot. Gesell.*, Bd. XIV, 1896, pp. 280-281, and "Die Plasmaverbindungen und die Fusionen der Pilze der Florideenreihe," *Bot. Zeit.*, Bd. LX, 1902, pp. 139-178.

<sup>2</sup> P.-A. Dangeard, "Second mémoire sur la reproduction sexuelle des Ascomycètes," *Le Botaniste*, T. V, 1897, p. 255; also "Structure et communications protoplasmiques dans le *Bactridium flavum*," *Le Botaniste*, T. VII, 1900, pp. 35-36, 39-40.

<sup>3</sup> M. Woronin, "Über *Sclerotinia cinerea* und *Sclerotinia fructigena*," *Mém. de l'Acad. Imp. des Sci. de Saint-Petersbourg, Classe-mathématique*, Bd. X, 1900. Cited from A. Meyer.

<sup>4</sup> F. Kienitz-Gerloff, "Neue Studien über Plasmodesmen," *Ber. d. D. bot. Gesell.*, Bd. XX, 1902, pp. 102-104.

<sup>5</sup> G. Poirault, "Les communications intercellulaires chez les Lichens," *Bull. Soc. Myc. France*, T. X, 1894, pp. 131-132; also in *Comptes Rendus*, T. CXVIII, 1894, pp. 1362-1363.

<sup>6</sup> A. Meyer, *loc. cit.*, pp. 142-143.

<sup>7</sup> E. Strasburger, "Über Plasmaverbindungen pflanzlicher Zellen," *Jahrb. f. wiss. Bot.*, Bd. XXXVI, 1901, p. 519.

<sup>8</sup> F. Kienitz-Gerloff, *loc. cit.*, pp. 102-106.

between the cells in trichogynes was observed : by Baur <sup>1</sup> (1898) in *Collema crispum* ; by Darbyshire <sup>2</sup> (1900) in *Physcia pulverulenta* ; and by Kienitz-Gerloff <sup>3</sup> (1902) in *Collema*.

In the summer of 1932, Mr. S. P. Wiltshire of the Imperial Mycological Institute, Kew, showed me central pores in the septa of multiseptate spores of *Stemphylium paxianum* (v. Szabo) Lindau (Baarn culture) and of *Alternaria circinans* (B.et C.) Bolle, mounted in lactic acid (Fig. 53). These pores could readily be seen in the middle of obliquely situated septa as rounded apertures through which light passed more freely than through the dark wall-substance. Doubtless, when the spores were living, protoplasmic strands passed through these pores and thus united the whole of the protoplasm of each spore into a single mass.

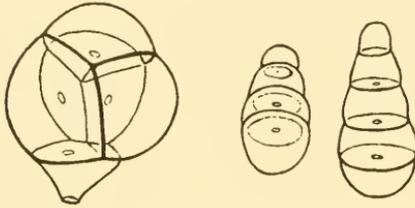


FIG. 53.—Central open pores in the middle of the septa of multiseptate spores : to the left, *Stemphylium paxianum* ; to the right, *Alternaria circinans*. Drawn by S. P. Wiltshire. Magnification, 1100.

In the autumn of 1932, with the help of Dr. E. S. Dowding, I succeeded with the iodine and chlor-zinc iodine method in observing quite clearly the protoplasmic bridges passing through the septa in mycelial hyphae of

*Fimetaria fimicola* and of *Rhizoctonia solani* (= *Corticium solani*). Drawings illustrating these bridges are reproduced in Fig. 54. Attempts to see the bridges in the inner stipe cells of *Psalliota campestris* and in the mycelium of *Coprinus sterquilinus* failed to give conclusive results, as the protoplasm, in consequence of imperfect fixation, usually became much contracted.

In the spring of 1933, Dr. P. H. Gregory, at my suggestion, sought for pores in the septa of certain dermatomycetes obtained from ring-worm patients at Winnipeg. With the help of the iodine and chlor-zinc iodine technique he had no difficulty in finding the

<sup>1</sup> E. Baur, "Zur Frage nach der Sexualität der Collemaceen," *Ber. d. D. bot. Gesell.*, Bd. XVI, 1898, p. 366.

<sup>2</sup> O. V. Darbyshire, "Über die Apothecienentwicklung der Flechte *Physcia pulverulenta*," *Jahrb. f. wiss. Bot.*, Bd. XXXIV, 1900, p. 336.

<sup>3</sup> F. Kienitz-Gerloff, *loc. cit.*, pp. 105-106.

pores in the multiseptate macroconidia (fuseaux) of *Microsporon felineum*, and he kindly demonstrated them to me (Fig. 55).

My own observations<sup>1</sup> on the mode of formation of particular septa in living mycelia of *Pyronema confluens*, *Rhizoctonia solani*, and *Rhizopus nigricans* have convinced me of the correctness of Wahrlich's conclusion that the septa of fungi in general are formed by annular ingrowths from a lateral wall. Further evidence of this mode of formation of septa is provided by Dr. Gregory's comparative observations on the structure of the macroconidia of *Microsporon felineum* in various stages of development. The spores were fixed in glacial-acetic acid, washed, and mounted in glycerine-jelly containing methylene-blue. The protoplasm of the spores took on a blue colour while the cell-walls remained colourless. Dr. Gregory, on examining spores treated in this way, found several in which the septa were only partially grown and in which it was evident that each of the septa had arisen in the first place as an annular ingrowth from the exterior spore-wall (Fig. 55, C).

From the investigations on protoplasmic continuity which have just been summarised we may conclude that Ascomycetes, Basidiomycetes, and Fungi Imperfecti resemble Phanerogamia, Pteridophyta, Muscineae, and multicellular Algae in that all the living cells which make up an individual plant are connected together so as to form a single mass of protoplasm. A realisation of this important fact

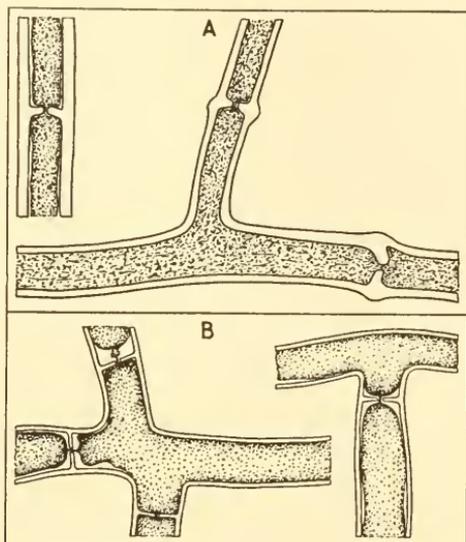


FIG. 54.—Protoplasmic bridges extending from cell to cell through the pores of septa in mycelial hyphae. A, *Fimetaria fimicola*, a Pyrenomycete; B, *Rhizoctonia solani* (= *Corticium solani*), a Hymenomycete. Hyphae treated with iodine and chlor-zinc iodine. Magnification, 1030.

<sup>1</sup> Vide infra.

helps us to understand not merely the phenomenon of protoplasmic streaming in the mycelium of certain Discomycetes, Pyrenomycetes,

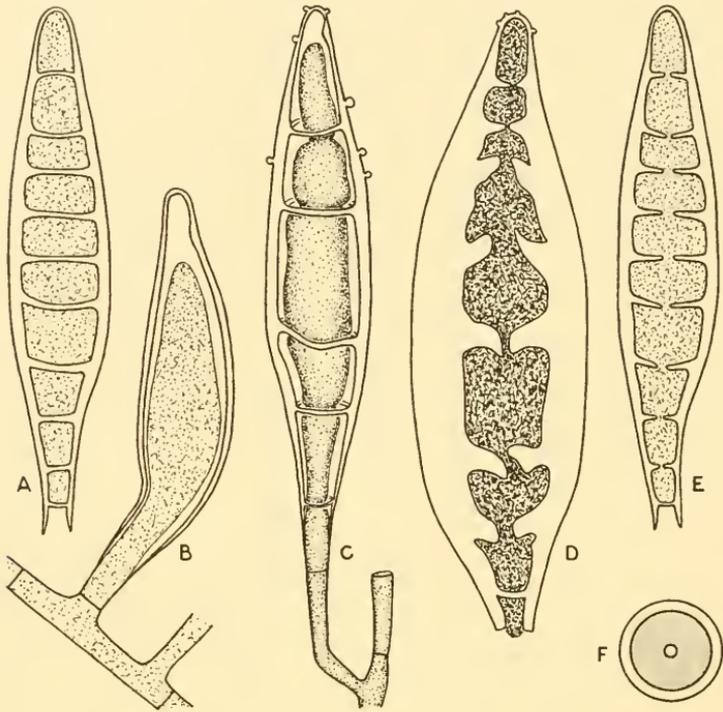


FIG. 55.—*Microsporion felineum*. The pore in the septum, and the mode of formation of a septum, in a macroconidium (fuseau). A, a macroconidium in water: the median pore in each septum cannot be seen. B and C, fixed in acetic acid and stained with methylene-blue. B, a very young macroconidium before septa have begun to develop. C, an older macroconidium: the septa are developing as annular ingrowths from the lateral wall; the protoplasm, slightly contracted from the lateral wall, is constricted at each partially formed septum. D, a large macroconidium with the outer wall and the septa much swollen with chlor-zinc iodine and the protoplasm darkly stained with iodine: the central pore in each septum can be readily seen. E, a diagrammatic representation of a median-longitudinal section of a living macroconidium showing a median pore in each septum. F, a diagrammatic cross-section of the macroconidium E, showing the outer wall, a septum, and a septal pore. Drawn by A. H. R. Buller and P. H. Gregory. Magnification, 1100.

and Hymenomycetes, but also how it is that a multicellular fungus can develop and react to external stimuli in a unitary manner.

In 1930, Köhler,<sup>1</sup> whilst studying the formation of hyphal fusions in *Neurospora sitophila*, *N. crassa* and *N. tetrasperma*, observed the streaming of protoplasm from cell to cell. He sowed some conidia of *Neurospora* (sp. ?) thickly in a film of agar and soon hyphal fusions took place between the germ-tubes. "The streaming of the protoplasm," says Köhler, "out of the connected germ-mycelia toward the growing conidia-forming edge of the mycelium could be splendidly seen. The cell-walls through which the stream moved were, except for traces attached to the inside of the membrane, re-absorbed so that the streaming, not confronted with any important opposition, attained a surprisingly high speed, almost comparable with the flow of blood through the capillaries of the tongue of a frog.<sup>2</sup> The streaming is in full progress about 20 hours after sowing the conidia at 18–20° C. It proceeds mostly by jerks, but at higher speeds proceeds steadily." In view of the investigations which have been made on the nature of the septa in the Higher Fungi by Wahrlich and others, and in view of my own observations on the rapid passage of protoplasm through the small central pore in the septa of *Fimetaria fimicola* and *Pyronema confluens*, I am inclined to think that Köhler was in error in stating that in hyphae of *Neurospora* in which streaming can be seen the septa have been re-absorbed. Probably the septa in *Neurospora* have a small central pore and are just as persistent as those in Higher Fungi generally.

**Investigations by the Author.**—In the spring of 1932, whilst studying hyphal fusions in the mycelium of *Fimetaria fimicola*, I

<sup>1</sup> E. Köhler, "Zur Kenntnis der vegetativen Anastomosen der Pilze. II.," *Planta*, Bd. X, 1930, pp. 505–506.

<sup>2</sup> The rate of flow of protoplasm in various fungi will be found in the Table on p. 109. The maximum rate observed was: for *Rhizopus nigricans*, 55  $\mu$  per second; and for the Pyrenomycete, *Fimetaria fimicola*, 16.5  $\mu$  per second. According to Michael Foster (*A Text Book of Physiology*, Part I, London, 1893, pp. 225–226), the observed rate of flow of blood is: through the capillaries of the web of a frog's foot, about 500  $\mu$  per second; through the capillaries of mammals generally, about 750  $\mu$  per second; and through the capillaries of man, about 1000  $\mu$  per second. The rate of flow of blood through the capillaries of a frog is therefore about nine and thirty times greater than that of the maximum rate of flow of protoplasm through the hyphae of *Rhizopus nigricans* and of *Fimetaria fimicola* respectively. It is evident that the rate of flow of blood through the capillaries of frogs, mammals, and man is far greater than the maximum observed rate of flow of protoplasm through the hyphae of any fungus whatsoever.

discovered that in this species the protoplasm often streams rapidly through the hyphae and sometimes so violently that it dislodges the vacuoles and carries them along. Subsequently I observed streaming in the mycelium of several other Higher Fungi, the names of which are set forth in the accompanying list.

*Higher Fungi in which Protoplasmic Streaming was observed  
by the Author.*

Ascomycetes	{	Pyrenomycetes { <i>Fimetaria fimicola</i> <i>Gelasinospora tetrasperma</i>
	{	Discomycetes { <i>Pyronema confluens</i> <i>Ascophanus carneus</i> <i>Ciboria</i> sp.
Basidiomycetes	Hymenomycetes	{ <i>Rhizoctonia solani</i> (= <i>Corticium solani</i> )

An account of my investigations on protoplasmic streaming in these fungi will be given in what follows and, in general, it will serve to verify and extend the observations on protoplasmic streaming in septate mycelia made by Ternetz and others whose contributions to this subject have been reviewed in the foregoing pages.

After the work on protoplasmic streaming in the Higher Fungi had been brought to a conclusion, I investigated streaming and the formation of septa in *Rhizopus nigricans* and thus obtained data which have enabled me to compare the mycelium of one of the Phycomycetes with that of the Ascomycetes and the Basidiomycetes. The results of the studies made on *Rhizopus nigricans* will be given toward the end of this Chapter.

***Fimetaria fimicola*.**—*Fimetaria fimicola* (Roberge) Griffiths and Seaver (= *Sordaria fimicola* Ces. and de Not.) is a fimicolous Pyrenomycete (cf. Fig. 56) which occurs on the dung of horses, cows, goats, rabbits, sheep, and deer, and also on old paper, in Europe and North America.<sup>1</sup> At Winnipeg it is frequent on horse dung.

<sup>1</sup> F. J. Seaver, "The Fimetariales," *North American Flora*, Vol. III, Part I, 1910, pp. 66-67.

Its fruit-bodies are flask-shaped, blackish, and beaked, and they often protrude in large numbers from the surface of the substratum. Their beaks are positively heliotropic (Figs. 57 and 58). The ascospores are black and are surrounded, except at the germ-pore, by a hyaline gelatinous envelope.

**Cultures.**—Some mycelium developing fruit-bodies of *Fimetaria fimicola* was found on horse-dung balls obtained from a stable. Some of this mycelium was then transferred to sterilised horse dung where it grew very rapidly over the substratum and soon fruited. The radial rate of growth of the mycelium on cleared dung-agar at room temperatures was found to be 2·8 cm. in 48 hours or 1·4 cm. per day.

**Protoplasmic Streaming.** — A hanging drop of cleared dung-agar was inoculated with some mycelium of *Fimetaria fimicola*, and 24 hours later the mycelium had spread freely in the culture medium. On observing the mycelium with the high power of the microscope, it was found that the protoplasm in the cells was distinctly granular and that it was flowing rapidly through certain of the hyphae. It appeared to pass through the septa without any difficulty, and it could also be seen making its way from one hypha to another through passage-ways formed by peg-to-peg fusions. A portion of the mycelium in which the direction of the flow of the protoplasm is indicated by a series of arrows is shown in Fig. 59. By reference to that illustration it will be seen that the protoplasm was flowing: acropetally through the cells of the hypha *a*; then through a passage-way *o* into a branch of the hypha *b*; then basipetally

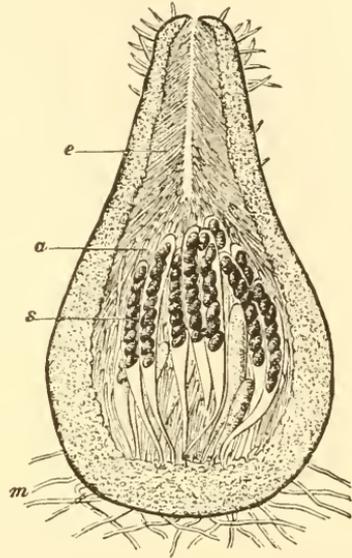


FIG. 56. — *Pleurage fimiseda* (= *Podospora fimiseda*), one of the coprophilous Pyrenomycetes. Its perithecium, here shown in longitudinal section, much resembles that of *Fimetaria fimicola*: *s*, asci; *a*, paraphyses; *e*, periphyses; *m*, hyphae of the mycelium. From von Tavel's *Vergleichende Morphologie der Pilze*.

through three cells of that branch ; then through a second passage-way *p* ; and then basipetally through five cells of the branched

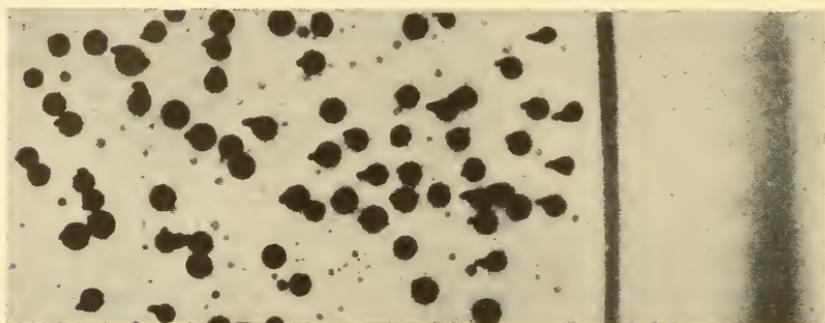


FIG. 57.—*Fimetaria fimicola*. Perithecia developing on nutrient agar in a Petri dish in a cupboard with a glass door. Light directed from left to right. The beaks of the perithecia are turned toward the source of light. Photographed by H. T. Güssow at Ottawa. Magnification, about 10.

hypha *c*. In another piece of mycelium the protoplasm was seen flowing through a series of hyphae for upwards of an hour without

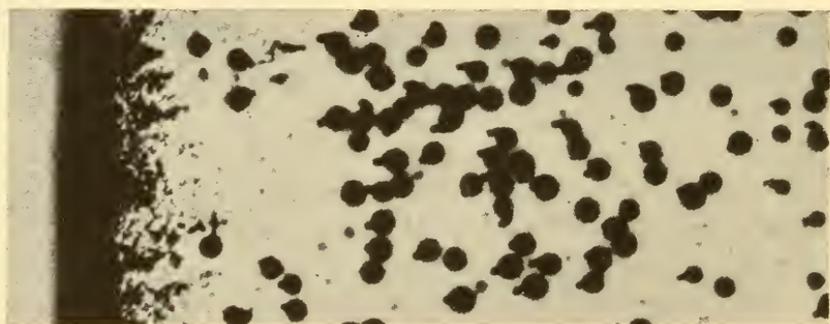


FIG. 58.—*Fimetaria fimicola*. Perithecia developing on nutrient agar in a Petri dish in a cupboard with a glass door (same culture as that shown in Fig. 57). Light directed from left to right. The beaks of the perithecia are positively heliotropic and, in consequence, the asci of the older perithecia have shot their spores toward the source of the light. A thick black spore-deposit can be seen on the side of the Petri dish and on the surface of the agar. Photographed by H. T. Güssow at Ottawa. Magnification, about 10.

any cessation, and the number of septa passed through exceeded twenty-eight.

Several other hanging-drop cultures of the mycelium of *F. fimicola* were made and in all of them protoplasmic streaming was

observed. When the flow is slow, the vacuoles remain fixed ; but, when it is relatively rapid, the vacuoles are torn from their places and are freely carried along in the stream.

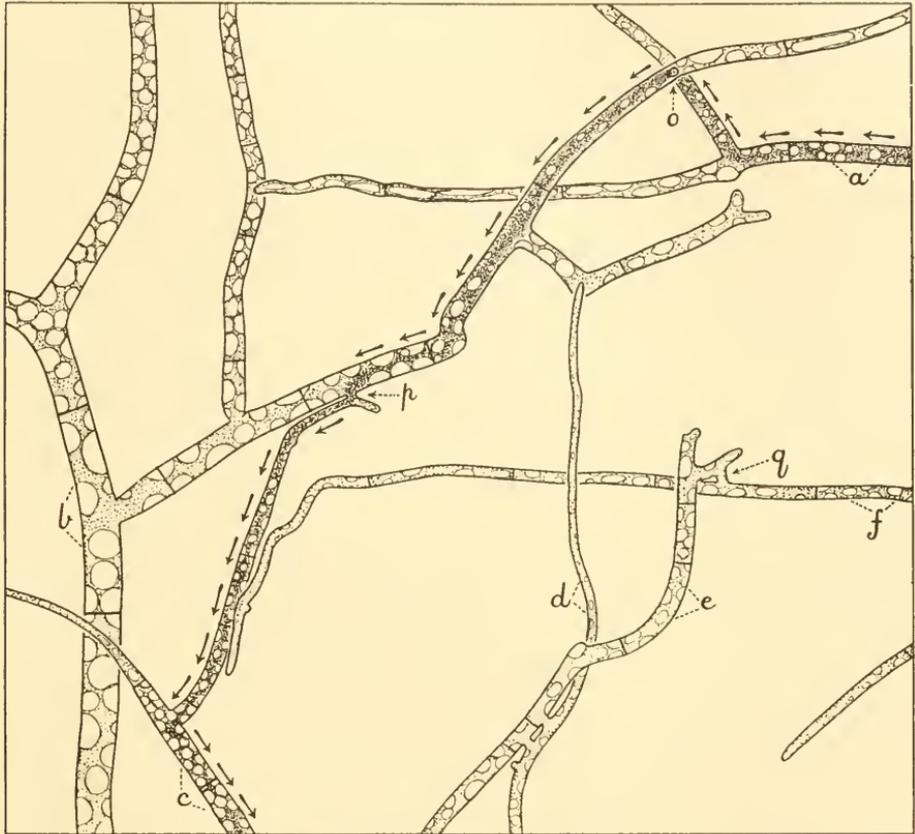


FIG. 59.—*Fimetaria fimicola*. Camera-lucida drawing of part of a mycelium in which protoplasmic streaming was taking place. The protoplasm is finely granular and vacuolated. The three hyphal systems *a*, *b*, and *c* are connected together owing to hyphal fusions having been formed at *o* and *p*. Protoplasm was flowing rapidly in the direction shown by the arrows: acropetally through three cells of *a*; through the fusion passage-way at *o*; basipetally through three cells of a branch of *b*; through the fusion passage-way at *p*; and basipetally through five cells of *c*. A few of the smaller vacuoles were moving with the stream, but all the larger vacuoles and most of the smaller ones remained fixed to the cell-walls. The hyphae *d*, *e* and *f* were connected together by hyphal fusions between *d* and *e* and at *q*, but no flow of protoplasm could be observed within them. Magnification, 434.

On first observing protoplasm streaming along a hypha, one very naturally desires to know how the protoplasm passes from cell

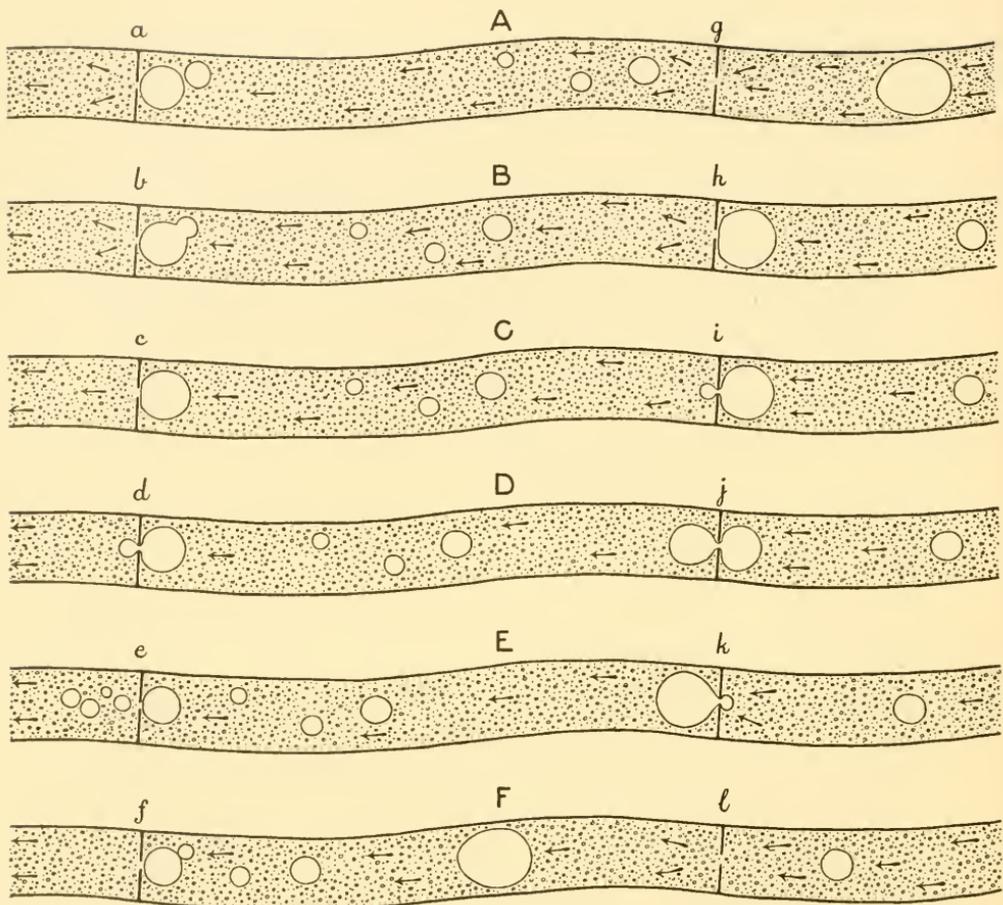


FIG. 60.—*Fimetaria fimicola*. Diagram illustrating successive stages in the passage of vacuoles through a hypha in which the protoplasm was streaming from cell to cell. A hanging drop of cleared dung-agar had been inoculated with mycelium from a stock culture and, three hours later, some of the new hyphae developed from the inoculum exhibited streaming. Part of one of these hyphae, including two septa, is here represented in median longitudinal section. A-F, successive stages in the appearance of the hypha during about 10 seconds: the protoplasm is distinctly granular and the direction of its flow is indicated by arrows; a-f, the fusion of two vacuoles and the pinching off of pieces of the enlarged vacuole through the pore of the septum; and g-l, the passage of a large vacuole as a whole through the pore of a septum; a, the larger vacuole is stationary, protoplasm is streaming around the front of it and through the pore in the middle of the septum, and a smaller vacuole which is being carried along by the protoplasm is about to meet and fuse with the larger vacuole; b, the two vacuoles are fusing to form a single vacuole; c, the vacuole has been pressed against the septum by the flowing protoplasm; d, a part of the vacuole has been pressed through the pore and is about to be constricted off; e, four pieces of the vacuole have been constricted off the parent vacuole and are being carried along by the protoplasm in the next cell, and the remaining part of the parent vacuole has

to cell. The solution of this problem is not difficult. It is true that, when one looks down on the septa of a hypha in a horizontally disposed mycelium, such as that shown in Fig. 59, each septum appears as a continuous plate seen edgewise. However, if streaming is taking place and one studies the movement of the numerous protoplasmic granules with a sufficiently high power of the microscope, one observes that the protoplasm passes through the septa in such a way as to leave no doubt that each septum is perforated centrally and that the perforation or pore is circular in outline and about  $1\ \mu$  in diameter. Often, when the vacuoles are not moving, one can perceive a cone of moving protoplasm passing toward a pore and a reverse cone passing away from the pore. Such cones are similar to those of *Pyronema confluens*, which will shortly be illustrated and described in detail. Very convincing evidence that each septum is normally perforated by a small open pore is afforded by the mode in which the vacuoles, when carried along by the protoplasm, pass from cell to cell.

**Passage of Vacuoles through the Pores in the Septa.**—When streaming in the mycelium of *Fimetaria fimicola* is relatively rapid, the vacuoles are forced out of their usual fixed positions and may be carried by the protoplasm for long distances. The way in which the vacuoles pass through the septa was studied in larger hyphae having a diameter of about  $10\ \mu$ , and it is illustrated in Fig. 60. At A in that Figure is shown diagrammatically in longitudinal median section a hypha in which a dense stream of granular protoplasm is flowing from cell to cell and carrying vacuoles with it. Each septum is perforated by a central open pore. A vacuole may pass through the pore of a septum in pieces (*a-f*) or as an unbroken whole (*g-l*). In the first case a vacuole may be held before a pore

FIG. 60—*cont.*

rounded itself off and is stationary, while other vacuoles are approaching it; *f*, the vacuole in front of the septum is about to be increased in size by fusion with another smaller vacuole which is now touching it; *g*, a larger vacuole is being carried by the protoplasm toward the septum; *h*, the vacuole has been brought to the septum and has been flattened out against it; *i*, part of the vacuole has been forced through the pore of the septum; *j*, one-half of the vacuole has passed through the pore; *k*, nearly the whole of the vacuole has passed through the pore; *l*, the vacuole has passed through the pore in its entirety and is being carried by the streaming protoplasm along the next cell. Magnification, about 1000; the hypha is about  $10\ \mu$  in diameter.

until another vacuole joins it and melts with it, thus increasing its size ( $a-c$ ); then the stream of protoplasm, instead of passing in front of the vacuole as it did when the vacuole was smaller, may press the vacuole against the septum ( $c$ ) and so cause it to constrict off pieces of itself through the pore of the septum ( $d$  and  $e$ ). The vacuole, after being thus diminished in size, may then round itself off, so that the protoplasm flows in front of it through the pore, and it may thus remain until it is joined by one or more other vacuoles ( $f$ ), when, having again increased its volume, the stream of protoplasm may once more press it against the septum and force pieces of it through the pore. In the second case, a larger or smaller vacuole may be pressed through a pore as a whole without being broken into pieces. This often happens when the stream of protoplasm is flowing very rapidly. The vacuole is carried to a septum ( $g$  and  $h$ ), is flattened out against the septum ( $h$ ), and is then forced through the pore ( $i-k$ ) without being broken up. Whilst passing through a pore, the vacuole is much constricted; but, as soon as the passage has been effected, it rounds itself off and resumes its original shape. A large vacuole, such as that shown in Fig. 60 at F, when being carried along by a dense stream of protoplasm, is usually much less convex behind than in front, thus assuming a form like that of the moving vacuoles of the Mucorineae.<sup>1</sup>

It sometimes happens that a vacuole, as it is passing through a septum under pressure from a rapid stream of protoplasm, instead of passing through the pore intact, breaks up in the course of its passage into two or more pieces.

The protoplasmic stream passing along a hypha is often a steady one; but in some hyphae in which the flow was very rapid it was seen to be somewhat pulsatory, in that it exhibited little jerks forward, the jerks being irregular, on the average about one per second. The unevenness of the flow appeared to be caused by large vacuoles being caught momentarily in front of septa or at sharp bends in the hyphae.

**Rate of Flow of the Protoplasm.**—The rapidity of flow of the protoplasmic stream in the mycelium of *Fimetaria fimicola* at about 20° C. was estimated by observing the rate of movement of

<sup>1</sup> Cf. Figs. 44 and 46, pp. 77 and 79.

particular vacuoles. In one cell which was 0·09 mm. long, vacuoles passed from one end of the cell to the other in 9 seconds, *i.e.* at the rate of 10  $\mu$  per second or 3·6 cm. per hour. A lateral hypha came off at an angle of about 45° from its parent hypha and was straight up to a sharp bend. This first straight part of the lateral hypha included two septa and was 0·115 mm. long. Vacuoles traversed this distance in 7 seconds, *i.e.* at the rate of 16·5  $\mu$  per second, or 1 mm. per minute, or 6 cm. per hour. This speed may well have been exceeded in other hyphae.

Data for the rate of flow of protoplasm through hyphae of four species of fungi, of which two are Ascomycetes and two Phycomycetes, are embodied in the accompanying Table.

*Rate of Flow of Protoplasm through Mycelial Hyphae.*

Group	Species	Per second in $\mu$	Per minute in mm.	Per hour in cm.	Temperature in C.°	Observer
Ascomycetes	<i>Fimetaria fimicola</i>	16·5	1·0	6·0	20°	Buller
	<i>Ascophanus carneus</i>	29	1·7	10·5	(?) room T°	Ternetz <sup>1</sup>
Phycomycetes	<i>Phycomyces nitens</i>	16·6-33·3	1-2	6-12	19°	Schröter <sup>2</sup>
	<i>Rhizopus nigricans</i>	16·6-33·3	1-2	6-12	19°	Schröter <sup>2</sup>
		55	3·3	19·8	28°	Arthur <sup>3</sup>

An examination of the Table enables us to conclude that the rate of flow of protoplasm in the septate and relatively thin hyphae of *Fimetaria fimicola* and *Ascophanus carneus*, at ordinary room temperatures, is about the same as that in the non-septate and relatively thick hyphae of *Phycomyces nitens* and *Rhizopus nigricans*. This fact suggests that the septa of the mycelium of the two ascomycetous fungi offer but little resistance to the flow of protoplasm from cell to cell, and that the protoplasm must pour through the open septal pores with great ease.

<sup>1</sup> C. Ternetz, *loc. cit.*, p. 284.

<sup>2</sup> A. Schröter, *loc. cit.*, p. 9.

<sup>3</sup> J. C. Arthur, *loc. cit.*, p. 495.

In *Fimetaria fimicola*, since a hypha with cells and septa 7–8  $\mu$  in diameter has septal pores only about 1.0–1.2  $\mu$  in diameter (cf. Fig. 60), it is clear that the area of a cross-section of a cell-lumen greatly exceeds the area of the open space of a pore and that, when streaming is very active and the vacuoles are in motion, the rate of flow of the individual protoplasmic granules must be far more rapid through the pores than through the cell-lumina.

**General Direction of the Protoplasmic Current.**—The general direction of flow of the protoplasm in the mycelium of *Fimetaria fimicola* is in one direction only, without any reversal, from older hyphae which have ceased to grow toward rapidly growing younger hyphae. There can be no doubt that this translocation serves to aid the younger hyphae in their growth.

A reversal in the direction of flow of protoplasm in any particular hypha, although not actually observed, must often take place; for, when a hypha is growing rapidly in length, protoplasm may flow into it from older hyphae which have ceased to grow but, if the hypha should cease to grow, its protoplasm may flow out of it into younger rapidly growing hyphae situated in another part of the mycelium. Doubtless, also, a reversal in the direction of flow in a particular hypha is sometimes brought about by changes in pressure suddenly arising from the establishment of a new anastomosis between two or more mycelia or systems of hyphae.

**Gelasinospora tetrasperma.**—*Gelasinospora tetrasperma*, like *Fimetaria fimicola*, is a coprophilous Pyrenomycete, but it differs from *F. fimicola* in having four spores in each ascus instead of eight and in having its spore-walls pitted instead of smooth. It was isolated by Dr. Dowding from ptarmigan dung collected in northern Manitoba and will shortly be described by her under the name here given.<sup>1</sup>

Hanging drops of malt-agar were inoculated with the mycelium of *Gelasinospora tetrasperma*. The mycelium grew rapidly in the drop and, within 24 hours, exhibited protoplasmic streaming. In hyphae in which the streaming was slow the vacuoles remained

<sup>1</sup> Eleanor S. Dowding, "Gelasinospora, a New Genus of Pyrenomycetes with Pitted Spores," *Canadian Journal of Research*, Vol. VII, 1933.

fixed, whereas in hyphae in which the streaming was rapid the vacuoles were torn away from the sides of the hyphae and were carried with the stream through the pores of the septa from cell to cell in the manner already described for *Fimetaria fimicola*. The maximum rate of flow of the protoplasm actually measured (determined by observing the rate of movement of the vacuoles) was approximately 0.5 mm. per minute or 3.0 cm. per hour.

In the mycelium of *Gelasinospora tetrasperma*, just as in the other septate mycelia investigated, the rate of movement of the protoplasm is proportional to the rate of growth of the hyphae: in other words, the more rapid the growth, the faster does the protoplasm move and, the slower the growth, the slower does the protoplasm move. When growth ceases, the movement of the protoplasm comes to a standstill.

A mycelium of *Gelasinospora tetrasperma* had grown throughout a shallow, widely spread, hanging drop of malt-agar and had largely exhausted the culture medium, so that the hyphae had almost ceased to elongate. As was to be expected, streaming of the protoplasm along the hyphae could no longer be observed. The cover-glass of the van-Tieghem cell was removed, three or four tiny drops of fresh culture medium were placed at several places on the surface of the old drop, and then the cover-glass was put back in its place. Within a few minutes, growth of the mycelium was resumed. Hyphae grew into each fresh drop of culture medium, thickened, branched and rebranched; and, in from one to two hours after the new drops had been added, the protoplasm in the mycelium could be seen streaming rapidly from cell to cell out of the older hyphae into the newly formed, rapidly developing systems of hyphae and toward their growing points. In a single much branched hyphal system, at each fork, the entering stream of protoplasm divided and on this account gradually slackened its pace, but its movement could still be detected even in the terminal hyphae.

**Pyronema confluens.**—*Pyronema confluens* (Fig. 61) is a well-known Discomycete with tiny pinkish confluent fruit-bodies, often seen on burnt ground. Its sexual phenomena have been investigated cytologically by Harper, Claussen, Gwynne-Vaughan, and others, and the conditions under which it grows and develops its fruit-bodies

have been elucidated by Robinson.<sup>1</sup> In the Dominion Rust Research Laboratory at Winnipeg it comes up freely on sterilised soil in pots used for the growth of wheat seedlings.

After protoplasmic streaming had been observed in *Fimetaria fimicola*, it occurred to me that it might be observed also in *Pyronema confluens*. A trial soon showed that this supposition was a good one; for, about 24 hours after sowing some spores of the fungus in



FIG. 61.—*Pyronema confluens*. Large irregular pale-orange apothecial masses formed by the confluence of numerous fruit-bodies 1-2 mm. in diameter. At maturity the apothecial masses puff well when suddenly exposed to dry air. Obtained on burnt ground in a wood in England and photographed by Somerville Hastings. Somewhat less than natural size.

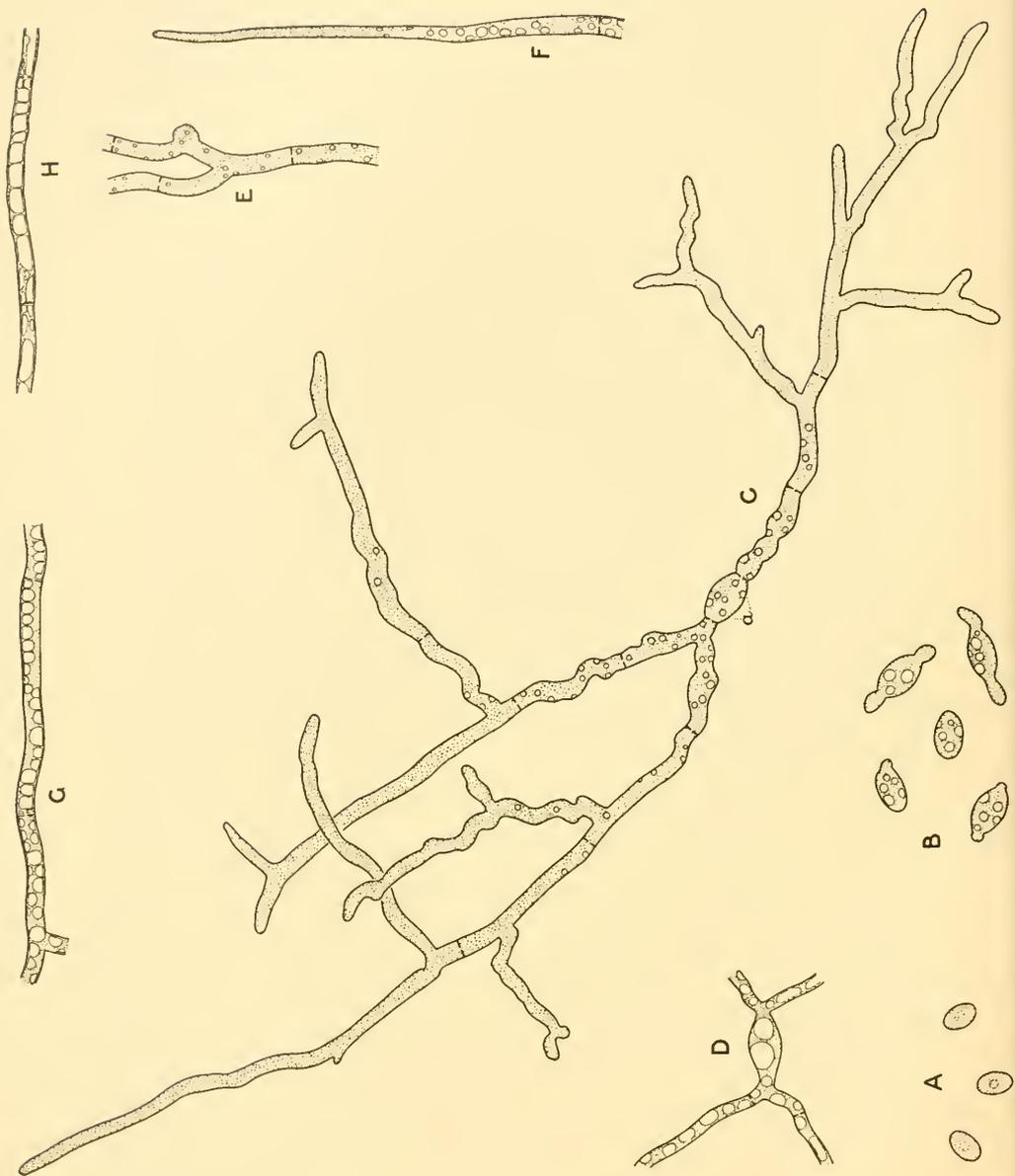
a van-Tieghem cell in a hanging drop of cleared dung-agar, protoplasm could be seen very clearly streaming through the cells of several of the thicker hyphae. Owing to the ease with which the spores can be sown, the certainty and rapidity with which the spores germinate, and the general structure of the mycelium, *P. confluens* proved to be excellent material for the study of protoplasmic streaming and, on this account, my attention became specially directed toward it.

<sup>1</sup> W. Robinson, "The Conditions of Growth and Development of *Pyronema confluens*," *Annals of Botany*, Vol. XL, 1926, pp. 245-272.

**Hanging-drop Cultures.**—Cultures of the mycelium of *Pyronema confluens* suitable for studying the streaming of the protoplasm through the hyphae were prepared as follows. Some fruit-bodies of the fungus, together with underlying soil, obtained from pots at the Dominion Rust Research Laboratory, were placed in closed Petri dishes and left there for a few hours. A van-Tieghem cell was then set up in the usual way: a glass ring was fixed to a slide with paraffin wax, a little water was poured into the cell, and vaseline was smeared over the upper surface of the ring. A drop of cleared dung-agar was now spread on the under surface of a sterilised cover-glass, the cover was removed from the Petri dish, and the cover-glass was held over a group of the fruit-bodies. Immediately some of the fruit-bodies puffed and thereby shot up a few spores into the hanging drop of dung-agar. The cover-glass was then set on the van-Tieghem cell. Thus the spores were sown without being handled by any instrument.

**Growth of the Mycelium and Development of Vacuoles.**—The spores of *Pyronema confluens* are oval and colourless (Fig. 62, A). After having been sown, they began to germinate in about four and a half hours (B), and in fifteen hours each spore had given rise to a rapidly growing, branched, septate mycelium (C). The spores before germination are filled with protoplasm, but small rounded vacuoles appear within them as they swell up and put out germ-tubes (*cf.* A and B). In a young mycelium, the younger hyphae are completely filled with protoplasm, but small rounded vacuoles develop in all the cells as these become older (C). As growth continues, the older cells and the younger hyphae which have ceased to grow in length become more and more vacuolated (*cf.* C and E with D, F, G, H), so that it is evident that in some way they lose a considerable amount of protoplasm.

**Protoplasmic Streaming.**—If a spore of *Pyronema confluens* germinates on one side of a hanging drop, the mycelium, within about twenty-four hours, spreads across the drop as a vigorous but somewhat sparsely-branched structure. In the middle of the drop in such a young mycelium there are leading radiating hyphae, like those shown at B in Fig. 63, composed of chains of cells, and in these hyphae the protoplasm may be



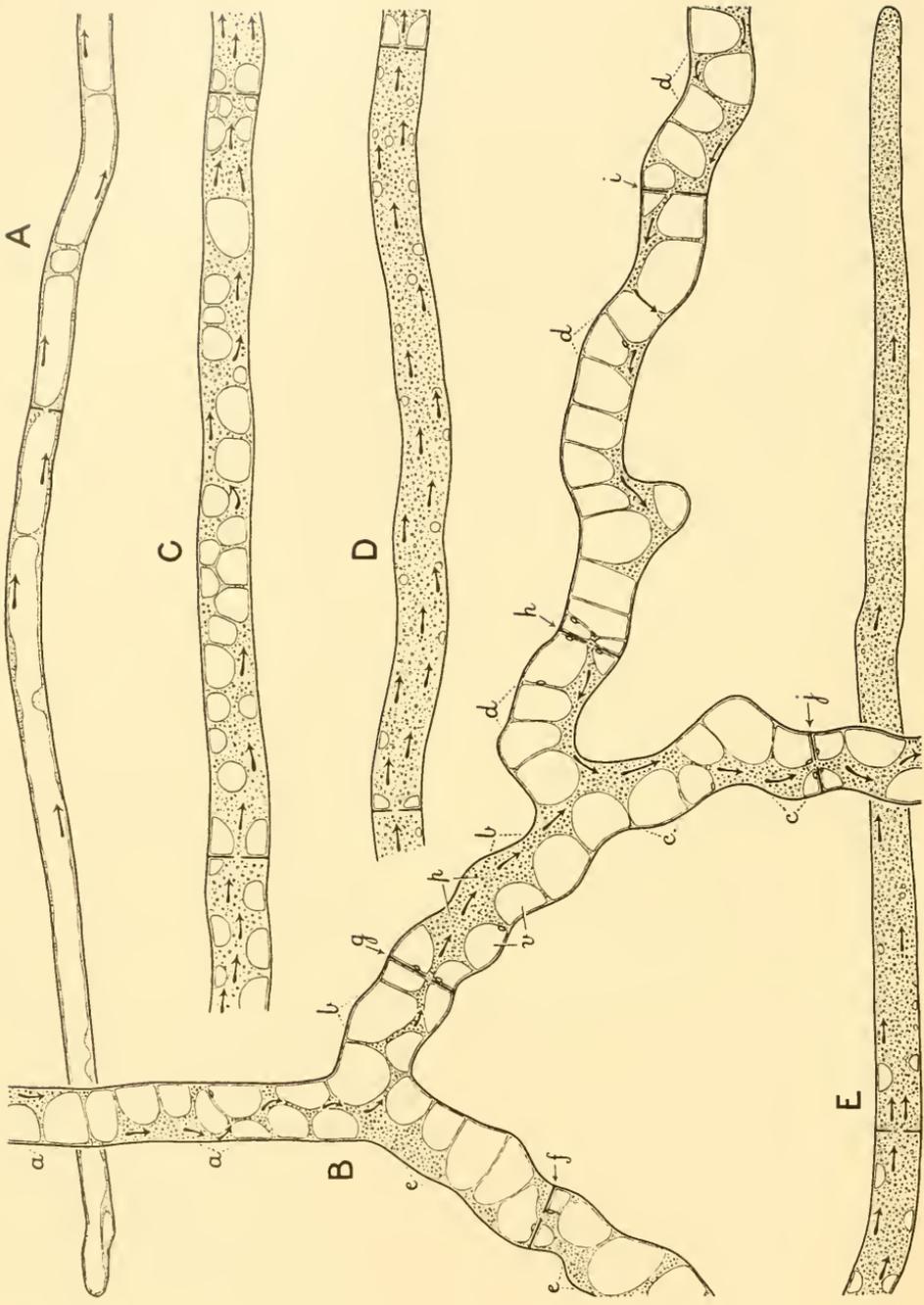
seen streaming either slowly or rapidly, in one direction only, from cell to cell.

The hyphae shown at B in Fig. 63, in which protoplasmic streaming was observed, were drawn with the *camera-lucida* and they have been represented in longitudinal median section. It will be seen that the cells are highly vacuolated. The vacuoles all have one wall adjoining the cell-wall and the other bulging forward into the cell-lumen and, at a bend in a cell, they are usually against the wall on the convex side. Probably one-half of the volume of each cell-lumen is occupied by vacuoles. The cytoplasm in each cell is decidedly granular, and it is the movement of the granules which attracts the eye and enables it to perceive the translocation of the protoplasm from cell to cell. Were the cytoplasm very homogeneous, as it is in the hyphae of *Coprinus sterquilinus*, protoplasmic streaming might be in rapid progress, but one would see nothing of it. The direction of the movement of the protoplasm is indicated by the arrows.

The translocation of protoplasm from cell to cell in the hyphae shown at B in Fig. 63 was watched for six and a half hours. During this time the vacuoles remained without any appreciable change in size. As indicated by the arrows, two streams of protoplasm from two different hyphae met and joined to form a single stream which poured through a third hypha. In another hypha shown on the left of B in Fig. 63, as might be inferred from the absence of arrows, no streaming could be seen.

Altogether in the hyphae shown at B in Fig. 63 five septa are included and in each of these a median pore has been represented.

FIG. 62.—*Pyronema confuans*. Development of the mycelium and formation of vacuoles. Culture medium, a hanging drop of cleared dung-agar. A: ripe spores just sown. B: four and a half hours later; spores which have swollen up and are putting out germ-tubes; small rounded vacuoles have been formed in the protoplasm. C: 15 hours after sowing the spores; the mycelium, developed from the spore *a*, already has many branches and is septate; the younger hyphae are full of protoplasm; small vacuoles have been formed in all the older cells. D: the middle part of an older mycelium; the vacuoles in the spore and adjacent hyphae are now much enlarged. E-H, enlargement of vacuoles in older cells or hyphae which have ceased to grow; E, younger cells shortly after their formation, the vacuoles are very small; F, a hypha which has ceased to grow, its vacuoles are enlarging; G, older cells, with much enlarged vacuoles; H, older cells which have very large vacuoles and have lost most of their protoplasm. Magnification, 300.



While the pore itself could not be directly seen, its width—about  $1\ \mu$ —was inferred from the width of the cone of protoplasm seen moving toward it or away from it. These cones, which were studied with a high magnification of the microscope (about 1000, dry system), are shown on a small scale at *g*, *h*, and *j* in Fig. 63, B, and on a much larger scale in Figs. 64 and 67 (pp. 118 and 131).

The streaming of the protoplasm through the hyphae shown at B in Fig. 63 was rapid when first observed and during the next three hours, but after six and a half hours its speed had declined considerably. The flow was continuous, without any halt, and always in one and the same direction.

As one watches hyphae like those represented at B in Fig. 63, one soon realises that a very considerable amount of protoplasm is flowing through the cells, an amount which must be many times the volume of the cells under observation. Whence comes so much

FIG. 63.—*Pyronema confluens*. Translocation of protoplasm along the hyphae in an older mycelium in a hanging drop of cleared dung-agar. Hyphae all drawn with the *camera-lucida*. The arrows everywhere indicate the direction of the flow of the protoplasm.

In general, the protoplasm is flowing: (1) slowly out of numerous hyphae which have ceased to grow, like that at A; (2) then rapidly through certain mature stable hyphae, like those at B; then less and less rapidly through younger and younger branched hyphae, like those of which parts are shown at C and D; and finally, very slowly into numerous rapidly elongating hyphae, like that at E.

A: protoplasm streaming slowly out of a hypha which has ceased to grow and has become much exhausted. B: part of a mycelium, 38 hours after sowing the spore from which it was derived, consisting of branched septate hyphae containing granular protoplasm *p* and large fixed persistent vacuoles *v* attached to the cell-walls; as indicated by the arrows, a stream of protoplasm is flowing through the hyphae *a a* and *b b* and is uniting with another stream which is flowing in an opposite direction through the hypha *d d d*, and the single stream thus being compounded is flowing through the hypha *c c*. In the hypha *e e e* the protoplasm, at the moment, is at rest. The five septa, *f-j*, each have a single small central open pore and through the pores of the septa *g*, *h*, *i*, and *j* the stream of protoplasm is freely passing. Owing to the conformation of the vacuoles at the ends of the cells by the septa, the moving protoplasm in approaching and in leaving a pore tends to have the form of a cone with the apex directed toward the pore. Examples of such afferent and efferent cones of protoplasm are more or less well displayed in connexion with the pores of the septa *g*, *h*, and *j*. Some highly refractive colourless oval particles are present on the walls of most of the vacuoles abutting on the septa, *e.g.* at *f*, *g*, *h*, and *j*, and also here and there on the walls of vacuoles at some distance from the septa, *e.g.* between the septa *g* and *j*, between the septa *h* and *i*, and in the hypha *a a* a little way above the fork. C: cells in a younger hypha, in which the vacuoles are enlarging and through which protoplasm is streaming slowly and acropetally. D: cells in a still younger hypha in which the vacuoles are as yet very small and through which protoplasm is streaming very slowly and acropetally. E: one of many young rapidly elongating hyphae in which protoplasm is flowing very slowly. Magnification, 933.

protoplasm and whither does it go? This question is easily answered. One has only to examine the mycelium as a whole, proceeding backwards and then forwards from such cells as those

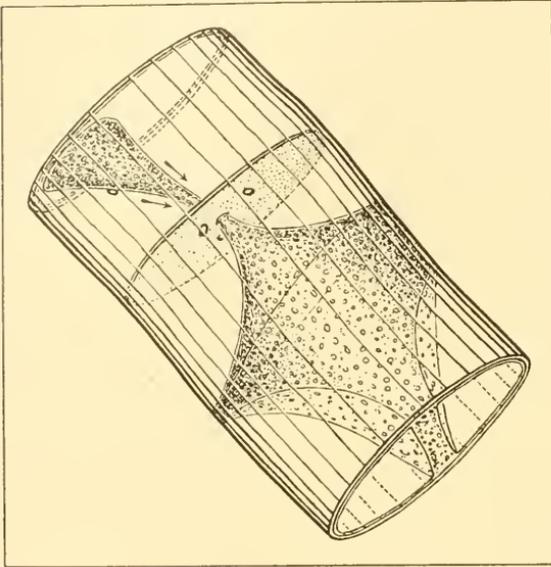


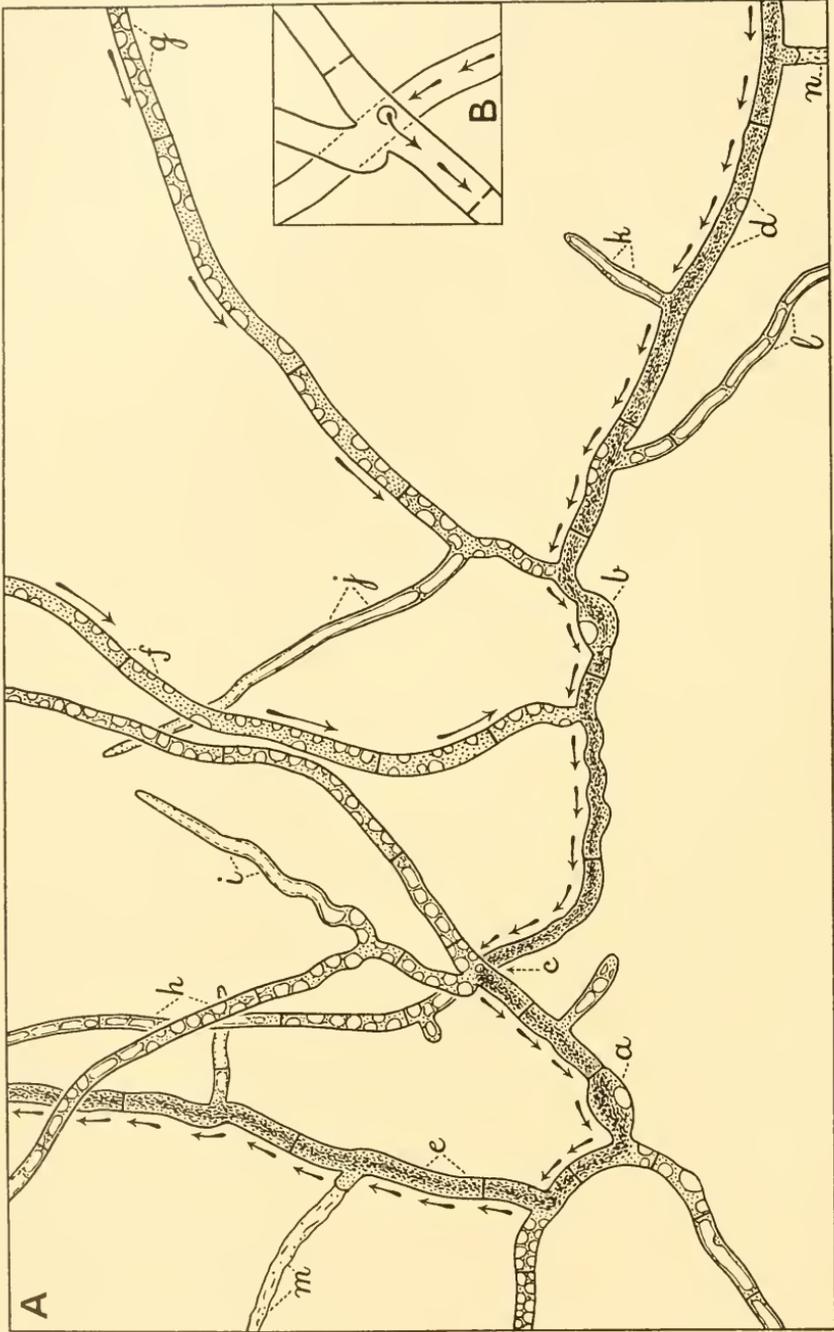
FIG. 64.—*Pyronema confluens*. Diagram of part of a mycelial hypha, drawn in perspective, to show protoplasm streaming from one cell to the next through the central pore of the septum. The main mass of the protoplasm is very granular and is so represented. The clear spaces are large vacuoles filled with cell-sap. There is an afferent cone of protoplasm flowing toward the pore, of which the direction of flow is indicated by two arrows, and there is a more obtuse efferent cone of protoplasm flowing away from the pore. The protoplasmic walls of the vacuoles are free from granules and are not moving. Attached to these walls in temporarily fixed positions are some tiny, oval, highly refractive bodies, three on the surface of the septum and one other to the left of the afferent cone of protoplasm. Magnification, 3240.

shown at B in Fig. 63 which were in the centre of the hanging drop of dung-agar. One finds that the protoplasm passing into the main stream is coming from numerous hyphae which have ceased to grow in length and are becoming more and more highly vacuolated. One of these hyphae is shown at A in Fig. 63. The outflow of protoplasm from any individual hypha takes place very slowly, but can be detected two or three cells back from the growing point as it passes beyond a septum. The protoplasmic streams of ultimate hyphae combine together, like the

streams in the upper reaches of a river, with the result that the main stream is produced. Since the hyphae conducting the main stream of protoplasm are of about the same diameter as the hyphae conducting the tributary streams, it naturally follows that the main stream must flow faster than the tributary streams. Thus, at B in Fig. 63, the main stream passing through the

hypha *c c* was more rapid than either of the two streams which combined to form it. When one traces a main stream forward, one finds that it is flowing toward a part of the mycelium which is branching and re-branching and has ultimate hyphae that are rapidly elongating. With the division and subdivision of the main stream, the protoplasm flows more and more slowly. In cells which are several cells back from a growing point and are still enlarging their vacuoles, like those at C in Fig. 63, the forward flow of the protoplasm could be readily observed; in very young cells, which are only two or three cells back from a growing point and as yet have very tiny vacuoles, the forward flow of protoplasm could be observed, but with difficulty, while in terminal cells with growing points, like that shown at E, the extremely slow forward movement past the last-formed septum could be detected with certainty only by watching the granular particles of the protoplasm as sharply as possible.

**Streaming of Protoplasm from One Mycelium to Another.**—Four spores which had been shot up into a drop of cleared dung-agar on one side of the drop germinated, and each spore gave rise to a mycelium. The four simple mycelia, by means of three hyphal fusions, soon became united to form a compound mycelium. Forty hours after the spores had germinated, protoplasm was observed to be flowing in a main channel of cells made up of parts of the four mycelia, including the four spores and the three fusion passage-ways, away from the side of the drop where the four spores had germinated and the hyphae had ceased to grow, across the drop to the other side and, finally, into a system of hyphae that were at the periphery of the drop and were branching, re-branching, and growing rapidly in length. Part of the main channel of flow is illustrated in Fig. 65. As indicated there by the series of short arrows, the protoplasm was flowing in a dense stream, interrupted by but few fixed vacuoles, along the hypha *d*, through the spore *b*, through the fusion passage-way *c*, through the spore *a*, and along the hypha *e*. This main stream was being fed by numerous tributary streams, of which those in the hyphae *f* and *g* were two. Streaming through the main channel was observed in 161 successive cells. The average length of these cells was at least 0.1 mm.; so that a simple calculation shows that the length of the main stream, as far as it was observed,



was approximately 1.61 cm. The stream was actually longer than this, as the first cells of the stream were not traced and the last cells could not be counted as they had become confused with vaseline and optically hidden at the side of the cover-glass adjacent to the glass ring of the van-Tieghem cell. Already many of the branch-hyphae which were connected with the main channel of flow had become highly vacuolated and had lost most of their protoplasm (Fig. 65, *h*, *i*, *j*, and *k*), while others had become completely exhausted and had died (*m* and *n*). The flow was watched for several hours; it went on continuously without any halt and without any reversal. Occasionally very small vacuoles were seen to move with the stream, but the free movement of large vacuoles through the pores, such as that already described for *Fimetaria fimicola*, was not observed.

In Volume IV of this work,<sup>1</sup> one of the functions attributed to hyphal fusions is that of facilitating the flow of food materials from one part of a simple or compound mycelium to another where the materials are needed for the production of fruit-bodies, etc. In the compound mycelium illustrated in part in Fig. 65, ocular evidence of this transfer of materials was provided, for the protoplasm was seen to flow through three fusion passage-ways in succession.

The flow of protoplasm through other fusion passage-ways than those just referred to was observed in other preparations.

FIG. 65.—*Pyronema confluens*. Translocation of protoplasm from one mycelium to another. A: four spores, of which *a* and *b* are two, were shot up into a drop of cleared dung-agar and there germinated. The drawing was made 40 hours after the spores were sown. The four mycelia, derived from the four spores, became united into a compound mycelium by means of three hyphal fusions of which one, connecting the mycelium *a* with the mycelium *b*, is shown at *c* (cf. B). Protoplasm flowed for several hours rapidly in the direction shown by the short arrows, through the hypha *d*, through the spore *b*, through the fusion passage-way *c*, through the spore *a*, and through the hypha *e*. The channel of flow *d b c a e* was part of a main channel consisting of more than 160 cells, including parts of all the four united mycelia, with a length of about 1.6 cm. The protoplasm was coming from numerous lateral hyphae which had ceased to grow and were exhausting themselves of protoplasm, and was going to a much branching system of young rapidly growing hyphae. In the part of the compound mycelium shown, the main stream of protoplasm was being fed by streams flowing slowly along the hyphae *f* and *g* in the direction shown by the arrows. Already the hyphae *h*, *i*, *j*, *k*, and *l* have lost most of their protoplasm and are highly vacuolated, while the hyphae *m* and *n* are collapsed and dead. B: is *c* in A, enlarged, to show more clearly the direction of flow of the protoplasm through the fusion passage-way between a hypha of mycelium *a* and a hypha of mycelium *b*. Magnification, 317.

<sup>1</sup> These *Researches*, Vol. IV, 1931, p. 182.

**Deformation of Vacuoles by Flowing Protoplasm.**—In a highly vacuolated hypha of *Pyronema confluens*, if the protoplasm is at rest or moving very slowly, the vacuoles are as a rule bilaterally symmetrical (Fig. 66, A) but, if the protoplasm is flowing very rapidly, the vacuoles, while still retaining their attachment to the cell-wall, become sloped in the direction in which the protoplasm is flowing (Fig. 66, B) and thus altered in shape. This deformation of the vacuoles is evidently due to the pressure of the stream of protoplasm.

The pressure of the streaming protoplasm in a hypha of *Pyronema confluens* is usually not sufficiently great to dislodge the vacuoles

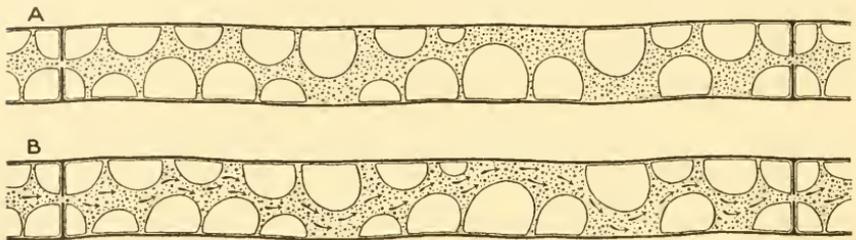


FIG. 66.—*Pyronema confluens*. Semi-diagrammatic drawing illustrating the effect of pressure of streaming protoplasm on vacuoles. A, a highly vacuolated cell in which the protoplasm is at rest or is moving very slowly. B, the same cell in which the protoplasm is streaming rapidly; the vacuoles, still attached to the cell-wall, are being pressed out of their original shape by the streaming protoplasm. The arrows indicate the direction of the stream. Each septum has a central pore. Magnification, about 1000.

from their attachment to the cell-wall. However, occasionally vacuoles are so detached. One such vacuole was seen to be carried in the streaming protoplasm to the nearest septum and to lodge there on one side without passing through. This vacuole then grew gradually smaller as if about to disappear altogether. It seems probable that the detached vacuoles which float along in the streaming protoplasm of *Fimetaria fimicola* and other ascomycetous species gradually become reduced in size and that sometimes they disappear altogether, but exact observations on the fate of floating vacuoles have yet to be made.

**Causes of Streaming.**—The causes of protoplasmic streaming in septate mycelia, such as that of *Pyronema confluens*, appear to be two: (1) vacuolar pressure and (2) increase in the amount of protoplasm.

(1) *Vacuolar pressure*.—As we have seen, Charlotte Ternetz, as a result of her investigations on *Ascophanus carneus*, came to the conclusion that the movement of protoplasm in the hyphae of that Discomycete is due to differences of osmotic pressure in the cells of the hyphae. My own observations on *Pyronema confluens*, *Fimetaria fimicola*, *Rhizoctonia solani*, etc., support the idea that osmotic pressure is a main cause of the flowing of protoplasm in septate mycelia, although not the only cause. The ultimate hyphae from which protoplasm is flowing away (*cf.* A in Fig. 63, p. 116) have all ceased to grow and have enlarging vacuoles. In every cell of such ultimate hyphae the protoplasm may be divided into two classes: (1) *fixed* protoplasm which, as an extremely thin non-granular clear layer, lines the cell-wall and surrounds each vacuole; and (2) *movable* protoplasm which, in the form of a granular mass, is enclosed by the fixed protoplasm. At first a cell is full of protoplasm. As it ceases to grow in length, vacuoles attached to the cell-walls appear in it and grow in size (*cf.* Fig. 63, E, D, C, and B, p. 116). Then the vacuoles may cease to grow and the cell may serve as a channel for the transportation of protoplasm (Fig. 63, B). Subsequently, the vacuoles may begin to grow again and the cell may lose all its labile protoplasm and, finally, die of exhaustion. The emission of protoplasm from a cell can be readily accounted for by supposing that *the pressure required for the process arises in the vacuoles*. If, through the addition of a soluble salt or other osmotic substance to the cell-sap, the osmotic pressure of the sap is increased, the vacuoles will press against the mobile granular protoplasm and this, unable to escape through the thin layer of clear fixed protoplasm lining the outer cylindrical cell-wall, must flow in the direction of least resistance, namely, through the pore of an adjacent septum and onwards toward younger growing hyphae, in which, presumably, the pressure on the granular protoplasm is much lower. Thus enlarging vacuoles drive the protoplasm out of certain hyphae which have ceased to grow into hyphae whose ends are full of protoplasm but are growing rapidly in length and so are making room for the protoplasm that is flowing into them.

(2) *Increase in the amount of protoplasm*.—In a young mycelium growing in a fresh culture medium the total amount of protoplasm

is constantly increasing in volume, and it may be readily supposed that this mass-increase of protoplasm must cause protoplasmic movement toward the growing points. As Reinhardt<sup>1</sup> showed forty years ago, a mycelial hypha elongates only *at its very tip*; and yet, as may be easily observed, a growing hypha, even when elongating very rapidly, has its end full of protoplasm (*cf.* Figs. 62, C, and 63, E, pp. 114 and 116). It is not to be supposed that the protoplasm which fills the end of a rapidly growing hypha is all manufactured at the hypha's tip. Rather we must suppose that the terminal cells of the hypha, surrounded as they are by an unexhausted nutrient medium, are manufacturing new protoplasm throughout their length and that, in consequence, there is a steady, although slow and scarcely perceptible, movement of the protoplasm toward the growing point, which results in the growing end of the hypha being kept full of protoplasm. In rapidly growing hyphae of *Pyronema confluens*, *Gelasinospora tetrasperma*, and *Rhizoctonia solani*, I have succeeded in observing the gradual pressing forward of the protoplasm past the last, just-formed septum, from the penultimate into the terminal cell, and toward the apical growing point; and, furthermore, in a species of *Ciboria* and in *Rhizoctonia solani*, as will be described more fully later on, I have observed that the last-formed septum (sometimes several of the last-formed septa) of a growing hypha becomes temporarily bulged forward toward the apical growing point as the somewhat viscous protoplasm presses against it, flows through its pore, and passes toward the end of the hypha (Fig. 82, p. 163).

In a mycelium which has exhausted its culture medium and which is forming fruit-bodies or producing hyphae that are growing in a film of water or in moist air, the cause of the rapid flow of protoplasm from the older hyphae which have ceased to grow into the newer hyphae which are elongating and branching vigorously is vacuolar pressure. In a young mycelium which is growing in a fresh culture medium and in which the vacuoles are still very small, the slow flow of protoplasm toward the apical growing points must be due to pressure arising from the general increase in volume of the proto-

<sup>1</sup> M. O. Reinhardt, "Das Wachsthum der Pilzhyphen," *Jahrb. f. wiss. Bot.*, Bd. XXIII, 1892.

plasm. However, in an older mycelium which has exhausted some parts of its substratum but not others, it is easily conceivable that the flow of protoplasm toward the apical growing points of the elongating hyphae may be due : in part to vacuolar pressure arising in older non-growing hyphae which are evacuating their labile protoplasm, and in part to the formation of new protoplasm in those cells which lie behind the growing points of the elongating cells and are surrounded by a nutrient medium that is still unexhausted.

The monoporous septum in the mycelium of a Higher Fungus is comparable with the polyporous septum or sieve-plate in a sieve-tube system of a Higher Plant ; and it may well be that the discovery of the means by which colloidal matter is rapidly transported from one part of a mycelium to another may help us to elucidate the means whereby colloidal matter is rapidly transported from one part of a sieve-tube system to another.

**Biological Significance of Streaming.**—As we have seen, de Vries, Arthur, and Schröter regarded streaming in the Phycomycetes as an important means of transferring food material to points of growth. Ternetz, in discussing the cause of the formation of apothecia in *Ascophanus carneus*, after insisting on the importance of a stimulus provided by a check in the supply of nutriment to the mycelium, says that the hyphae which form the apothecia receive nutriment from the mycelium in the substratum and that in this process “the copious streaming of the protoplasm plays no small rôle.”

With the views expressed by my predecessors as to the biological significance of the streaming I am in entire agreement. My own observations have taught me that, in *Pyronema confluens* and *Fimetaria fimicola*, protoplasm is transported as such from hyphae which have ceased to grow into hyphae which are growing vigorously. I have not studied the development of the fruit-bodies of either of these species but, with Ternetz, I am strongly of the opinion that, during the growth of the fruit-bodies, the transference of the food-materials from the vegetative mycelium into the fruit-bodies is largely carried out by protoplasmic streaming. If this view is correct, the translocation of protoplasm from cell to cell in the mycelium by the process of streaming results in great advantage to the species in which it occurs.

**Rate of Growth of the Mycelium in a Dung-agar Plate.**—On a plate of cleared dung-agar at about 20° C., the mycelium of *Pyronema confluens* grew radially at the rate of 5·4 cm. (approximately 2·1 inches) per day of 24 hours. This is an extraordinarily rapid rate of growth which, combined with quick germination of the spores<sup>1</sup> and early fruiting,<sup>2</sup> doubtless helps to explain why it is that the fungus spreads so rapidly, and at first often dominates other fungi, on burnt ground, sterilised soil, etc. There is every reason to believe that the rapid growth of the mycelium is aided by the free flow of the protoplasm from cell to cell toward the ends of the growing hyphae. It was found that in plate cultures the leading radial hyphae, on reaching the edge of the dung-agar, grew up the glass sides of the dish and on to the glass cover for a distance of about 2 cm. before their further development was checked. This advance over the surface of non-nutrient glass must have been at the expense of a supply of nutriment sent from the hyphae in the dung-agar, and in all probability protoplasmic streaming played a chief part in the translocation process.

**Observations with Dark-field Illumination.**—With the help of Dr. P. H. Gregory, I made a few observations on the mycelium of *Pyronema confluens* with a Leitz dark-field condenser. A hanging drop of Sabouraud's medium, as used for cultivating ring-worm fungi and other dermatophytes, was inoculated with mycelium, and the next day the mycelium had spread in the drop. The cover-glass with the hanging drop was now removed from the van-Tieghem cell and set on a drop of water on a glass slide, and the mycelium was examined under the microscope with the aid of the dark-field condenser. The objective was a one-twelfth inch oil immersion, fitted with a diaphragm, and the ocular was 10 ×. The magnification was about 1,000.

The hyphae stood out as bright objects against a black background. The vacuoles were clear black, but the massive granular protoplasm in the interior of the cells everywhere glittered and

<sup>1</sup> *Vide supra*, p. 113.

<sup>2</sup> According to Robinson (*loc. cit.*, p. 247) "a culture from a single spore carries through its developmental cycle to the ascus and mature ascospore on a suitable medium, if illuminated at a favourable temperature, in from eight to fourteen days."

twinkled in the most extraordinary manner. Where streaming was taking place, the twinkling points seemed to be moving along in the direction of protoplasmic flow ; but, even where the protoplasm was not moving and right up to the ends of the growing points of all the hyphae, continuous twinkling was in progress. Doubtless the twinkling was due to light coming from the protoplasmic granules and the granules being in Brownian movement. On focussing just within the cell-wall, I observed a layer of protoplasm containing granules at rest. Possibly the granules were like those of the ordinary massive granular protoplasm but were fixed in position owing to their being in contact with the thin fixed hyaline plasmic layer lining the cell-wall. For comparison with *Pyronema confluens*, some mycelium of *Coprinus sterquilinus* was examined with the dark-field condenser. The hyphae of this fungus looked very empty. The protoplasm was almost invisible ; only here and there could a twinkling particle be observed. A few larger white particles could be seen in the cells, some of them on the cross-walls, and these moved about irregularly. With ordinary transmitted light one perceives that, whereas the protoplasm of *Pyronema confluens* is very distinctly granular, that of *Coprinus sterquilinus* is relatively homogeneous, and doubtless this difference accounts for the fact that, when the dark-field condenser is employed, the protoplasm of *P. confluens* twinkles very much more than that of *C. sterquilinus*.

**Woronin Bodies and their Movements.**—In 1886, Woronin discovered in the cells of *Ascobolus* (= *Lasiobolus*) *pulcherrimus* certain highly refractive particles a few of which can usually be seen on one side or both sides of each septum. Charlotte Ternetz, in 1900, again called attention to these particles as they occur in *Ascophanus carneus*, and she described them as follows : they are rounded in form, do not contain tannin, are not stained by iodine, and in stained preparations they take on nuclear dyes ; they appear in the apical cells of hyphae, irregularly dispersed in the protoplasm, and there they move every now and then here and there, and then they settle down against a longitudinal wall or a septum ; even when streaming is taking place, they may move independently of the granular protoplasm, sometimes in the direction of flow and

sometimes against it ; as the septa become fully formed, the particles gather upon them, the number on a septum being eventually one to seven and usually two to four ; they belong to the protoplasm and are not thickenings of the cell-walls, as is shown by the fact that, in a cell undergoing plasmolysis, they retire from the cell-wall in the contracting plasma membrane.

Highly refractive particles, like those of *Lasiobolus pulcherrimus* and *Ascophanus carneus*, also occur in *Ascodesmis nigricans*,<sup>1</sup> *Helvella elastica*,<sup>2</sup> *Ascobolus magnificus*,<sup>3</sup> *Pyronema confluens*, and doubtless in many other Discomycetes. Since they are such definite structures, for convenience in reference they need a special name. By Claussen,<sup>4</sup> Faull,<sup>5</sup> and others they have been referred to as metachromatic bodies ; but, as this designation seems to me to be an unsatisfactory one, I propose to call them *Woronin bodies*.

The Woronin bodies of *Pyronema confluens* (Figs. 63, B, 64, and 67, pp. 116, 118, and 131), like those of *Ascophanus carneus*, occur sparsely scattered in the protoplasm filling the terminal cells of elongating hyphae, and there they move about irregularly in the manner described by Ternetz. In older cells they settle down *in the walls of the vacuoles* and, while at rest in that position, they can be readily studied with the microscope. They are not rounded but elongated-oval in shape. When turning on their long axis, they sometimes appear to become thicker or thinner, so that possibly they are oval in cross-section. In any single cell, their number is small—perhaps less than one dozen. While a few of them occur

<sup>1</sup> P. Claussen, "Zur Entwicklungsgeschichte der Ascomyceten. Boudiera," *Bot. Zeit.*, Jahrg. LXIII, 1905, p. 6 (his Boudiera was wrongly identified ; it was *Ascodesmis nigricans*).

<sup>2</sup> W. A. McCubbin, "Development of the Helvellineae. I. *Helvella elastica*," *Botanical Gazette*, Vol. XLIX, 1910, p. 198.

<sup>3</sup> B. O. Dodge, "Artificial Cultures of *Ascobolus* and *Aleuria*," *Mycologia*, Vol. IV, 1912, Plate LXXII, Figs. 7-8.

<sup>4</sup> P. Claussen, *loc. cit.*

<sup>5</sup> J. H. Faull, "The Cytology of *Laboulbenia chaetophora* and *L. Gyridarum*," *Annals of Botany*, Vol. XXVI, 1912, p. 333. Faull found in these Laboulbeniaceae "rather coarse, deeply staining granules (a brilliant red with Fleming's triple stain), some of which are closely in contact with the 'Schliesshaut' (Fig. 7)," and he adds "these are probably the 'metachromatic' granules observed by Claussen in the mycelium of *Ascodesmis* and by McCubbin in *Helvella*."

scattered on the walls of vacuoles distant from the septa (Fig. 63, B, above the fork in *a a*, between the septa *g* and *j*, and between the septa *h* and *i*), they tend to congregate on the walls of vacuoles adjacent to the septa (Figs. 63, B, 64, and 67).

When streaming is active in a hypha (Fig. 63, B) the Woronin bodies usually remain more or less fixed in position in the walls of the vacuoles, and I have never yet seen them leave those walls, join the granular protoplasm, and be carried off by the current. When they move, they do so spasmodically and their direction of motion is along the wall of a vacuole. If one of them is on the wall of a vacuole occupying the corner of a cell (*cf.* Fig. 67), its movements along the vacuolar wall may cause it to approach and come up to the septum or to leave the septum. Whilst the majority of Woronin bodies at a septum have their long axes parallel to the septum (Fig. 63, B, *g* and *h*, and Fig. 64), some have their long axes more or less perpendicular to the septum (Fig. 63, B, *f* and *j*, and Fig. 67). These relative positions are determined by the shape of the vacuole. If a Woronin body moves along a vacuolar wall bulging into the cell-lumen and comes up to a septum, on arrival its axis may be perpendicular to the septum, as shown at *f* in Fig. 63, B, and to the right in Fig. 67. If then it continues to move and passes on to a vacuolar wall lying against the septum, its axis becomes parallel to the septum. A Woronin body at one moment may be seen in side view as a short rod (Fig. 67) and then it may move round through a right angle and be seen in end view as a rounded structure. An attempt to show Woronin bodies as solid bodies in perspective has been made in Fig. 64 (p. 118). Woronin bodies are less than  $1\ \mu$  in diameter and are therefore quite small enough to pass through the pore of a septum. Perhaps they do actually pass through pores occasionally. I once observed a Woronin body on one side of a septum near a pore. After some time, it was found to have disappeared from that position; and in a vacuolar wall on the other side of the septum in the next cell there were now three Woronin bodies, instead of two as seen previously. The inference is that the Woronin body under discussion had in some way passed through the pore.

It is not to be supposed that Woronin bodies have any powers

of locomotion of their own. Doubtless they are moved passively by the cytoplasm of the vacuolar walls.

The true nature of Woronin bodies and what their function is remain for the present unknown. Since they are present in apical cells, it is possible that they are specialised parts of the protoplasm. When once formed they are very persistent for, as Ternetz<sup>1</sup> has pointed out, they can still be seen on the walls in exhausted cells.

**Pore Plugs and their Formation under Experimental Conditions.**—Observations on the manner in which protoplasm streams from cell to cell in *Fimetaria fimicola* and *Pyronema confluens* justify us in concluding that in these and other similar fungi each septum in a mycelium is provided with a small, central, circular, open pore through which protoplasm can pass, and often does pass, with the greatest ease. The acceptance of these conclusions suggests the following questions: when a cell in a living hypha dies naturally or is killed by manipulation, does protoplasm flow through the pores of the septa from the adjacent living cells into the dead cell; and, if not, what prevents it from doing so? The answer to these questions was obtained by means of some special observations which will now be recorded.

Some spores of *Pyronema confluens* were sown in a hanging drop of cleared dung-agar, and they soon germinated. On one of the next few days, when the mycelium was well grown, some of its hyphae were operated upon with the help of a beading needle—a slender cylindrical needle about two inches in length. The cover-glass was removed from the van-Tieghem cell, inverted, and set on a glass slide. Then the pointed end of the needle was heated in a flame, and, whilst still very hot, was drawn rapidly across the preparation in such a way as to pass through the dung-agar and sever several of the long main hyphae (*cf.* Fig. 70, A, p. 137). Immediately after the operation had been performed, the cover-glass was set back in its old position on the van-Tieghem cell and the broken hyphae were examined with the microscope.

The operation in other hanging-drop preparations was performed with an improved technique. The glass slide bearing the inverted cover-glass was set under the low-power objective of the microscope

<sup>1</sup> C. Ternetz, *loc. cit.*, p. 279.

and the pointed end of the beading needle, without being heated, was brought into contact with a particular cell and this was pressed upon, with the result that its protoplasm died and became disorganised, often without its cylindrical cell-wall being ruptured (cf. Fig. 71, A and B, p. 138). The average length of the cells in the hyphae to which the needle was applied was 0.13 mm.

It was found that the protoplasm of a cell injured by the needle always died as a whole, *i.e.* throughout the length of the cell and right up to its two septa (cf. Fig. 71, B), and that the injury was not transmitted to the two adjacent cells, which remained living.

Also, immediately after a cell has been killed, it was found :

(1) that the two septa separating the dead cell from the two adjacent living cells, which previously were plane, become convexo-concave with the convex side directed toward the lumen of the dead cell ; and (2) that the pores of the two septa become blocked by a plug in consequence of which the escape of living protoplasm from the adjacent living cells into the dead cell is prevented.

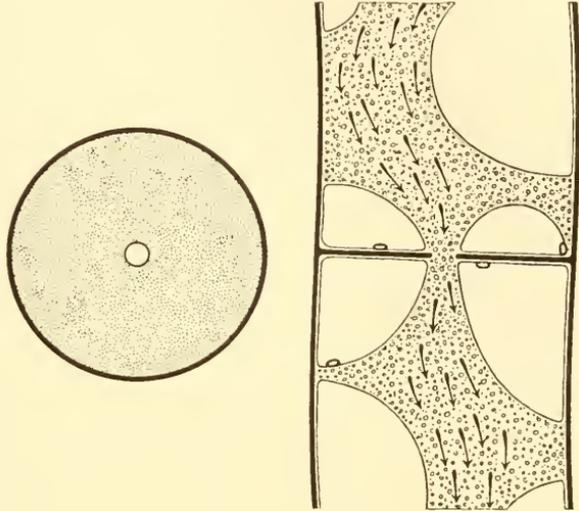


FIG. 77.—*Pyronema confluens*. Diagrams to illustrate the septal pore and its employment as a passage-way for protoplasm as this streams in a mycelium from cell to cell. To the left, a septum in face view : it is about 10  $\mu$  in diameter and its central pore about 1  $\mu$  in diameter. To the right, parts of two adjacent cells of a hypha in median longitudinal section. As indicated by the arrows, the main mass of the protoplasm, which is finely granular, is streaming from one cell to the next through the pore of the septum. The vacuoles are large and have very thin non-granular protoplasmic walls which are not moving. Attached to the walls of the vacuoles in temporarily fixed positions are some tiny, oval, highly refractive bodies. These at times change their position on the wall of a vacuole, without reference to the direction of the current of the granular protoplasm. Magnification, 3240.

The bulging forward of a septal wall and the formation of a pore plug are illustrated semi-diagrammatically in Figs. 67 and 68. In Fig. 67 are shown: to the left, a septum with an open pore, seen in face view; and, to the right, parts of two adjacent cells of

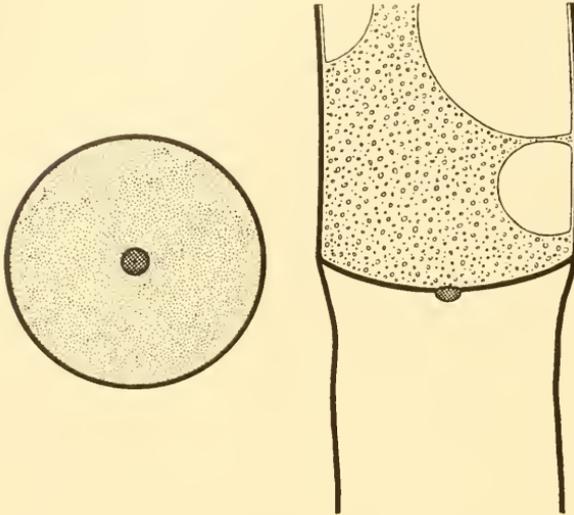


FIG. 68.—*Pyronema confuens*. Diagrams illustrating the plugging of a pore of a septum immediately after a cell on one side of the septum has been killed. To the right, parts of two adjacent cells of a hypha in median longitudinal section. The two cells were both living and the septum was straight and provided with an open pore, as shown in Fig. 67. The lower cell was then broken open and killed by a mechanical operation with a needle. As an immediate result, as here shown, the turgid living cell pressed its cell-wall convexly forward toward the dead cell, and the pore of the septum became plugged, so that the escape of protoplasm from the living cell into the dead cell was prevented. To the left, in face view, a septum with a plugged pore. Magnification, 3240.

a hypha, seen in median longitudinal section. In these cells, as indicated by the arrows, the main mass of the protoplasm, which is finely granular, is streaming from one cell to the next through the pore of the septum. Let us now suppose that by means of a mechanical operation with a needle the lower cell is broken open and killed. Then, as shown in Fig. 68, the septum is pressed forward by the living cell into the dead cell, the pore becomes blocked by a plug,

and the protoplasm of the living cell ceases to flow through the pore because the passage-way is no longer open.

Other drawings showing convexo-concave septa provided with plugs, facing cells which have been killed by mechanical operations, are reproduced in Figs. 69, 70, 71, and 72.

A septal plug closing a pore in a septum separating a living and a dead cell of *Pyronema confuens* is a discoid, somewhat plano-

convex body with a diameter of about  $1\ \mu$ . Its more convex side bulges forward from the pore into the lumen of the dead cell (Fig. 68). The substance of which a plug is composed appears homogeneous, colourless, and highly refractive, and may well be nothing more than a coagulum of protoplasmic origin. We may suppose that the death and disorganisation of the protoplasm of a cell instantaneously causes those parts of the living protoplasm of the two adjacent cells which happen to be in the pores of the two septa and are beginning to press through them to solidify and thus form plugs.

Ordinarily, in a living hypha, the pressure on the two sides of a septum is equal or nearly so and the septum is plane. The bulging forward of a septum when one of the cells adjacent to it is killed is due to the turgidity of the living cell. As is well known, a similar bulging of septa takes place in a *Spirogyra* filament whenever one of the cells in the chain ceases to live.

**Formation of Pore Plugs in Old Mycelia when Individual Cells Die.**—When spores of *Pyronema confluens* are sown in a hanging drop of dung-agar, the mycelium resulting soon spreads throughout the drop. At first all the numerous cells of which it is composed are living; during the next day or two certain hyphae lose their protoplasmic contents and die; and, in the course of two or three weeks, certain cells of the larger main hyphae die and thus the living parts of the hyphae become separated from one another. As a cell in the middle of a living hypha dies, its septa become pressed into it by the adjacent living cells and the septal pores become plugged just as when a cell is killed suddenly on being injured with a needle. We may therefore conclude that, under natural conditions, the plugging of the pores of septa which divide living from dead cells is a normal phenomenon.

**Pore Plugs in Other Fungi.**—In all probability pore plugs are formed between dead and living cells in the mycelium of *Discomyces* in general. It is true that they were not recorded as being present in *Lasiobolus pulcherrimus*, *Ascophanus carneus*, and *Ascobolus magnificus* by Woronin,<sup>1</sup> Ternetz,<sup>2</sup> and Dodge<sup>3</sup> respectively,

<sup>1</sup> M. Woronin, *loc. cit.*

<sup>2</sup> C. Ternetz, *loc. cit.*

<sup>3</sup> B. O. Dodge, "The Life History of *Ascobolus magnificus*," *Mycologia*, Vol. XII, 1920, pp. 115-134.

but they are easily overlooked unless one is interested in them. Ternetz,<sup>1</sup> if one may judge by her silence about plugs and by one of her illustrations, probably saw plugs but confused them with Woronin bodies.

Plugs are formed in *Fimetaria fimicola* in just the same way as they are in *Pyronema confluens*. This observation suggests that the plugging of pores is a phenomenon of wide occurrence in the Pyrenomycetes.

Some cells of a mycelium of *Coprinus sterquilinus* growing in a hanging drop of cleared dung-agar were killed with a needle. Septal walls dividing a living cell from a dead cell were then examined and, although the hyphae were much thinner than those of *Pyronema confluens* and *Fimetaria fimicola*, in a number of cases a very tiny plug was seen crowning the apex of the bulging wall. The inference from these observations is that each septum of *C. sterquilinus* has a central pore normally open but readily closed when one of the two cells adjacent to it dies. In *Rhizoctonia solani* (= *Corticium solani*), another Hymenomycete, the formation of a plug at the pore of a septum at the moment when one of two adjacent living cells died was actually observed (*vide infra*, Fig. 74, D and E, p. 145).

**The Origin of Intrahyphal Hyphae from Septal Walls and their Growth through Older Dead Hyphae under Experimental Conditions.**—*Intrahyphal hyphae*, *i.e.* younger more slender hyphae running through the cavities of older thicker dead parts of hyphae, have been observed by many investigators. In 1881, Zopf<sup>2</sup> recorded them as occurring in *Chaetomium Kunzeanum*, one of the Pyrenomycetes. In his description of the germination of the gemmae, which are formed by the concentration of protoplasm in certain cells in an old mycelium, he says: the germ-tubes originating from the septa "take their way through the emptied neighbouring cells of the mycelial hyphae, boring through the cross-walls where they find them (Figs. 24, 25, A and B). Often empty hyphae for wide stretches are quite filled with germ-tubes bent here and there. Fig. 25, B, gives an idea of this. On seeing such illustrations one

<sup>1</sup> C. Ternetz, *loc. cit.*, Taf. VII, Fig. 5. This should be compared with my Fig. 72.

<sup>2</sup> W. Zopf, "Zur Entwicklungsgeschichte der Ascomyceten: *Chaetomium*," *Nova Acta Acad. Cues. Leop.-Carol.*, Bd. XLII, 1881, p. 243, Taf. XVI, Figs. 23-25.

might almost believe that one has before one a foreign organism which is living as a parasite in the *Chaetomium* hyphae." Intrahyphal hyphae have also been recorded as present in old cultures : of *Ascophanus carneus*. by Ternetz<sup>1</sup> (1900); and of *Ascobolus*

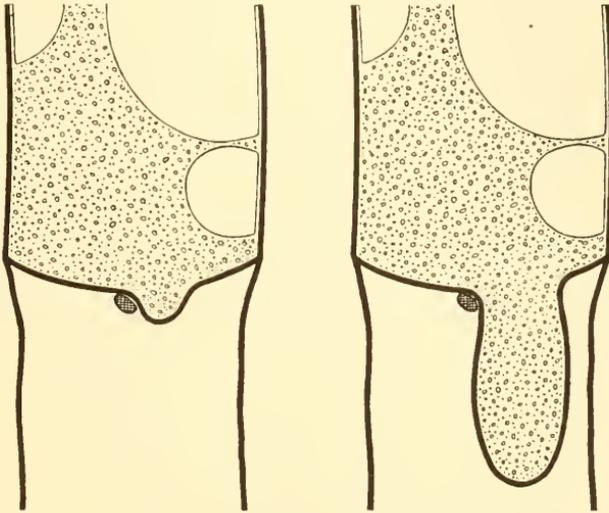


FIG. 69.—*Pyronema confluens*. Diagrams illustrating the outgrowth of a hypha from a living cell into a dead cell. Parts of two cells are shown in median longitudinal section. The lower cell was killed by a mechanical operation with a needle, with the result that, immediately, the septum became convexly bulged into the dead cell and its pore closed by a plug, as shown in Fig. 68. Half an hour later, as shown here on the left, a hypha began to grow from the living cell into the dead cell as an extension of the septal wall. A few minutes later, it had attained the length shown in the drawing on the right. During its growth, it pushed aside the plug which formerly closed the pore of the septum. Magnification, 3240.

*magnificus*, by Dodge. At first (1912), Dodge<sup>2</sup> thought that the intrahyphal hyphae of *A. magnificus* were those of a parasite but, in a subsequent communication (1920), he<sup>3</sup> corrected this

<sup>1</sup> C. Ternetz, *loc. cit.*, p. 280, Taf. VII, Fig. 5. Ternetz gives many references to the literature concerned with intrahyphal hyphae.

<sup>2</sup> B. O. Dodge, "Artificial Cultures of *Ascobolus* and *Aleuria*," *Mycologia*, Vol. IV, 1912, pp. 220-221, Plate LXXII, Figs. 7 and 8.

<sup>3</sup> B. O. Dodge, "The Life History of *Ascobolus magnificus*," *Mycologia*, Vol. XII, 1920, pp. 125-126, Plate VIII, Figs. 1-7.

misconception. A list of fungi in which intrahyphal hyphae have been seen, compiled by Dodge in 1920, includes *Alternaria*, *Botrytis cinerea*, *Sclerotium hydrophilum*, *Dematium pullulans*, *Rhizoctonia*, *Morchella esculenta*, *Gloeosporium nervisequum*, and *Gymnosporangium*.

Intrahyphal hyphae can be found in old hanging-drop cultures of both *Pyronema confluens* and *Fimetaria fimicola*. Just as in other similar fungi, the mycelium, as it grows older in an exhausted medium, tends to concentrate its protoplasm in certain cells, thus forming gemmae. Here and there in a hypha one or more cells die. The remaining living cells then often produce slender hyphae which grow through adjacent dead cells intrahyphally. These new hyphae, by means of hyphal fusions, may then serve to reconnect two older living cells which previously, owing to the death of one or more intermediate cells, had become separated from one another. It was found possible to cause new hyphae to grow out from septa intrahyphally as a result of operating upon the older hyphae of a mycelium. The production of intrahyphal hyphae under experimental conditions will now be described.

Some spores of *Pyronema confluens* were sown in a shallow drop of cleared dung-agar. Two days later, when the mycelium had spread across the drop, the pointed end of a beading needle was drawn across the drop in the manner already described. Thereby several of the main hyphae were cut into two parts. The end of one part of one of the hyphae operated upon is shown in Fig. 70, A. As usual, each septum between a living and a dead cell became pressed forward convexly into the dead cell and its pore plugged (*cf.* Figs. 67 and 68). These convexo-concave septa remained unchanged for about half an hour and then from the side or middle of some of them, as shown semi-diagrammatically in Fig. 69 (p. 135) and in an actual case in Fig. 70, B, a new and relatively slender hypha began to push its way forward within the cavity enclosed by the collapsed and cylindrical wall of the dead cell. The new hyphae grew rapidly in length, and the one illustrated in Fig. 70 attained the length represented at C about two and one-half hours after the hypha had been severed. The new hyphae frequently branched and formed hyphal fusions with other hyphae.

In the experiments just described and in others made later, it was observed that an intrahyphal hypha, as it grows out from a septum, pushes the pore plug aside and occasionally carries it to

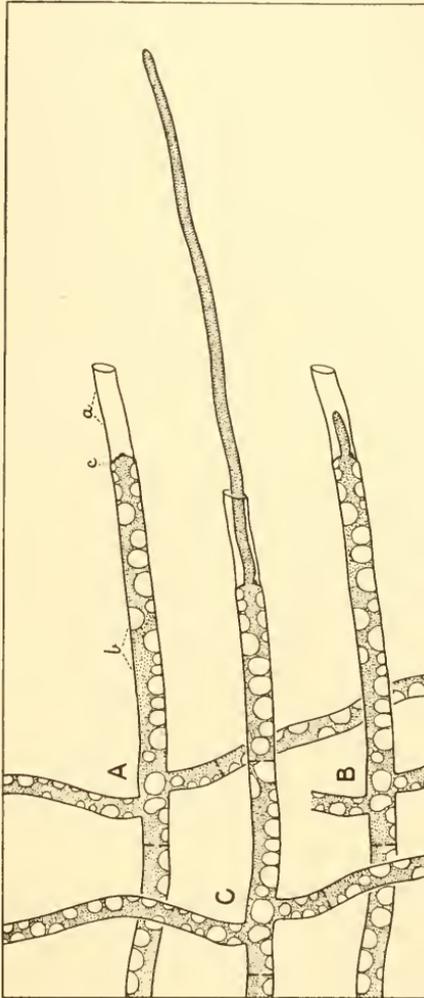


FIG. 70.—*Pyronema confuens*. The outgrowth of a hypha from a living cell through a broken dead cell. A : by means of a mechanical operation with a needle, the cell *a* has just been broken across and killed, with the result that the living cell *b* has pressed the septum *c* convexly toward the dead cell and the pore of the septum has become plugged. B : about half an hour later ; a hypha is growing from the living cell into the dead cell. C : two hours after B ; the new hypha has grown considerably in length. Magnification, 420.

some distance from the septum where it was formed. One may suppose that, when a cell on one side of a septum is killed or dies, the pore is first blocked by the instantaneous formation of a plug of coagulum and, very soon afterwards, is still more effectively sealed by the formation of cell-wall substance across it.

In a further set of experiments with mycelia growing in hanging drops of dung-agar, the cover-glass was removed from a van-Tieghem cell, inverted, and placed on a slide under the microscope. Then, with a beading needle, one or more particular cells, without their walls being broken, were pressed upon or bent until they were dead. As a cell died, its vacuoles disappeared, its protoplasm

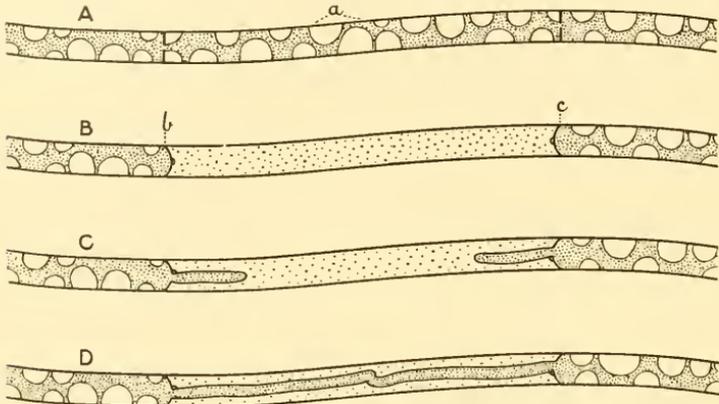


FIG. 71.—*Pyronema confluens*. Diagram showing stages in the union of two living parts of a mycelium brought about by the growth of hyphae through a cell killed by a mechanical operation. A: three cells in a mycelium, all containing granular protoplasm and large clear vacuoles attached to the cell-walls. B: the middle cell *a* has just been killed as a result of being touched and moved by a needle; its protoplasm has become disorganised; the two septa, *b* and *c*, which in A were plane and had open pores, have now become convexly bulged into the dead cell and their pores have become plugged. C: about 40 minutes after B; a hypha has grown from each of the remaining living cells into the dead cell. D: about two hours after B; the two new hyphae have met and fused. Magnification, 446.

became disorganised, its turgidity became reduced to zero, and its bounding septa became concavo-convex and crowned with a plug (Fig. 71, A and B). About half an hour after a cell with an intact cell-wall had been killed, often an intrahyphal hypha began to grow out from one of the septa, or from both, into the cavity of the dead cell (Fig. 71, C). When two intrahyphal hyphae grew toward one another simultaneously, as shown diagrammatically in Fig. 71, D, and in an actual case in Fig. 72, A, they usually met near the middle of the dead hypha and fused with one another. In this way

an injury to a hypha which resulted in the death of a cell and the separation of living cells from one another was healed up.

Two *camera-lucida* drawings illustrating the appearance of certain hyphae a few hours after some of their cells had been killed are reproduced in Fig. 72. In Fig. 72, A, a branched cell *a b c* was

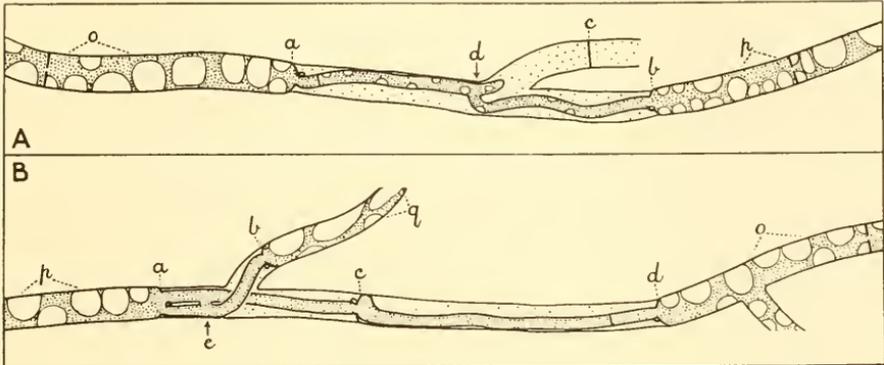


FIG. 72.—*Pyronema confluens*. *Camera-lucida* drawings showing the union of two living parts of a mycelium brought about by the growth of hyphae through cells killed by a mechanical operation. A: the branched cell *a b c* was killed as a result of being touched and moved by a needle; at the moment when the cell *a b c* died, the septa *a* and *b*, which were previously plane, became convexly extended into the lumen of the dead cell and their pores became blocked with a plug. Two and a half hours later, as here shown, two hyphae had grown out from the living cells *o* and *p* into the dead cell and had fused together at *d*. B: the branched cell *a b c* and the adjacent unbranched cell *c d* were both killed as a result of being touched and moved by a needle; at the moment when these two cells died, the septa *a*, *b*, and *d* bulged outwards from the living cells *p*, *q* and *o* into the dead cells. Four and a half hours later, as here shown, the living cell *o* through the septum *d* had sent out a hypha which had grown through the dead cell *c d*, had pierced the septum *c*, had grown through the dead cell *a b c*, and had united with the living cell *p*; while the living cell *q* through the septum *b* had sent out a hypha which had grown through the dead cell *a b c*, had united with the living cell *p*, and had also united at *c* with the other new hypha. Magnification, 446.

killed, with the result that two intrahyphal hyphae grew out from the septa, pushed the pore plugs aside, met in the middle of the dead cell, and fused. In Fig. 72, B, the branched cell *a b c* and the adjacent unbranched cell *c d* were both killed. Four and a half hours later, as shown in the illustration, the living cell *o* through the septum *d* had sent out an intrahyphal hypha which had grown through the dead cell *c d*, had swollen out against and had pierced the septum *c*, had grown through the dead cell *a b c*, and had united with the living cell *p*, while the living cell *q* through the septum *b*

had sent out an intrahyphal hypha which had grown through the dead cell *a b c*, had united with the living cell *p*, and had also united with the other intrahyphal hypha at *e*. Thus in this instance a relatively complicated injury to a hypha, resulting in the death of two cells and the division of the mycelium into three separated living parts, was soon healed and the unity of the mycelium restored.

There can be but little doubt that, under natural conditions, owing to the growth movements of Phanerogams and other plants, locomotory and other movements of small animals, etc., the mycelium of such fungi as *Pyronema confluens* and *Fimetaria fimicola* must often be injured, so that some of the cells die. Doubtless many of these injuries are healed up by the production of intrahyphal hyphae and the fusion of these hyphae with one another or with other older cells. Fruit-bodies can be produced only when the mycelium as a living unit has attained a sufficient mass. Any growth reaction, such as the production and fusion of intrahyphal hyphae, which tends to restore the unity of a mycelium which has been accidentally divided into parts, must therefore tend to promote reproduction and, indirectly, to be of advantage to the fungus species concerned.

**Ascophanus carneus.**—As we have seen, streaming in this fungus was discovered and investigated by Charlotte Ternetz in 1900.

Some tiny reddish fruit-bodies were found on old hay which had been kept for a long time partially immersed in water in the laboratory, and they were identified by my colleague, Dr. G. R. Bisby, as belonging to *Ascophanus carneus*. One of the fruit-bodies was cut up, and some pieces of it were put in a hanging drop of cleared dung-agar. Very soon hyphae began to grow from the pieces of fruit-body into the agar and, next day, the mycelium which developed from these hyphae was examined under the microscope. In several of the hyphae protoplasmic streaming was in active progress. In one part of the culture, in a hypha lacking vacuoles, the protoplasm was seen streaming through fourteen cells in succession, away from an older part of the mycelium where vacuoles were developing and toward younger rapidly elongating hyphae. These observations confirm similar ones made by Ternetz.

A brief study of protoplasmic streaming in the mycelium of

*Ascophanus carneus* served to convince me that the process takes place in this fungus in essentially the same manner as in *Fimetaria fimicola* and *Pyronema confluens*.

**A Ciboria on Male Birch Catkins.**—Dr. G. R. Bisby and the writer found the stalked apothecia of a species of *Ciboria* on old fallen male catkins of the Birch (*Betula alba* var. *papyrifera*) in woods at Victoria Beach, Lake Winnipeg, on May 8, 1933. Some of the fruit-bodies were photographed next day in my laboratory (Fig. 73), and others were used by Dr. Bisby for inoculating nutrient media with spores and thus obtain-

ing pure cultures. Dr. H. H. Whetzel of Cornell University, to whom specimens of the fungus were sent, has informed me that in his opinion the species differs from *Sclerotinia* (*Ciboria* ?) *betulae* which he has found on seeds of the Birch and that, in due course, he intends to describe it as new. The mycelium turns malt-agar black, and several of the fruit-bodies puffed vigorously when being gathered from the ground.

A hanging drop of nutrient gelatine was inoculated with some mycelium of the *Ciboria* one afternoon ; and, next morning, it was found that many hyphae had grown out into the drop and that, in some of the hyphae, protoplasmic streaming was clearly visible : the protoplasm was slowly creeping toward the growing points.

The formation of a new septum as an annular ingrowth from the lateral wall of the terminal cells of certain hyphae was watched. Each septum took about six minutes to form. At first a septum was plane ; but, shortly after it had been formed, doubtless owing to the pressure of the protoplasm in the sub-terminal cell, it bulged forward slightly toward the growing point. The protoplasm could

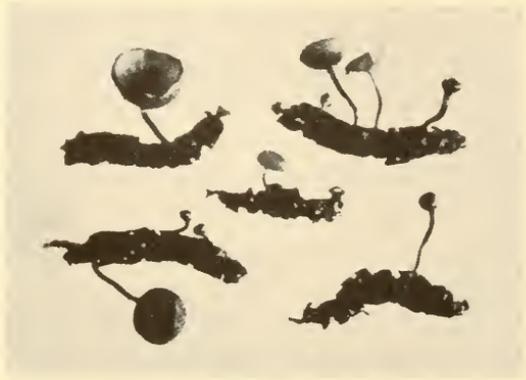


FIG. 73.—Apothecia of a species of *Ciboria* growing on fallen male Birch catkins. Gathered at Victoria Beach, Lake Winnipeg, May 8, 1933. Natural size.

be seen streaming slowly past the pore of a newly formed, convexly bulged septum, along the terminal cell, and almost up to the growing point. In some of the hyphae several of the terminal septa were seen to be bulged forward toward the growing point. The phenomenon of the bulging forward of the terminal septum in a hypha, which apparently does not occur in *Fimetaria fimicola* and *Pyronema confluens*, was first observed in *Rhizoctonia solani* and it will be treated of more fully in connection with that species.

In a *Ciboria* hypha in which streaming was fairly rapid a vacuole was seen to be torn loose from the side of a cell and to be carried by the protoplasm up to the next septum.

**The Hymenomyces.**—The general structure of the mycelium of the Basidiomyces resembles that of the Ascomycetes in being cylindrical, branched, and divided up into cells by means of septa each of which has a small central pore occupied by a protoplasmic bridge. Since we know that protoplasmic streaming takes place in Pyrenomycetes and Discomycetes, there seems to be good reason to suppose that it also takes place in Hymenomyces, Gymnomyces, Uredineae, Ustilaginaceae, and Tilletiaceae. With this in mind and realising that protoplasmic streaming and not mere diffusion may well be the means by which the mycelium of Agaricaceae or Polyporaceae, etc., sends to a rapidly growing fruit-body great quantities of building substances, I determined to seek for streaming in the mycelium of some typically basidiomycetous fungi. The species chosen were Hymenomyces and, in the end, my efforts were crowned with success.

A search for streaming in the Hymenomyces at first yielded negative results. Mycelia of *Coprinus sterquilinus* and *C. lagopus*, grown in hanging drops, were repeatedly and carefully examined, but no definite streaming movement could be observed in them. It is true that the vacuoles were seen to be constantly changing their shape and that the Woronin bodies individually often moved from one to six diameters of a hypha up or down a cell very rapidly, but nothing was observed to suggest a mass flow of the cytoplasm in any one direction. The vacuoles were attached to the cell-walls. In these Coprini, the general cytoplasm, except for the sparsely distributed Woronin bodies, appears extremely hyaline when viewed

either with ordinary or with dark-field illumination. When water flows through a glass tube, one can see nothing of its motion because there are no visible particles in it. There was therefore the possibility that, even if protoplasm were streaming rapidly through a hypha of *C. sterquilinus* or *C. lagopus*, one would not be able to detect its flow.

The mycelial hyphae of *Coprinus sterquilinus* and of *C. lagopus*, relatively to those of *Fimetaria fimicola* and *Pyronema confluens* in which streaming has actually been observed, not only have a hyaline instead of a granular cytoplasm but are also very thin. In a further attempt to observe streaming in the Hymenomycetes, a hymenomycetous species with thicker hyphae and more granular protoplasm than in Coprini was sought, and the desired material was found in *Rhizoctonia solani*.

*Rhizoctonia solani* is the cause of the *black scurf and stem canker* disease of potato tubers. The "dirt that will not wash off" on affected tubers consists of small black sclerotia. Although the fungus is often placed in the Fungi Imperfecti, it is now well known that, under favourable conditions in the field, it gives rise to a thelephoraceous fructification, known as *Corticium solani*, which produces basidia in abundance. I myself have seen these fructifications around the base of living potato stems at Kew, England. The strain of *R. solani* used was kindly supplied by my colleague, Dr. G. R. Bisby, who had isolated it from soil in the course of his studies of the soil fungi of Manitoba.

The young vegetative hyphae of *Rhizoctonia solani* are often about  $8\ \mu$  thick, have colourless walls, and are septate at intervals of 100–200  $\mu$ . The cytoplasm is not quite hyaline, for it is very faintly clouded with fine almost imperceptible particles. The vacuoles resemble those of *Pyronema confluens*: they arise peripherally and remain attached to the cell-wall. The septa all have a small central pore,  $1\ \mu$  or less in diameter, through which a bridge of protoplasm passes (Fig. 54, B, p. 99).

A hanging drop of cleared dung-agar was inoculated with mycelium from a stock culture of *Rhizoctonia solani*. On the following day the hyphae, which had grown outwards to the periphery of the drop, were illuminated by daylight and examined with

the high power of the microscope.<sup>1</sup> No protoplasmic movement could be detected. A powerful electric lamp with a focussing screw was then used as a source of light. After much manipulation of the light, the mirror, and the condenser, streaming of the protoplasm in several hyphae was observed. The streaming protoplasm appeared as a very faint grey cloud moving forward in one direction (Fig. 74, A). In some of the hyphae the movement was very rapid, just as in *Fimetaria fimicola* and *Pyronema confluens*. As the protoplasm came up to a septum it seemed to pass through it with the greatest ease, and streaming was observed in one direction through about a dozen long cells in succession. The hyphae in which streaming was very active appeared to be less vacuolated than those in which the protoplasm was at rest or moving very slowly.

Protoplasmic streaming, like that just described, was seen in a number of hanging-drop preparations made on different days. Usually artificial light was employed, but it was found that streaming could be observed even by daylight when this is obtained from white clouds strongly illuminated by the sun.

As it is not easy to illuminate a hypha in which streaming is taking place so that the movement of the protoplasm can be actually observed and as, in consequence, other investigators may have difficulty in detecting the phenomenon, I shall here record that Dr. E. S. Dowding, Dr. G. R. Bisby, and two other observers, after examining my cultures, all declared that they also had been able to perceive the faintly visible, cloudy protoplasm flowing *en masse* rapidly from cell to cell.

In a hypha of *Rhizoctonia solani* in which streaming is in progress, usually no large vacuoles are in contact with the septa; and hence an afferent cone of protoplasm approaching a septal pore and an efferent cone of protoplasm proceeding away from a septal pore, such as have been described for *Pyronema confluens*, were not observed. However, careful focussing at the level of a pore when protoplasm is streaming through it enables one, under favourable conditions, to observe that the stream of protoplasm, immediately after passing through a pore, rapidly widens out in the next cell.

In hanging-drop cultures of *Rhizoctonia solani*, *Coprinus*

<sup>1</sup> Zeiss microscope, 1900, Ocular No. 4, Objective FF, dry system.

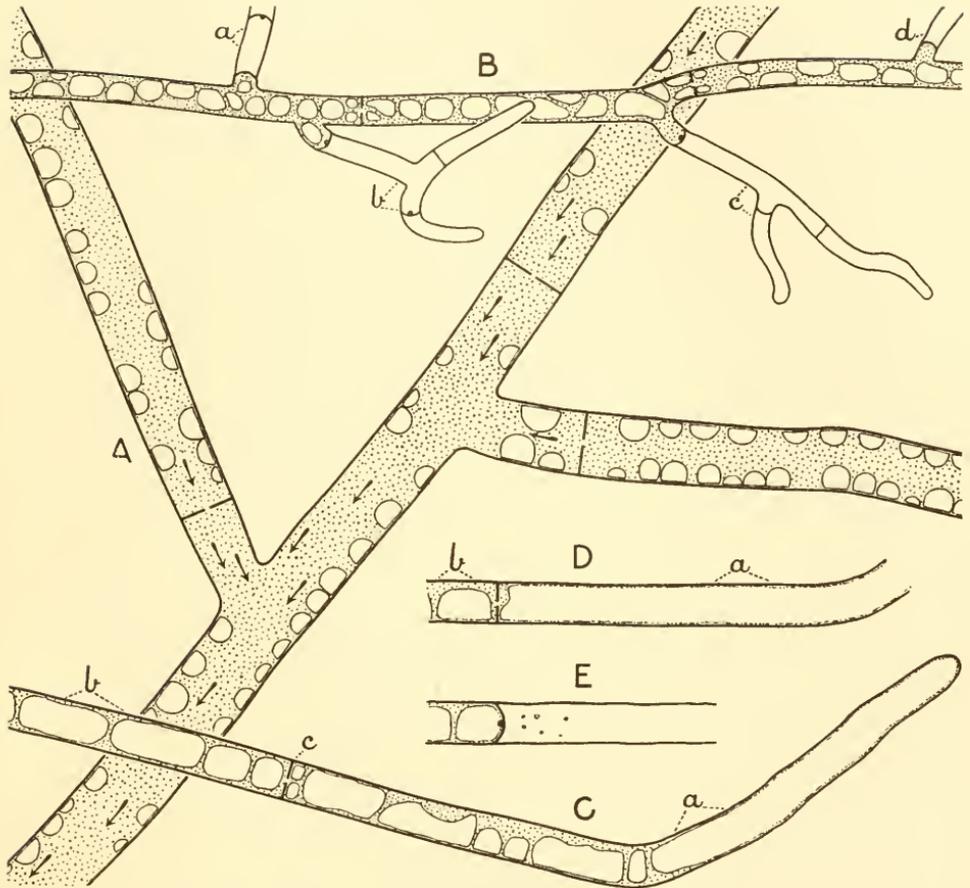


FIG. 74.—*Rhizoctonia solani* (= *Corticium solani*), a Hymenomycete. To illustrate the enlargement of vacuoles in mycelial hyphae which have ceased to grow and the translocation of protoplasm from cell to cell. A, a main hypha and two lateral branches; the vacuoles are small and fixed to the cell-wall; the protoplasm was observed flowing *en masse* along the hyphae and through the pores of the septa in the directions indicated by the arrows. B, part of a mycelial system from which protoplasm was passing out; the lateral hyphae *a*, *b*, *c*, and *d* have already evacuated their protoplasm and are now dead; the main hypha is highly vacuolated and its vacuoles are growing in size. C, D, and E, three stages in the evacuation of protoplasm from a terminal cell. C, the terminal cell *a* and the subterminal *b*, separated by the septum *c*, are evacuating their protoplasm; there are nine vacuoles in *a*, the largest at the apex of the cell. D, about two hours later; the vacuoles in the terminal cell have now become reduced to one; a little heap of protoplasm is passing through the pore of the septum from *a* to *b*. E, about half an hour later; the terminal cell has evacuated its little heap of protoplasm; it died less than two seconds ago, at which time it lost its turgidity; the subterminal cell has blocked up the pore of the septum and has pressed the septum out convexly into the dead terminal cell. Magnification: A, C, D, and E, 1000; B, 467.

*sterquilinus*, and *C. lagopus*, when one focusses with the high power of the microscope on the middle part of a septum, one can see something of the pore. At first one gets the impression that on each side of a pore there is a more or less hemispherical or discoid pad of material which possibly might block up the pore. That this appearance is not due to the cell-wall around the edge of the pore being slightly thickened is indicated by the fact that no such thickening is revealed when hyphae are treated with iodine and chlor-zinc iodine (Fig. 54, B, p. 99); and that the appearance is not due to pads of specially dense protoplasm is indicated by the fact that in *Rhizoctonia solani* it persists even when protoplasm can be seen streaming rapidly through the pore. The "pads" in all probability are due to an optical illusion. Doubtless light impinges on the edge of each pore and the appearance of the "pads" may well be due to the peculiar way in which light passes from the edge of a pore to the eye.

In *Rhizoctonia solani*, just as in *Pyronema confluens*, there can be but little doubt that the movement of the protoplasm along the hyphae is caused (1) by the increase in volume of the vacuoles in certain cells and (2) by the increase in the mass of the protoplasm. In an older mycelium it was observed that the end-cell of a side branch becomes emptied of protoplasm first, and then the penultimate cell, and so forth (Fig. 74, B). When a cell has lost nearly all its protoplasm and has come to contain one large vacuole, it suddenly dies. Its turgidity is lost and it contracts in breadth. At the same instant the adjacent living cell pushes forward the dividing septum into the dead cell and plugs up the septal pore. Thus parts of the mycelium become entirely exhausted of protoplasm and thus the streaming of protoplasm along main hyphae to other hyphae which are continuing growth is provided for.

The emptying and dying of the end-cell shown in Fig. 74, C, was actually observed. At first the cell, which had already evacuated most of its protoplasm, contained nine vacuoles. The walls of protoplasm separating the vacuoles from one another disappeared one by one, so that at length one large vacuole was left (Fig. 74, D). The protoplasm gradually passed out of the cell through the septum until it became reduced to an extremely thin imperceptible layer

lining the cell-wall and preserving the cell's turgidity. About two and one-half hours after the cell first came under observation, it suddenly died. As death took place, the septum had its pore plugged up and was pressed forward convexly into the dead cell by the adjacent living cell (Fig. 74, E). At the same time, the dead cell lost its turgidity, for it suddenly shortened by about 9 per cent. of its original length and also became narrower.

The mycelium of *Rhizoctonia solani* grows rapidly in a radial direction in a dung-agar plate (4-5 mm. per day) and, doubtless, this rapidity of growth is a factor in permitting one to see streaming through the hyphae. The observations of myself and of others indicate that in the Phycomycetes, the Discomycetes, and the Pyrenomycetes, the rate of growth in length of the mycelial hyphae and the rate of flow of the protoplasm are correlated: the faster the growth, the faster the flow, and the slower the growth, the slower the flow. Doubtless this rule holds for the Hymenomycetes also.

When *Rhizoctonia solani* is growing under natural conditions in the field, it may well be that the protoplasm of the ordinary long hyphae which branch and grow over the surface of the Potato tubers streams to the short irregularly anastomosing cells which loosely or compactly make up the tissues of the sclerotia.

The streaming of protoplasm along the cells of a hymenomycetous fruit-body has not yet been observed, but there is every reason to suppose that it takes place there just as in mycelia. The mechanism by which the cytoplasm and the four nuclei of a basidium-body of a *Coprinus*, a *Collybia*, or other agaric are transferred through the narrow channels of the sterigmata into the four spores is essentially the same as that by which protoplasm is transferred from some hyphae of a mycelium of *Rhizoctonia solani* to others, for it depends on the increase in size of a vacuole. Young basidia about to produce spores are full of protoplasm (Fig. 75, A). When the spores are being formed, a number of small rounded peripheral vacuoles come into existence in the stalk of the basidium-body (B) and fuse together to form a single vacuole (C). This vacuole enlarges and, *pari passu*, the cytoplasm and the four nuclei are pushed out of the basidium-body into the four spores (C). Finally, the basidium-body

loses all its protoplasm except a very thin layer lining the cell-wall which is necessary for preserving the cell's turgidity. Within a few minutes after the spores have been shot away, the basidium-dies. Although the slow passage of the cytoplasm and the four nuclei from a basidium-body of a single individual living basidium into the four spores has not been actually observed in detail, from comparative observations on living and on fixed and

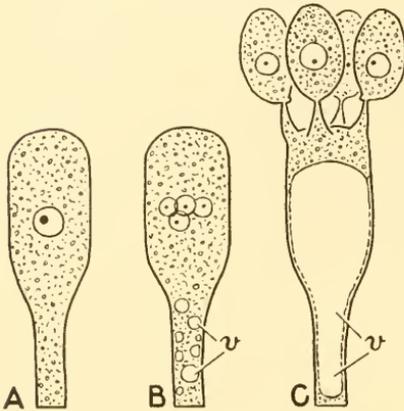


FIG. 75.—*Coprinus sterquilinus*. A, a young basidium full of protoplasm; above, a fusion nucleus. B, a slightly older basidium with vacuoles *v* below and four haploid nuclei above. C, a much older basidium; the small vacuoles have united to form one large vacuole *v* which is expanding and driving the labile protoplasm into the spores. Magnification, 612.

stained basidia in all stages of development we are justified in believing that it actually occurs. By inference, therefore, we are entitled to regard it as a definite instance of protoplasmic streaming in hymenomycetous fruit-bodies.

The biological significance of protoplasmic streaming in the Hymenomycetes is the same as in the Pyrenomycetes and Discomycetes, *i.e.* it serves to supply protoplasm to rapidly growing hyphae wherever these may be situated.

With the discovery that, in the Hymenomycetes, protoplasm can flow rapidly from cell to cell through the pores of the septa,

light is thrown on the means whereby hymenomycetous fruit-bodies in general obtain the nutriment required for their upbuilding. Everyone with an interest in living things is apt to be astonished at the rapidity with which such large fruit-bodies as those of *Polyporus squamosus*, *Boletus edulis*, *Lepiota procera*, etc., grow to maturity; and, when confronted by the phenomenon, a plant physiologist naturally asks: how is the required nutriment transferred so quickly from the mycelial hyphae to the sporophores? Formerly there was no satisfactory answer to this question. Now we can answer it as follows. When a *Polyporus squamosus* or other large

hymenomycetous fruit-body begins its development, the protoplasm within the mycelium begins to be driven toward it by vacuolar pressure. As the fruit-body rudiment increases in size, more and more mycelial hyphae become highly vacuolated and, as a result, the amount of protoplasm per hour forced into the growing hyphae of the rudiment increases correspondingly. When the fruit-body is growing in size most rapidly, the increase in size of the vacuoles of hyphae in the mycelium goes on most rapidly too, and the rate of flow of the protoplasm along certain mycelial hyphae and into the hyphae of the fruit-body attains its greatest speed, probably at least 10 cm. per hour. As the rate of growth of the fruit-body slows down, so also slows down the rate of flow of the mycelial protoplasm into the fruit-body; and, when the fruit-body ceases to grow, the mycelium ceases to supply it with protoplasm. By the time this stage of development has been attained, the mycelium as a whole or in large part has become fully exhausted and a great many of its hyphae are already dead.

Thus, in general, a hymenomycetous fruit-body does not receive its nutriment from the mycelium by the slow process of diffusion or by conduction through special persistent channels like sieve-tubes or wood vessels, but in the form of protoplasm forced toward it by vacuolar pressure arising in a system of tubular mycelial hyphae progressively exhausting themselves and progressively dying.

The growth of the vacuoles in mycelial hyphae engaged in evacuating their labile protoplasm is doubtless due to the protoplasm increasing the amount of the osmotic substances contained in the vacuoles and to the absorption by the vacuoles of more water from the substratum.

Large vacuoles may be formed in a fruit-body even while it is developing and before it has begun to shed its spores. Thus, in *Coprinus sterquilinus*, the rapid intercalary growth in length of the upper part of the stipe before and during the expansion of the pileus is correlated with an increase in the size of the vacuoles in the elongating cells. Since there are continuous columns of watery protoplasm stretching from the mycelium in the moist substratum to the most distant hyphae of the fruit-body, including those elongating in the stipe just beneath the pileus, the water required to fill

the enlarging vacuoles of the elongating stipe-cells can be readily drawn up by suction pressure from the mycelium in just such amounts as are required.

The ripe spores of a hymenomycetous fruit-body are always full of dense protoplasm and, as a fruit-body discharges more and more of its millions of spores, it becomes more and more exhausted of protoplasm. At last, when spore-discharge is ceasing or has ceased, most of the space in every cell of the stipe, the pileus-flesh, the trama, the subhymenium, and the hymenium (paraphyses) is occupied by one or a few very large vacuoles. Doubtless, as these vacuoles enlarge and drive out protoplasm toward the basidia, much of the water required to increase the volume of the cell-sap is drawn into the vacuoles through the protoplasm of adjacent cells from a distance. The ultimate source of the water which enters these enlarging vacuoles may sometimes be water in other cells of the fruit-body, sometimes water stored between the pileus-flesh cells, and, sometimes, possibly, water contained within the mycelium in contact with the substratum.

**Mycetozoa and Fungi.**—The flow of protoplasm in a plasmodium of a Mycetozoon toward some place where the sporangium or sporangia are to be formed is comparable to the flow of protoplasm in the mycelium of Phycomycetes, Discomycetes, Pyrenomycetes, and Hymenomycetes toward the sporophores. In both Mycetozoa and Fungi, the protoplasm formed in the vegetative part of the organism often flows a distance of several inches or feet to the place where the spores are to be developed.

**Phycomycetes compared with Ascomycetes and Basidiomycetes.**—We now know that protoplasmic streaming takes place in the mycelium of species belonging to all the three great groups of fungi: Phycomycetes, Ascomycetes, and Basidiomycetes.

In the Mucorineae streaming is characterised: (1) by frequent reversals in the direction of flow of the protoplasm; (2), at least sometimes, by a peripheral current of protoplasm flowing in a direction opposite to that of the main stream; and (3) by a general movement of the vacuoles.

On the other hand, in the Higher Fungi streaming is characterised: (1) by the flow of the protoplasm for an indefinitely long time

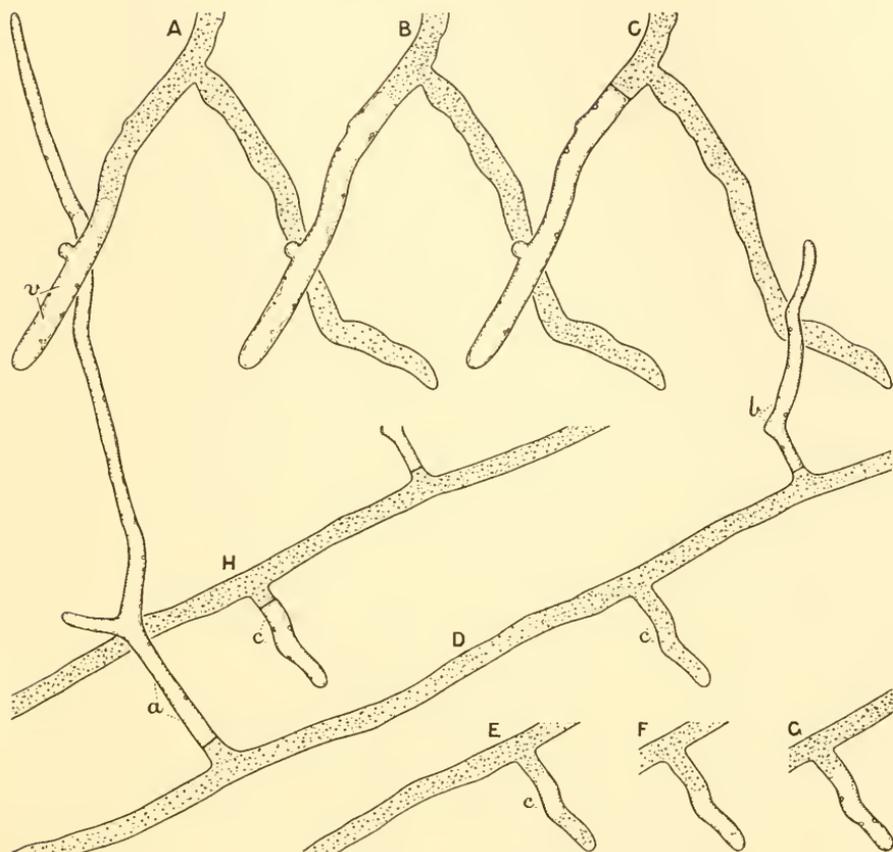
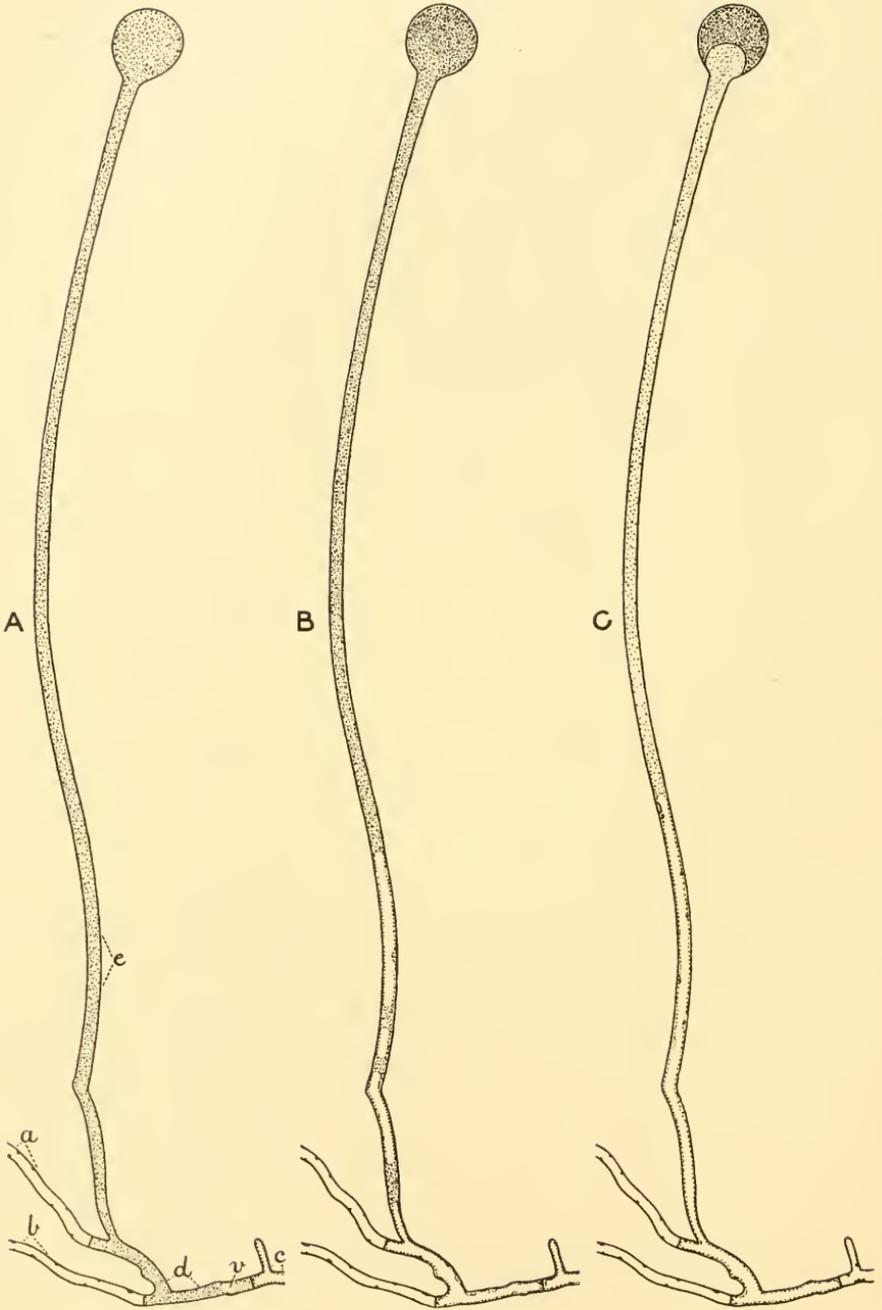


FIG. 76.—*Rhizopus nigricans*. The evacuation of protoplasm from, and the subsequent walling-off of, parts of a mycelium during the formation of sporangio-phores and sporangia. The mycelium had developed in a hanging drop of nutrient gelatine, and the hyphae shown had ceased to grow in length. A, a large fixed vacuole *v* has arisen in the left-hand hypha and is growing in size and thereby pushing the massive labile protoplasm backwards toward a sporangiophore. B, two hours later; the vacuole has increased greatly in size. C, an hour after B; a septum (without any central pore) has been formed near the base of the vacuole. The terminal cell, thus cut off, is about to die. Streaming was observed in the protoplasm in the right-hand hypha and irregular longitudinal rotatory movement in the protoplasm bounding the vacuole. D, a main hypha with three side-branches. Through vacuolar pressure, the massive labile protoplasm has been evacuated from the branches *a* and *b*, and these exhausted hyphae, which are soon to die, have been walled-off from the main hypha. The branch *c* is as yet full of protoplasm. E, F, G, and H, four successive stages in the branch *c*, passed through in about 12 hours, showing the formation of a large vacuole, the consequent evacuation of the massive protoplasm, and the walling-off of the exhausted hypha. Drawn by A. H. R. Buller and C. C. Neufeld. Magnification, 332.



in one direction only, without any more or less rhythmic reversals in direction ; (2) by the absence of peripheral currents of protoplasm flowing in a direction opposite to that of the main stream ; and (3) by the fact that the flow of protoplasm often takes place through a cell for long periods of time without the vacuoles being involved in the movement.

In the Higher Fungi, the vacuoles appear to be strongly attached to the immovable outermost non-granular layer of protoplasm lining the cell-wall ; whereas, in the Mucorineae, the vacuoles appear to be not so attached or not attached so strongly. Hence perhaps it is that, in the Higher Fungi, the vacuoles are only torn loose and carried off by the streaming protoplasm when the current is very strong ; whereas, in the Mucorineae, the vacuoles are translocated by the streaming protoplasm even when the current is very weak.

In the Mucorineae, the streaming takes place in a non-septate mycelium, so that the flow of the protoplasm from the mycelium into the young sporangia is not hindered by any cross-walls. The absence of septa facilitates the flow of protoplasm to the growing points and may be correlated with the rapid growth in length of the vegetative hyphae and of the sporangiophores. Septa are eventually formed : (1) across the base of lateral hyphae, etc., which have evacuated their main mass of protoplasm and are about to die (Fig. 76) ; and (2) across the base of sporangia after these have swollen up and have been filled with protoplasm (Fig. 77). Thus, in the Mucorineae, septa are formed only *after the flow of protoplasm out of or into an organ has finally ceased*.

FIG. 77.—*Rhizopus nigricans*. The translocation of protoplasm from a mycelium into a sporangiophore, the accumulation of protoplasm in the sporangium, and the subsequent walling-off of the sporangium by the wall of the columella. The mycelium developed in a hanging drop of gelatine ; the sporangiophore was parallel to the hanging drop and finally touched its surface. A : the hyphae *a* and *b*, now dead, and the hypha *c*, still living, have evacuated their massive labile protoplasm into, and have been walled-off by septa from, the hypha *d*. The hypha *d* has grown out into the sporangiophore *e* which, in turn, has become swollen at its apex so as to form a sporangium. A vacuole *v* has been formed in the cell *d* and is growing in size. B, several hours after A : the hypha *d* and the lower part of the sporangiophore have now become highly vacuolated and protoplasm has accumulated (become denser) in the sporangium. C, about five hours after B : the dense protoplasm in the sporangium has now become cut off from the much less dense protoplasm of the sporangiophore by a columella-wall. Drawn by A. H. R. Buller and C. C. Neufeld. Magnification, 113.

In the Higher Fungi, on the other hand, the streaming takes place in the mycelium after this has become divided up into cells by septa. Each septum, as we have seen, has a small central pore through which the moving protoplasm must pass. There can be but little doubt that the septa do to some extent diminish the rate of flow of the protoplasm from cell to cell although, as will be shown in another Section of this Chapter, their presence in the mycelium is fraught with important advantages.

In the Higher Fungi, as Wahrlich discovered,<sup>1</sup> each septum grows from the periphery of the hypha inwards but never becomes complete; so that, like a "closed" iris diaphragm of a microscope,

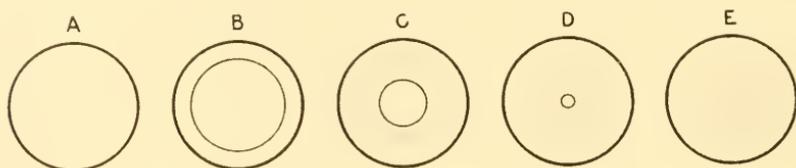


FIG. 78.—Diagram to illustrate the formation of a septum in a hypha: A–D, Ascomycetes and Basidiomycetes: A–E, Phycomycetes as represented by *Rhizopus nigricans*. A, a cross-section of a lateral wall of a hypha at a place where a septum is to be formed. B–E, successive stages in the formation of a septum by means of an annular ingrowth from the lateral wall. The end-stage for the Ascomycetes and Basidiomycetes is shown at D, where the septum has a small central pore through which protoplasm extends from cell to cell; and the end-stage for the Phycomycetes, as represented by *Rhizopus nigricans*, is shown at E where the septum is complete.

there is a small hole or pore left at its centre (Fig. 78, D). In the Mucorineae, as the researches of Harper<sup>2</sup> and Swingle<sup>3</sup> have shown, the wall of the columella is formed by the deposition of wall-substance in a cleavage plane between two masses of protoplasm.<sup>4</sup> There was the possibility that the septa formed across hyphae in the evacuating

<sup>1</sup> *Vide supra*, p. 95.

<sup>2</sup> R. A. Harper; in respect to the formation of the columella in *Pilobolus*, *vide* copies of his illustrations in the forthcoming Volume VI of this work.

<sup>3</sup> D. B. Swingle, "The Formation of Spores in the Sporangia of *Rhizopus nigricans* and *Phycomyces nitens*," *U. S. Dept. of Agric., Bureau of Plant Industry*, Bull. XXXVII, 1903.

<sup>4</sup> It remains to be determined whether the wall of the columella is formed by an even deposition of wall-substance throughout the cleavage plane or whether the wall begins as an annular ingrowth from the lateral wall of the base of the sporangium and then grows upwards in the cleavage plane to the apex of the columella.

mycelia of the Mucorineae might be formed like the wall of the columella, but a special investigation carried out with the help of my research assistant, Mr. C. C. Neufeld, has shown that the septa in the mycelium are developed in the same way as those of *Spirogyra*, *i.e.* by the closing-diaphragm process carried to completion (Fig. 78, A-E). In *Rhizopus nigricans* a septum starts as a circular rim and grows inwards toward the centre of the cell. During this process protoplasm has been observed passing through the diminishing pore from one cell to the next (Fig. 79, B). However, the pore, just as in *Spirogyra*, quickly becomes closed up (C), so that a mature septum of *R. nigricans* differs from a mature septum of one of the Higher Fungi in being entire and imperforate instead of having a pore at its centre. In *R. nigricans* protoplasm has never been seen streaming through the centre of a mature septum and this, doubtless, is due to the fact that such a septum has no central pore. Soon after its formation, a septum in an evacuating mycelium of *R. nigricans* becomes concavo-convex (D and E), owing to the turgidity of the terminal cell having become greater than that of the subterminal cell. At the moment when the terminal cell dies, the subterminal cell presses the septum into the terminal cell (F), and thus the bulge in the septum becomes reversed in direction. When one of two cells in the mycelium of *R. nigricans* is killed or dies, there is no instantaneous formation of a plug in the middle of the septum. The absence of a pore in the septum renders the formation of such a plug unnecessary. If we accept the view that a mature septum in the mycelium of *R. nigricans* is imperforate, the fact that septa are formed only in an older evacuating mycelium and only where they cut off a piece of the mycelium which has become emptied of its massive labile protoplasm from a piece which has an abundance of such protoplasm becomes intelligible: the septa in this species are never formed in places where they would hinder the flow of the labile protoplasm from smaller ultimate hyphae into the larger hyphae or from these larger hyphae into the sporangiophores and sporangia.

In *Rhizopus nigricans*, as the labile protoplasm in an evacuating mycelium passes out of a terminal hypha or system of hyphae (*cf.* Figs. 76 and 79), the place of the labile protoplasm is taken by

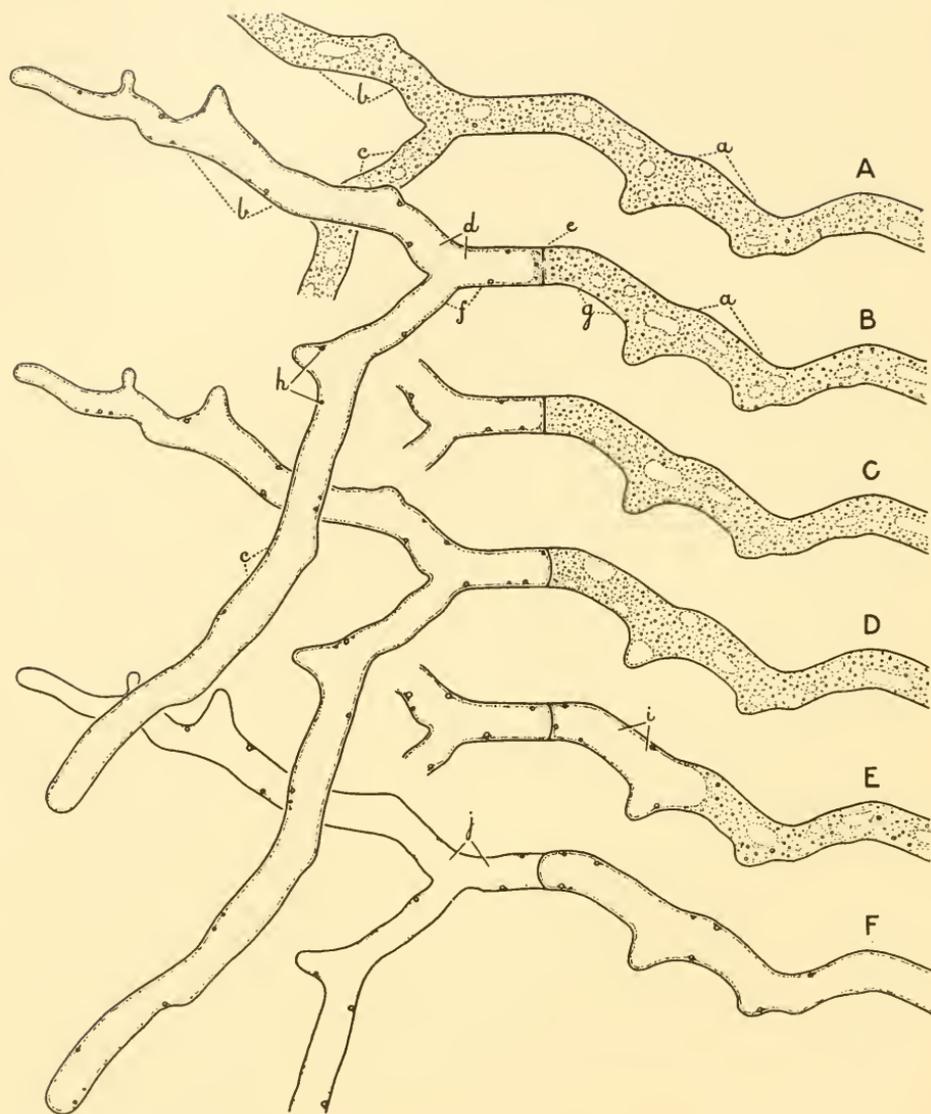


FIG. 79.—*Rhizopus nigricans*. Evacuation of protoplasm, the formation of a septum, and changes in turgor in a two-days-old mycelium grown in a hanging drop of 10 per cent. cane-sugar and gelatine. A: part of the mycelium consisting of the hypha *a* and its two terminal branches *b* and *c* (shown full-size in B), represented as if seen in a median-longitudinal section; at present *a*, *b*, and *c* are filled with dense protoplasm and are non-septate. B, about 12 hours after A: the hyphae *b* and *c*, concomitantly with the formation of, and presumably with the aid of the pressure of, the very large vacuole *d*, evacuated their massive protoplasm which passed into the hypha *a* and so

enlarging vacuoles which soon fuse together and form one great central vacuole bounded by a thin layer of hyaline protoplasm pressed against the cell-wall. There can be but little doubt that here, just as in *Pyronema confluens* and the Higher Fungi generally, the evacuation of the labile protoplasm from the hyphae is accomplished by vacuolar pressure.

In *Rhizopus nigricans*, terminal hyphae or systems of hyphae which, after evacuating their contents, have been cut off by a septum usually live for some hours and, during this period, small rounded highly refractive droplets looking like oil-drops can be seen moving irregularly along the surface of the hyaline protoplasm lining the cell-wall (Fig. 79, B, *h*). Finally, the hyphae die of exhaustion. As they do so, they contract somewhat in diameter and the movement of the droplets abruptly ceases (Fig. 79, F).

In a mycelium of *Rhizopus nigricans* in which a rhythmic change in direction of the streaming protoplasm was taking place about every three or four minutes, it was observed : (1) that the vacuoles in that part of the mycelium to which the protoplasm was flowing were diminishing in size ; (2) that the vacuoles in that part of the mycelium from which the protoplasm was flowing were increasing in size ; and (3) that, when the current of protoplasm changed its direction, the vacuoles which had decreased in size began to increase

FIG. 79—cont.

toward a developing sporangiophore and sporangium ; then, as shown at *e*, a septum began to be formed as an annular ingrowth from the lateral wall ; at the centre of the septum is a still large, but rapidly diminishing, aperture, through which particles of protoplasm were seen to pass from the cell *f* to the cell *g* ; *h*, small rounded highly refractive globules which move irregularly from place to place along the surface of the layer of protoplasm lining the cell-wall. C, 10 minutes after B : the septum, owing to annular ingrowth to the centre of the hypha, has become complete, and now protoplasm can no longer pass from *f* to *g* ; the time taken from the beginning to the end of septum-formation was about 20 minutes ; it will be seen that the septum has cut off a highly-vacuolated part of the mycelium from a part which is filled with protoplasm. D, half an hour after C : the turgor pressure in the cell *f* has become greater than that in the cell *g*, as is shown by the fact that the septum now bulges convexly into *g*. E, half an hour after D : a large vacuole *i* has come into existence in the cell *g*, and, presumably by its pressure, the protoplasm in *g* is being evacuated. F, 24 hours after E : the cell *f* has died and collapsed, its shrinkage is especially noticeable at *j*, its protoplasm has become disorganised, and its small rounded highly refractive granules have ceased to move ; the cell *g* is still living, but the part in view, as a result of the growth of the vacuole *i*, has evacuated all its massive protoplasm ; the living cell *g*, by turgor pressure, has caused the septum to bulge convexly into the dead cell *f*. Drawn by A. H. R. Buller and C. C. Neufeld. Magnification, 400.

in size and the vacuoles which had increased in size began to decrease in size. Whether or not in general in *Rhizopus nigricans* and other Mucorineae the rhythmic backward and forward flow of protoplasm is normally associated with a rhythmic waxing and waning of vacuoles remains to be determined by further more detailed observations.

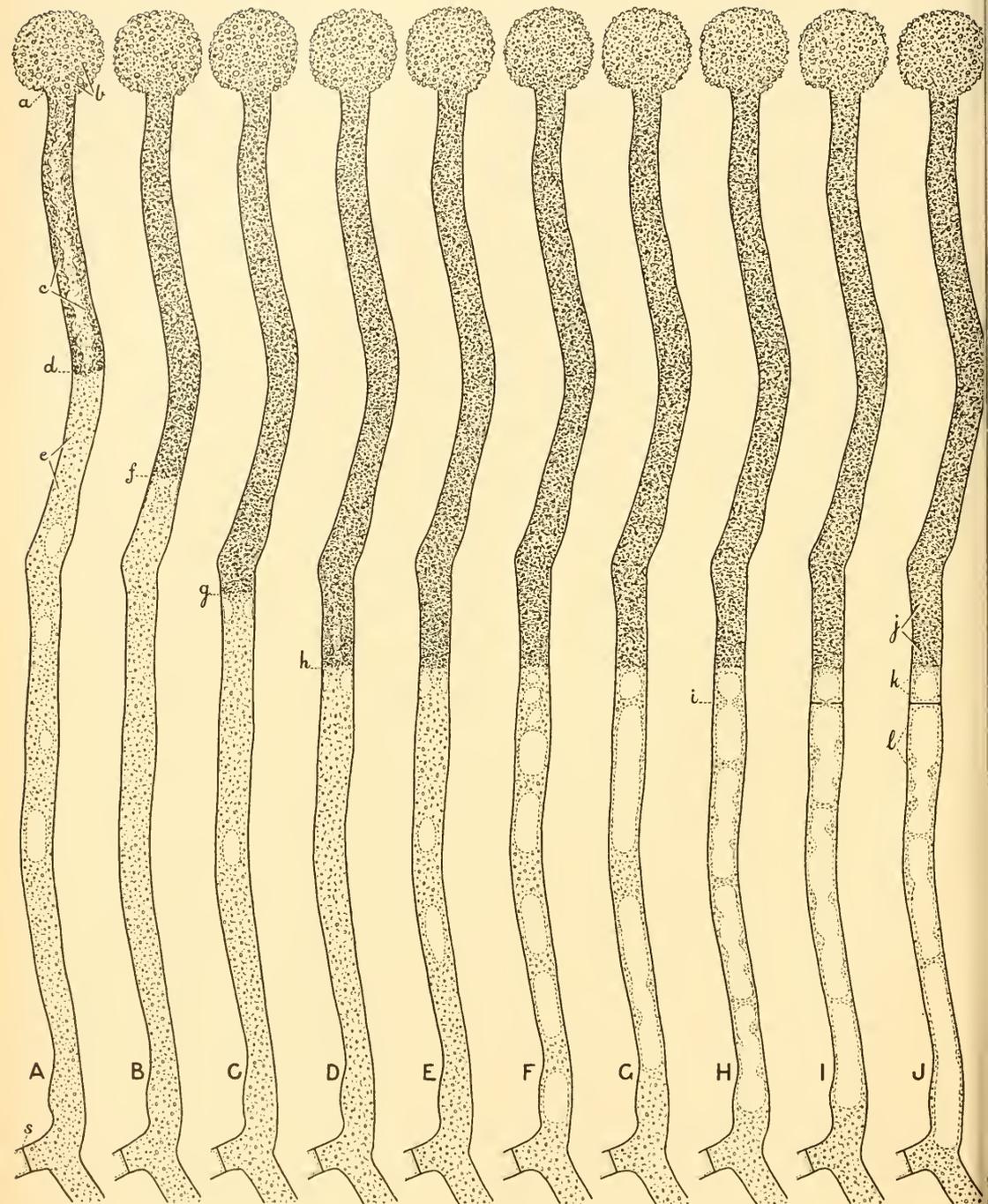
**The Biological Significance of Septa in the Mycelium of the Higher Fungi.**—The cylindrical wall which bounds the hyphae in the Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti has several functions : (1) it serves as a sort of exoskeleton in that it supports the protoplasm and maintains the form of the hyphae ; (2) it protects the protoplasm from small mechanical injuries ; and (3) as a firm elastic membrane it opposes the outward pressure of the cell-sap and thus permits hyphae to become turgid.

The younger mycelia of the Phycomycetes are non-septate ; whereas, in the mycelia of the Ascomycetes, Basidiomycetes, and Fungi Imperfecti, septa are developed from the first. In these septate mycelia each septum is a thin flat circular plate with a relatively small central open pore (*cf.* Figs. 67 and 78, D, pp. 131 and 154). In most septate hyphae the septa are situated at considerable distances from one another. Thus in some leading hyphae of *Pyronema confluens*, with a diameter of about  $8\ \mu$  and having thirty-two septa, the average distance between two successive septa was  $133\ \mu$ , so that the cells were about seventeen times as long as they were thick (*cf.* Fig. 63, C and D, p. 116). Taking into account the peculiar structure and the location of septa we may ask : what is the biological significance of a septum or, in other words, how do septa promote the welfare of the living hyphae in which they are situated ? An attempt to answer this question will now be made.

Septa must strengthen hyphae mechanically by increasing the resistance to bending or breaking where they are situated ; but, as the cells of septate hyphae are often 10–20 times as long as they are wide, the mechanical advantage accruing to hyphae through being septate must on the whole be but slight. Evidently we must look in another direction for the chief biological significance of septa.

In all fungi, whether high or low, the protoplasm in a hypha is, normally, a continuous mass. In a hypha of one of the Phycomycetes the mass of protoplasm is more or less cylindrical ; whilst, in

a hypha of one of the Ascomycetes, Basidiomycetes, or Fungi Imperfecti, the mass of protoplasm is much constricted at intervals owing to the presence of the small-pored septa. The presence or absence of septa in a hypha is correlated with differences in the reaction of the protoplasm in respect to an injury which causes part of the protoplasm to die. To elucidate this statement let us suppose that one breaks into two pieces first a hypha of a Phycomycete, *e.g.* *Rhizopus nigricans*, and then a hypha of one of the Higher Fungi, *e.g.* *Pyronema confluens*, and compares the reactions of the remaining living portions of the two hyphae. When a hypha of a Phycomycete is broken across, from each broken part some of the protoplasm escapes and forms a rounded mass at the opening. This ball of protoplasm and also some of the protoplasm remaining in the end of the hypha quickly coagulates and dies (Fig. 80, A). The dying back of protoplasm in the hypha continues for some time (B, C, D) and then ceases, with the result that the broken end of the hypha becomes plugged up with an elongated cylindrical coagulum. The living and the dead protoplasm in the hypha remain in contact with one another for a long time and no septum is formed between them (E, F, G). Finally, when the living part of the hypha has become highly vacuolated, a septum may be formed within it near to (H, I, J), or at some distance from, the junction of the living and the dead protoplasm. How far along the hypha the effect of the wound will travel in any particular case can not be predicted. On the other hand, when a hypha of an Ascomycete, a Basidiomycete, or a Fungus Imperfectus is broken across, as we know from experiments already described, the protoplasm of the broken cell dies as a whole up to each septum and at the two septa the effect of the injury ceases (*cf.* Fig. 70, A, p. 137): the two cells adjacent to the broken cell remain living. Evidently, a septum in these fungi serves to protect a living cell against the effects of the death of an adjacent cell. When one cell in a septate hypha is broken across and killed, the two living masses of protoplasm left behind are each bounded and protected from the first by an old septum, and no new cross-walls are constructed. We thus see that the septa in the mycelia of the Higher Fungi play a very important part in limiting the deleterious effects of wounds.



The advantage in each septum of a Higher Fungus being provided with a *small pore* seems to lie in this : that (1) the pore permits of protoplasm passing readily out of one cell into the next and thus being moved to places where it is needed, as may actually be seen where the protoplasm is very granular and streaming is rapid ; while, (2) the pore can be closed instantaneously when one of the cells adjacent to it is killed or dies (*cf.* Fig. 68, p. 132). The septum in the Higher Fungi is therefore constructed in such a way that it interferes but little with the passage of materials from one cell to the other so long as the cells on each side of it are living normally, and yet in such a way that it can have its pore blocked the moment one of the cells adjacent to it is killed or dies, the escape of living protoplasm through a pore being thus rendered impossible.

A septum serves not only to protect the protoplasm of one cell against the deleterious effects of the death of the protoplasm in an adjoining cell, but it also plays an important part in initiating the process (already described) by which the living parts of a mycelium

FIG. 80.—*Rhizopus nigricans*. The reaction of a hypha to a mechanical wound : the plugging of the end of a broken hypha with coagulated protoplasm and the subsequent formation of a septum by the living protoplasm near the dead protoplasm. A : a portion of a three-days-old mycelium grown in nutrient gelatine in a van-Tieghem cell, represented as if seen in a median-longitudinal section ; the hypha was cut across at *a* by pressing upon it with a sharp scalpel ; immediately some of the protoplasm escaped from the hypha and collected at the hypha's mouth so as to form the ball *b* ; the ball of protoplasm and the adjoining protoplasm *c* in the hypha coagulated and died ; at *d* the living protoplasm *e* is in direct contact with the coagulated protoplasm *c* ; the septum *s* divides a lateral part of the mycelium which has already evacuated its massive protoplasm from the main unexhausted hypha here shown. B, about 10 minutes after A ; C, 10 minutes after B ; and D, 10 minutes after C : protoplasm in the wounded hypha has continued to die, so that the junction between the coagulated and the living protoplasm has passed down the hypha successively from *d* to *f*, *f* to *g*, and *g* to *h* ; after the protoplasm had died down to *h*, further coagulation of the protoplasm ceased as may be seen by comparing D with E-J. E, 40 minutes after D : the living part of the hypha has begun to evacuate its massive mobile protoplasm as is shown by the growth of the two large vacuoles. F, 10 minutes after E : the vacuoles have increased in number and in size. G, 20 minutes after F : the vacuoles are still increasing in size. H-J : the formation of a septum across the living part of the hypha a short distance from the dead part. H, 95 minutes after G : a circular ridge of protoplasm has appeared at *i*. I, 30 minutes after H : in the ridge of protoplasm a septum is developing as an annular ingrowth from the lateral wall. J, 26 minutes after I : the septum has now become complete and it divides a smaller living cell *k* from a larger living cell *l* ; the living protoplasm in *k* is bounded at one end by the plug of dead coagulated protoplasm *j* and at the other end by the newly-formed septum ; the cell *l* has evacuated most of its massive labile protoplasm, presumably by vacuolar pressure. Drawn by A. H. R. Buller and C. C. Neufeld. Magnification, 706.

which have become isolated from one another by the death of one or more intervening cells become re-united (*cf.* Figs. 71 and 72, pp. 138 and 139); for, shortly after a septum has come to separate a living from a dead cell, it is frequently used as a point of departure for the growth of a new hypha (Figs. 69 and 70, pp. 135 and 137).

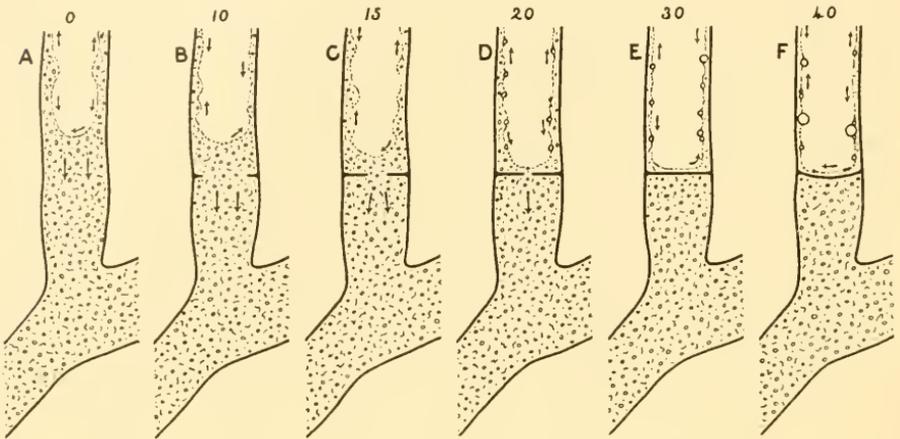


FIG. 81.—*Rhizopus nigricans*. Successive stages in the formation of a septum at the base of a hypha which has evacuated most of its protoplasm, represented in optical median-longitudinal sections. A, a septum is about to be formed near the end of the vacuole; the vacuole in the branch-hypha is enlarging and pushing the protoplasm slowly toward the main hypha; the two larger arrows represent the direction of movement of the labile protoplasm as a whole, while the smaller arrows indicate that irregular longitudinal rotatory movements are going on in the protoplasm bounding the vacuole. A was drawn at the zero of the time-scale, and the time in minutes at which each of the other stages was drawn is indicated above each of the drawings B–F. The total time in which all the changes were observed was 40 minutes. B, the septum, in the form of an annular ingrowth from the lateral wall of the branch-hypha, has begun to form. C, the septum is more than half-formed. D, the septum is almost complete; particles of protoplasm are still passing through the pore in the direction indicated by the arrow. E, the septum is now complete; its pore has been completely closed up. The septum has taken 20–25 minutes to form. F, as a result of the continued expansion of the large vacuole, the septum has been pressed forward, so that it now bulges toward the main hypha. Drawn by A. H. R. Buller and C. C. Neufeld. Magnification, 1007.

**Time taken for the Formation of a Septum.**—The formation of a septum as an annular ingrowth from the lateral wall of a hypha takes a certain time. The formation of particular septa from their first beginning to completion was watched under the microscope, and it was found that the time taken for the formation of a septum, on the average, is: in *Rhizopus nigricans*, 20–25 minutes (Fig. 81);

in *Rhizoctonia solani* (Fig. 82, A-D), about 10 minutes; and in the Ciboria that grows on male Birch catkins, about 6 minutes.

Temporary Bulging of Septa toward Growing Points.<sup>1</sup>—A

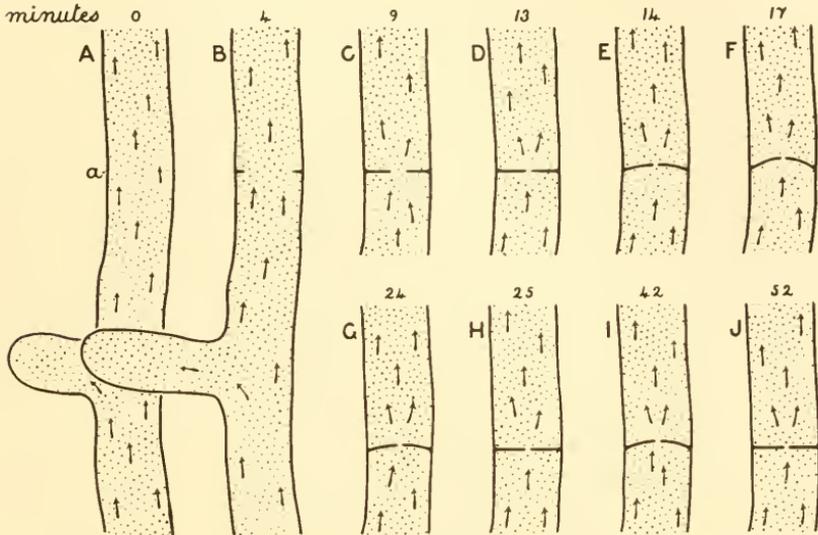
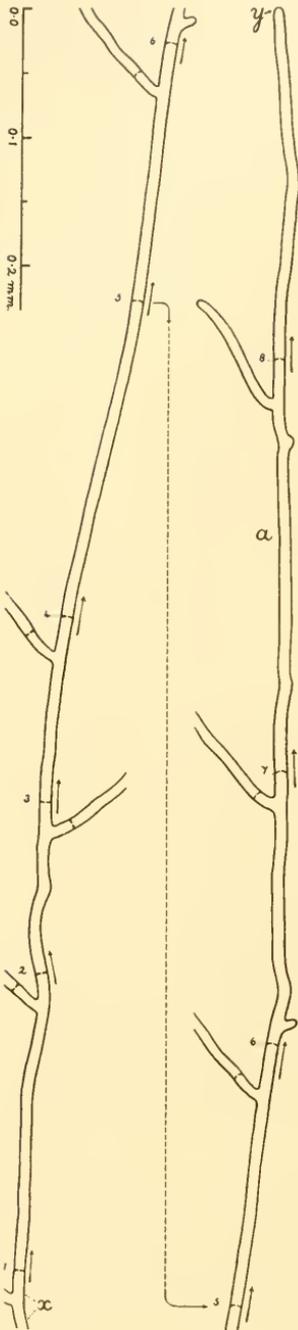


FIG. 82.—*Rhizoctonia solani* (= *Corticium solani*). Successive stages in the formation and temporary bulging forward of a septum, represented in optical median-longitudinal sections. A, the middle part of the terminal cell of a rapidly-growing hypha in which a new septum is about to be formed at the level *a*; the protoplasm, as indicated by the arrows, is very slowly flowing toward the apical growing-point of the main hypha and toward the apical growing-point of the main hypha's lateral branch. A was drawn at the zero of the time-scale, and the time in minutes at which each of the other stages was drawn after A is indicated above each of the drawings B-J. The total time in which all the changes were observed was 52 minutes. B, the septum, in the form of an annular ingrowth from the lateral wall, has begun to form. C, the septum is more than half-formed. D, the septum is complete: it has taken about ten minutes to form and has an open pore left at its centre; through the pore protoplasm is slowly passing from the subterminal cell to the growing-point of the terminal cell. E, the septum is becoming bulged in the direction of the flow of protoplasm. F, the septum is now bulged forward to the maximum extent. G, the septum is flattening again. H, the septum is now plane. I, the septum has become bulged a second time. J, the septum has become plane again. Drawn by A. H. R. Buller and C. C. Neufeld. Magnification, 1007.

septum of *Rhizoctonia solani*, whilst being formed, is plane (Fig. 82, D); but, very soon after its formation, it bends forward toward

<sup>1</sup> This phenomenon was first observed and then brought to my attention by my research assistant, C. C. Neufeld.



the growing point of the hypha and thus becomes decidedly concavo-convex (E, F). After a few minutes it flattens again (G, H). A second bulging forward followed by a second flattening may also take place (I, J); and even a third repetition of this process has been observed. Finally, the septum remains plane. Sometimes one may observe that in a single hypha behind the growing point the last 2-5 septa are bulged forward toward the growing point (Fig. 83), and that these septa, one by one, in succession, beginning with the oldest, become plane again. In the older parts of a mycelium the septa are all plane (Fig. 83).

Careful observation with the high power of the microscope revealed that whilst a terminal septum is changing its shape in the manner shown in Fig. 82 :

FIG. 83.—*Rhizoctonia solani* (= *Corticium solani*). Temporary bulging forward of a series of younger septa in a main hypha, as a result of protoplasm being pressed toward the apical growing-point: *x*, the basal part, and *y*, the apical growing-point of a main hypha of which the piece shown is 1.84 mm. in length. The hypha was growing rapidly in a hanging drop of nutrient gelatine. The four youngest septa, nos. 5-8, are considerably bulged forward; the next youngest septum, no. 4, is slightly bulged forward; whilst the oldest septa, nos. 1-3, are all plane. When the hypha was first observed, its apex was at *a* and the septum no. 3 was distinctly bulged forward. After an interval of one hour, the apex had grown to *y* and the septum no. 3 had become plane. In general, both in the main hypha and in its side-branches the older septa are all plane and the younger ones are all curved forward. Each septum has a small central open pore, and protoplasm was seen moving slowly forward through each of the eight septa of the main hypha as indicated by the arrows. Drawn by A. H. R. Buller and C. C. Neufeld. Magnification, 167.

(1) the apical cell of the hypha is growing steadily and evenly in length ; and (2) the protoplasm in the hypha is flowing continuously through the pore of the septum into the apical cell and onwards toward the growing point.

In view of the observations just recorded we may conclude that the cause of the temporary bulging forward of a newly formed septum of *Rhizoctonia solani* is not the blocking of the septal pore, but rather the pressure of the viscous protoplasm on the septum becoming temporarily and considerably greater in the penultimate cell than in the terminal cell. There can be no question but that a septum with a tiny central pore must offer some resistance to the flow of protoplasm from one cell to another, and in the bulging of a newly formed septum we seem to be afforded evidence of this fact. In an older part of the mycelium where the septa are straight, it is possible that the septa are slightly thicker and stiffer than the septa near a growing point and it may be that, in such a region of the mycelium, the massive protoplasm is more labile than near the growing points ; but all this remains to be decided by exact investigation.

An actively growing hypha of *Rhizoctonia solani* in which the protoplasm is slowly streaming *en masse* toward the growing point and in which two to five of the terminal septa are simultaneously bulged forward toward the growing point presents us with evidence which shows that protoplasm is being pressed by a series of cells with considerable force toward the growing point and that there is a decreasing pressure gradient for the protoplasm from the oldest cell to the one last formed.

After the temporary bulging-forward of newly formed septa had been discovered in *Rhizoctonia solani*, the phenomenon was looked for in the mycelia of other Higher Fungi. It could not be observed in *Fimetaria fimicola*, *Gelasinospora tetrasperma*, or *Pyronema confluens*, but it was distinctly seen in the species of *Ciboria* that grows on male Birch catkins. In this *Ciboria* a new septum was seen to bulge forward slightly about six minutes after it had begun to form and immediately after it had completed its development. The bulging forward of newly formed septa is not so marked in the *Ciboria* as it is in *Rhizoctonia solani* ; but, once again, in the *Ciboria*, just as

in *R. solani*, it was found that protoplasm flows through the pore of a bulged septum toward the hypha's growing point.

**The Passage of Nuclei through the Haploid Mycelium of Hymenomycetes during the Diploidisation Process.**—In Volume IV of this work, I showed that, in *Coprinus lagopus*, when a large haploid mycelium 6 cm. in diameter is becoming diploidised by a small haploid mycelium of opposite sex, nuclei pass out of the small mycelium and through the large mycelium from one side to the other in the course of three or four days.<sup>1</sup> Since such a large mycelium has its hyphae divided up into cells by thousands of septa, this question arises: how do nuclei of opposite sex pass from cell to cell along the hyphae which they are diploidising? To consider a concrete case, let us suppose that the haploid mycelium becoming diploidised is (*AB*) and that the nuclei diploidising its hyphae have come from a haploid mycelium (*ab*). Then our problem is: how do (*ab*) nuclei pass from cell to cell along the (*AB*) hyphae?

Lehfeldt,<sup>2</sup> as a result of a cytological investigation upon *Typhula erythropus*, states that, in a haploid mycelium that was becoming diploidised, he observed a number of septa which had been dissolved on one side and reduced to "three-quarter" or "half" septa, thus permitting nuclei to pass through them; and, in his Text-fig. 1 *a*, he shows individual nuclei drawn-out and, as it were, in the act of threading themselves through the hole in a partially-dissolved septum. He also states that the lateral wall of a hypha at the side of a partially-dissolved septum was sometimes bulged outwards slightly, as if to increase the width of the septal opening and thus to facilitate the movement of a nucleus when passing through it.

If Lehfeldt's observations were correctly made, the answer to our question concerning the diploidisation of an (*AB*) mycelium of *Coprinus lagopus* by (*ab*) nuclei would be as follows. An (*ab*) nucleus, on approaching a septum in an (*AB*) hypha, in some way causes the septum to break down partially or causes the (*AB*) hypha to bulge and so leave a space between the septum and the

<sup>1</sup> These *Researches*, Vol. IV, 1931, pp. 213-229.

<sup>2</sup> W. Lehfeldt, "Über die Entstehung des Paarkernmyzels bei heterothallischen Basidiomyceten," *Hedwigia*, Bd. LXIV, 1923, pp. 13-51.

cylindrical wall ; a passage-way having thus been made, the (*ab*) nucleus passes from one cell to the other ; and this mode of making and using a passage-way is employed in respect to each of a long series of septa which must be passed through or passed by as the (*ab*) nucleus moves along (*AB*) hypha several centimetres in length.

When watching haploid hyphae of *Coprinus lagopus* being diploidised,<sup>1</sup> I never once observed a bulging of hyphae at a septum which had to be passed by a nucleus, nor did I see any septa disappear.

In my Volume IV, in discussing the diploidisation process, I accepted Lehfelddt's conclusions ; but now, as a result of my own observations upon, and a study of the literature upon, the structure of septa in the Higher Fungi, I feel that Lehfelddt's work needs verification before it can be accepted.

The work of Wahrlich and others (already reviewed), supported by my own observations, has taught us that there is an open pore in the middle of each septum, not only in Ascomycetes, but also in Hymenomycetes such as *Coprinus atramentarius*, *Merulius lacrymans*, and *Rhizoctonia solani* (= *Corticium solani*) (Figs. 51, 52, and 74, pp. 95, 96, and 145). If cytoplasm can pass through these septal pores there seems to be no reason why nuclei should not pass through also. Passage of a nucleus through an open pore would be the line of least resistance ; and I now wish to offer the suggestion that, during the diploidisation process in the Hymenomycetes, the open pores are actually used for the passage of nuclei from one cell to another.

We know that, in a basidium, the four haploid nuclei resulting from the division of the fusion nucleus pass up from the basidium-body through the exceedingly narrow necks of the four sterigmata (*cf.* Fig. 75, p. 148) into the four spores. During their passage the nuclei become extraordinarily constricted and drawn-out. Since nuclei can pass through sterigmatic necks, *a priori* there seems to be no reason why they should not pass through septal pores during the diploidisation process.

<sup>1</sup> W. Lehfelddt, "Über die Entstehung des Paarkernmyzels bei heterothallischen Basidiomyceten," *Hedwigia*, Bd. LXIV, 1923, pp. 237-241.



## PART II

THE PRODUCTION AND LIBERATION OF SPORES IN  
CERTAIN NON-HYMENOMYCETOUS BASIDIOMYCETES



## CHAPTER I

### SPOROBOLOMYCES, A BASIDIOMYCETOUS YEAST-GENUS

Introduction—Cultures—Spore-deposits—Method of Observing the Development and Discharge of the Spores—Observations on the Development of Sporobolomyces Colonies—The Production and Violent Discharge of the Spores—Abnormal Drop-excretion—The Successive Production of Spores on One and the Same Sterigma—The Successive Production of Spores on Two or More Sterigmata—Nuclear Phenomena—The Taxonomic Position of Sporobolomyces

**Introduction.**—In 1924, Kluver and van Niel<sup>1</sup> gave the name *Sporobolomyces* to certain yeast species which have the peculiarity of producing conidia at the surface of the culture medium and of violently discharging them into the air; and these authors described the genus *Sporobolomyces* as follows:

“Red or salmon-pink yeast-like organisms which multiply themselves by budding. The metabolism is altogether of an oxidative kind, fermentation not taking place. A part of the cell produces on well-formed aerial sterigmata typical kidney-shaped or sickle-shaped spores which, when ripe, are discharged into the air by means of a peculiar mechanism.”<sup>2</sup>

Kluver and van Niel placed three species of yeasts in *Sporobolomyces*, namely, *S. salmonicolor*, *S. roseus*, and *S. tenuis*; and they distinguished these species from one another by criteria concerned with the size and shape of the yeast cells and conidia, colour, appearance of the colonies in culture media, relations with carbonaceous and nitrogenous food-materials, etc.

Previously to 1924, yeast species now included in *Sporobolomyces*

<sup>1</sup> A. J. Kluver and C. B. van Niel, “Über Spiegelbilder erzeugende Hefenarten und die neue Hefengattung *Sporobolomyces*,” *Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten*, Abt. 2, Bd. LXIII, 1924–25, pp. 1–20, Taf. I and II.

<sup>2</sup> *Ibid.*, p. 19.

had been observed by Lasché (1792), Fischer and Brebeck (1894), Janssens and Mertens (1903), Schimon (1911), and Hansen (1911). Whilst studying them, Fischer and Brebeck, Janssens and Mertens, and Schimon observed that, when a Petri-dish culture of any one of them is inverted, in the course of a few days a sharp picture of the inverted colony is gradually formed on the glass cover owing to the fall of certain cells from the colony, and they referred to this phenomenon as mirror-picture-formation (*Spiegelbilderzeugung*). Janssens and Mertens recognised that mirror-picture-formation is due to the fall of conidia which are produced on little pedicels.<sup>1</sup>

Kluyver and van Niel<sup>2</sup> cleared up the phenomenon of mirror-picture-formation by showing that the pedicel and conidium produced by a *Sporobolomyces* cell resemble respectively the sterigma and the basidiospore of one of the Hymenomycetes and that each conidium is discharged by a drop-excretion mechanism of the same nature as that found in the Hymenomycetes and the Uredineae.

In 1924, as a result of their observations, Kluyver and van Niel<sup>3</sup> suggested that the *Sporobolomyces* species are Basidiomycetes which may be included in the Hemibasidii. In 1926, Lohwag<sup>4</sup> published a paper called *Sporobolomyces—kein Basidiomyzet* in which he refused to accept Kluyver and van Niel's suggestion that *Sporobolomyces* is of basidiomycetous origin. In 1927, Kluyver and van Niel<sup>5</sup> replied to Lohwag in a second paper called *Sporobolomyces—ein Basidiomyzet?*, in which they maintained that there is no evidence that *Sporobolomyces* is of ascomycetous origin and that the question whether or not this genus is basidiomycetous is still an open one. Finally, in 1927, Guilliermond<sup>6</sup> announced the results of his cytological and taxonomic study of *Sporobolomyces*. He found

<sup>1</sup> For a fuller account of the history of *Sporobolomyces* yeasts previous to 1924 and for references to the literature cited in this paragraph *vide* Kluyver and van Niel, *loc. cit.*, pp. 1-5.

<sup>2</sup> A. J. Kluyver and C. B. van Niel, *loc. cit.*, pp. 12-17.

<sup>3</sup> *Ibid.*, pp. 19-20.

<sup>4</sup> H. Lohwag, "Sporobolomyces—kein Basidiomyzet," *Annales Mycologici*, Vol. XXIV, 1926, pp. 194-202.

<sup>5</sup> A. J. Kluyver and C. B. van Niel, "Sporobolomyces—ein Basidiomyzet?" *Annales Mycologici*, Vol. XXV, 1927, pp. 389-394.

<sup>6</sup> A. Guilliermond, "Étude cytologique et taxinomique sur les Levûres du genre *Sporobolomyces*," *Bull. Soc. Myc. France*, T. XLIII, 1927, pp. 245-258.

that the yeast cells in all stages of their development contain but a single nucleus and never exhibit karyogamy, so that in this respect they differ from typical basidia ; and he concluded : (1) that there is no cytological justification for placing the *Sporobolomyces* yeasts in the Hemibasidii ; (2) that these yeasts deserve a special place in the classification of fungi ; and (3) that their affinities are still unknown.

In 1930, Derx<sup>1</sup> divided the yeasts which produce and violently discharge conidia into two genera, *Sporobolomyces* and *Bullera* ; and he placed these genera in a new family which he called the *Sporobolomycetes*. The species included in *Sporobolomyces*, *S. alborubescens*, *S. gracilis*, *S. odoratus*, *S. roseus*, *S. salmoneus*, *S. salmonicolor*, and *S. tenuis*, are rosy, red, or flesh-coloured and have reniform, sickle-shaped, or pyriform spores which are asymmetrical ; whereas the species included in *Bullera*, *B. alba* and *B. grandispora*, are colourless, creamy, yellowish, or brownish, without any trace of red, and have rounded, ovoid, or globular spores which are symmetrical.<sup>2</sup> *Sporobolomyces salmoneus* in culture gave rise to a colourless mutation, *S. salmoneus* var. *albus*. Several species are markedly mucilaginous, but this character may disappear in culture. As a means for differentiating and determining the known species of *Sporobolomycetes*, Derx has provided a key.<sup>3</sup>

Among Derx's<sup>4</sup> general observations on the *Sporobolomycetes* are the following. The *Sporobolomycetes* are very widely distributed in nature and they are essentially epiphyllous. They occur on damaged leaves, on straw, on grains of wheat, etc., and on stems covered with sugary exudations ; and, in particular, they are nourished by honey-dew. Consequently, they are often in the company of leaf-parasites and of sooty moulds such as *Dematium pullulans* and *Cladosporium herbarum*. To obtain species of *Sporobolomycetes* in culture one has only to suspend moist leaves or

<sup>1</sup> H. G. Derx, " Étude sur les *Sporobolomycètes*," *Annales Mycologici*, Vol. XXVIII, 1930, pp. 1-23, Plate I.

<sup>2</sup> The spores of both *Sporobolomyces* and *Bullera* develop asymmetrically on their sterigmata (cf. this Chapter, Fig. 90, nos. 2-5). The terms asymmetrical and symmetrical, as employed by Derx, refer to the shape of the mature spores detached from their sterigmata.

<sup>3</sup> H. G. Derx, *loc. cit.*, pp. 20-21.

<sup>4</sup> *Ibid.*, pp. 2-5, 22.

stems, etc., above a Petri dish containing malt-agar; and then, if any of the species desired are present on the under side of the material, they will soon discharge some of their spores into the air and these will fall upon the agar and germinate there.

There can be but little doubt that certain species of Sporobo-



FIG. 84.—*Sporobolomyces roseus*. An S-shaped yeast colony on 2.5 per cent. malt-sugar in a Petri dish. Photographed 14 days after inoculation. Reduced to seven-eighths the natural size.

lomyces are of common occurrence in Western Canada. At Winnipeg, by means of the spore-fall method, in 1928, Hanna<sup>1</sup> repeatedly isolated from rusted wheat and oat straw *Sporobolomyces roseus* Kl. et v. N. and *S. albus* Hanna, now known as *Bullera alba* (Hanna) Derx. In 1929, he<sup>2</sup> found *S. salmonicolor* occurring as a contamination in a plate culture of a Pleospora; and, in 1932, he

<sup>1</sup> G. R. Bisby, A. H. R. Buller, and J. Dearness, *The Fungi of Manitoba*, London, 1929, p. 80. Here *S. albus* is described by W. F. Hanna as a new species.

<sup>2</sup> W. F. Hanna, Dominion Rust Research Laboratory, personal communication.

again obtained *S. roseus*, on this occasion from Water Lily leaves gathered at Kenora on the Lake of the Woods.

In 1925, Kluyver and van Niel, at my request, kindly sent me cultures of *Sporobolomyces roseus*, *S. salmonicolor*, and *S. tenuis* so that I have been able to study the production and liberation of the



FIG. 85.—*Sporobolomyces roseus*. Spore-deposit made from the yeast colony shown in Fig. 84 by inverting the Petri dish for a few days. To prevent condensation of water-vapour, the base of the dish was raised above the cover by means of two glass slides placed on opposite sides of the cover. The whole was then covered with a large crystallising dish. The spore-deposit is S-shaped and is a mirror-picture of the colony which produced it. Photographed by reflected sunlight against a black background. Reduced to six-sevenths the natural size.

spores of these fungi at first hand. My observations on the drop-excretion mechanism confirm those of Kluyver and van Niel. These authors<sup>1</sup> stated that only one spore is produced on each sterigma as in the Hymenomycetes; but, by watching single yeast cells for

<sup>1</sup> A. J. Kluyver and C. B. van Niel, *loc. cit.*, p. 18.

many hours continuously, I have obtained clear evidence that one and the same sterigma often produces not one spore only, but two or three, or possibly four, spores in succession. My cytological investigations, completed before Guilliermond's paper came to hand, confirm his findings in respect to the nuclear condition of the yeast cells and spores. An account of my own observations, which have been carried through with the assistance of Miss Ruth Macrae, will now be given.

**Cultures.**—The species chosen for special investigation was *Sporobolomyces roseus*. It was cultivated on 2·5 per cent. malt-agar in Petri dishes, and in the course of a few weeks the colonies, which are bright pink-red in colour, spread widely over the plates. The yeast cells duly produced sterigmata and discharged numerous spores.

New Petri-dish cultures were made from old ones, often by the following method. The base of a Petri dish containing malt-agar bearing a *S. roseus* colony was inverted over the base of another Petri dish containing sterilised malt-agar. The conidia discharged from the sterigmata into the air fell downwards on to the agar in the lower plate and thus sowed themselves on the new culture medium.

**Spore-deposits.**—Spore-deposits can be collected readily from colonies of *Sporobolomyces* species, as already known to previous workers, by simply inverting a Petri dish in which a vigorous colony is growing on malt-agar. The spores, on being discharged, then fall on to the glass cover and there, in the course of a few days, form a mirror-picture of the colony. An S-shaped colony of *S. roseus* and its mirror-picture spore-deposit are shown in Figs. 84 and 85 respectively. A spore-deposit is white and the individual spores in it are colourless.

**Method of Observing the Development and Discharge of the Spores.**—In *Sporobolomyces*, just as in the Hymenomycetes, the Uredineae, and *Tilletia*, it is somewhat difficult to observe the development and discharge of the spores, owing to the fact that it is necessary to use the high power of the microscope and make continuous observations for some hours. If the yeast cells are kept in a small closed chamber, the air soon becomes saturated with water vapour, and then the spores are often not discharged in a

normal manner. The drop excreted from the spore-hilum may grow abnormally large, run up the side of the spore and more or less enclose the spore, and then the spore is not discharged from its sterigma. The drop may then dry up and, later, a second spore may be developed on the end of the same sterigma. Then there are

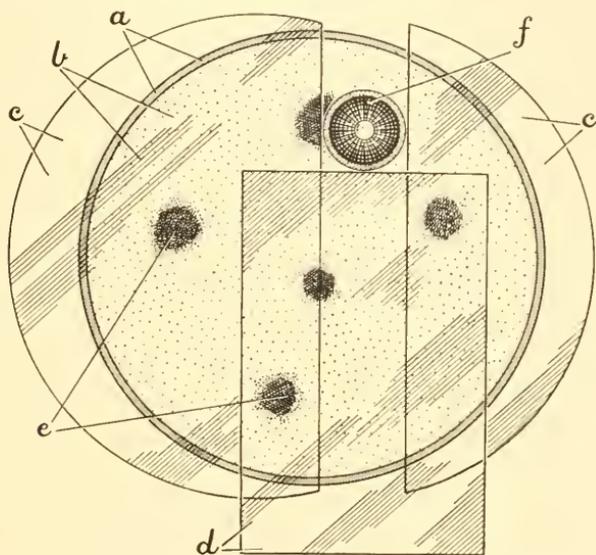


FIG. 86.—Apparatus used for observing the development of yeast colonies of *Sporobolomyces roseus*; the Petri dish *a* contains malt-agar *b* and is covered almost completely by a split glass plate *c* and by a rectangular glass plate *d*. Scattered in the agar are several yeast colonies *e*, and to the edge of one of them the objective of the microscope is applied, as shown at *f*. Drawn by A. H. R. Buller and Ruth Macrae. Reduced to about two-thirds the natural size.

two spores sticking together on the end of one and the same sterigma. This is clearly an abnormality due to unsuitable atmospheric conditions.

To provide ventilation and, at the same time, to prevent undue desiccation of the culture medium and yeast cells whilst observations were being made with the high-power objective, the following method was employed.

*Sporobolomyces roseus* was grown on malt-agar in a Petri dish in the usual way (Fig. 86, *e*). Spores were shot upwards from the older

yeast cells, and some of them fell on unoccupied agar at the periphery of the yeast colony and there budded and initiated the formation of new colonies. These new small colonies, which soon began to produce sterigmata and spores on their own account, proved very suitable for observation. The Petri-dish cover was removed and replaced by a glass plate which had been cut across into two unequal parts (Fig. 86, *c c*). The two pieces of the glass plate were placed over the culture-dish right and left of the yeast colony which was to be observed with the microscope. Then the yeast colony was brought into focus with the high power of the microscope, whereupon most of the gap between the two pieces of glass plate was covered over with a third piece of glass (Fig. 86, *d*). At intervals between observations, the objective of the microscope was raised and the two pieces of the plate were pushed together so as to completely cover the culture-dish. The temperature of the laboratory was usually about 24° C. With the apparatus arranged as just described, the development of certain yeast cells was successfully watched for 10 or more hours (in one instance for 24 hours) before it became appreciably abnormal.

**Observations on the Development of *Sporobolomyces* Colonies.**—

In Fig. 87, *a*, is shown a spore which had been shot away from its sterigma and had settled at a distance from the parent cell on the surrounding malt-agar of the same plate. Stages in its germination and in the production of a colony of 13 yeast cells in the course of 22 hours are shown from *b* to *m* in the same Figure. It will be observed that the spore produced two buds, one from each end, and that these daughter buds budded in their turn. In the last stage shown, drawn 22 hours after the first stage, it will be seen that three of the yeast-cells are producing sterigmata. These sterigmata, doubtless, produced and discharged spores in the course of the next two hours; but, in this particular case, spore-formation was not followed. The appearance of a spore on one of the sterigmata, as it might have appeared in side view, is indicated semi-diagrammatically in Fig. 87 at *n*.

Spores behave somewhat differently according to whether they are sown on (1) a fresh medium or (2) an old medium.

(1) When a spore germinates on agar in a new plate, it and its

progeny develop a colony of very numerous yeast cells before any

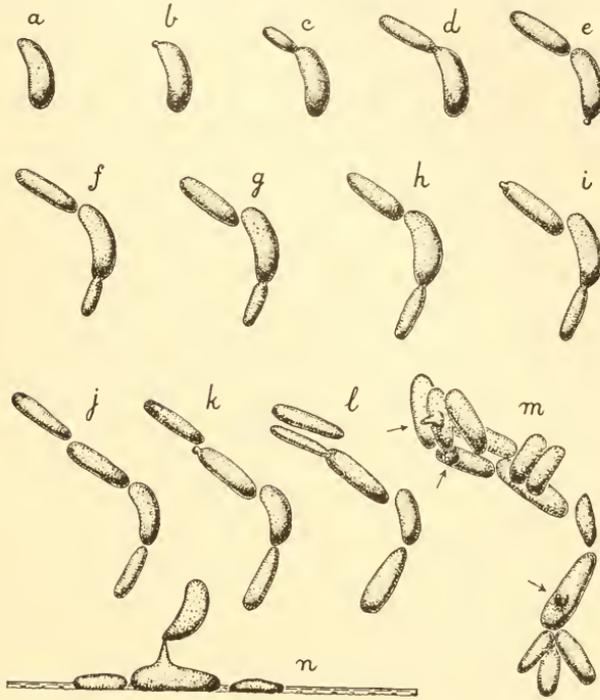


FIG. 87.—*Sporobolomyces roseus*. At *a*–*m*, successive stages in the development of a yeast colony from a spore in the course of 22 hours on the surface of malt-agar; *a*, a spore which fell on the agar; *b*, after 1 hour 10 minutes; *c*, after 1 hour 40 minutes; *d*, after 3 hours 10 minutes; *e*, after 4 hours 30 minutes; *f*, after 5 hours; *g*, after 5 hours 30 minutes; *h*, after 5 hours 45 minutes; *i*, after 6 hours 15 minutes; *j*, after 8 hours; *k*, after 8 hours 45 minutes; *l*, after 10 hours; and *m*, after 22 hours. The arrows in *m* point to yeast cells which have developed a sterigma and are about to produce a spore. At *n* three of the lower cells of the colony *m* are represented semi-diagrammatically in side view as they would have appeared about 2 hours after *m* was drawn; the cells are partly embedded in agar; the sterigma now bears a mature spore like that shown at *a*; the spore is aerially situated and shortly would be discharged. The right-hand cell is shrunken; it seems to have died between the stages *k* and *m*. Total time from stage *a* to stage *n* is 24 hours. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 756.

of the yeast cells produce sterigmata and spores, and spore-production takes place in what may be considered as a normal manner. The

yeast cells remain oval or ellipsoid in form and do not become branched like hyphae or irregular in shape, and the sterigmata are short, conical, simple and unbranched, like the sterigmata on the basidia of the Hymenomycetes.

(2) When a spore is produced from a yeast cell at the edge of

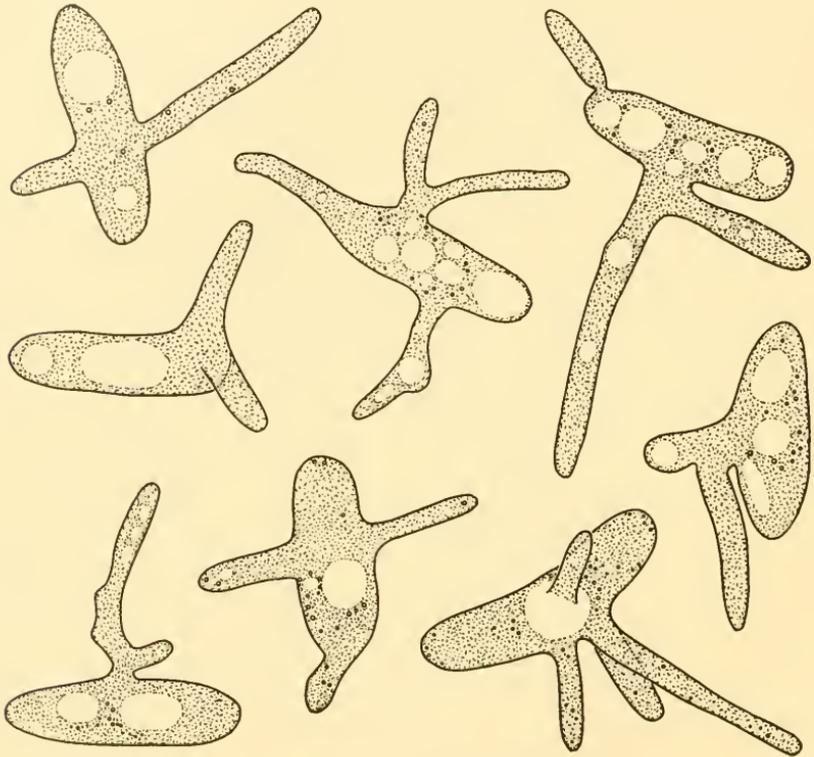


FIG. 88.—*Sporobolomyces roseus*. Yeast cells of rather large size which, in an old malt-agar culture, have produced hypha-like outgrowths of limited growth in length. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2234.

a large colony on malt-agar in a Petri dish and is shot upwards so as to fall on to the agar close to the colony, it germinates on the agar, but it and its progeny show evidence of being affected by the exhaustion of the medium or by staling products; for (a) it and its progeny form only a relatively small colony of yeast cells before the yeast cells begin to produce fresh spores, and (b) some or

many of the yeast cells, instead of retaining the oval or ellipsoid form, become elongated like hyphae or variously irregular in form (Fig. 88). Moreover, the sterigmata often become abnormally elongated, two or more in number instead of one only, and frequently bifurcated (Fig. 89). In very exhausted media, presumably rich in staling substances, a spore, when germinating, gives rise not to a yeast cell but to another spore (Fig. 97, p. 193).

**The Production and Violent Discharge of the Spores.**—The normal method of spore-production and spore-discharge, which was first observed by Kluyver and van Niel, is illustrated in Fig. 90, nos. 1–8. The sterigmata are produced by yeast cells only when the cells are in contact with the air, and every sterigma grows away from the substratum into the air. A sterigma, after beginning its development, becomes fully formed in 30–50 minutes, at the end of which time a spore begins to develop at its summit (Fig. 90, no. 3). At first the spore is a minute spherical body but, with further development, it becomes asymmetrically situated on the end of the sterigma just as do the basidiospores of the Hymenomycetes, the Uredineae, and Tilletia. The development of a spore from its first rudimentary beginning to full size takes about 30 minutes (Fig. 90, nos. 3–5). After full size has been attained, the spore remains on its sterigma for 30–50 minutes and is then violently discharged. Just before discharge, a drop of liquid appears at the hilum of the spore and in 3–5 seconds its diameter increases until it is about equal to that of the spore (Fig. 90, nos. 6 and 7). The spore and the drop are then suddenly shot away together (Fig. 90, no. 8). In one instance, when the sterigma was inclined away from the vertical, the spore after being shot into the air settled on the surface of the agar at a horizontal distance of 0.11 mm. from the parent

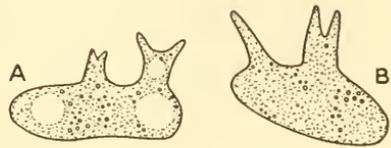


FIG. 89.—*Sporobolomyces roseus*. Yeast cells with branched sterigmata, from an old malt-agar culture. A, with two branched sterigmata, which presumably produced four spores in succession, one from each point. B, with one simple and one branched sterigma, which presumably produced three spores in succession, one from each point. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2234.

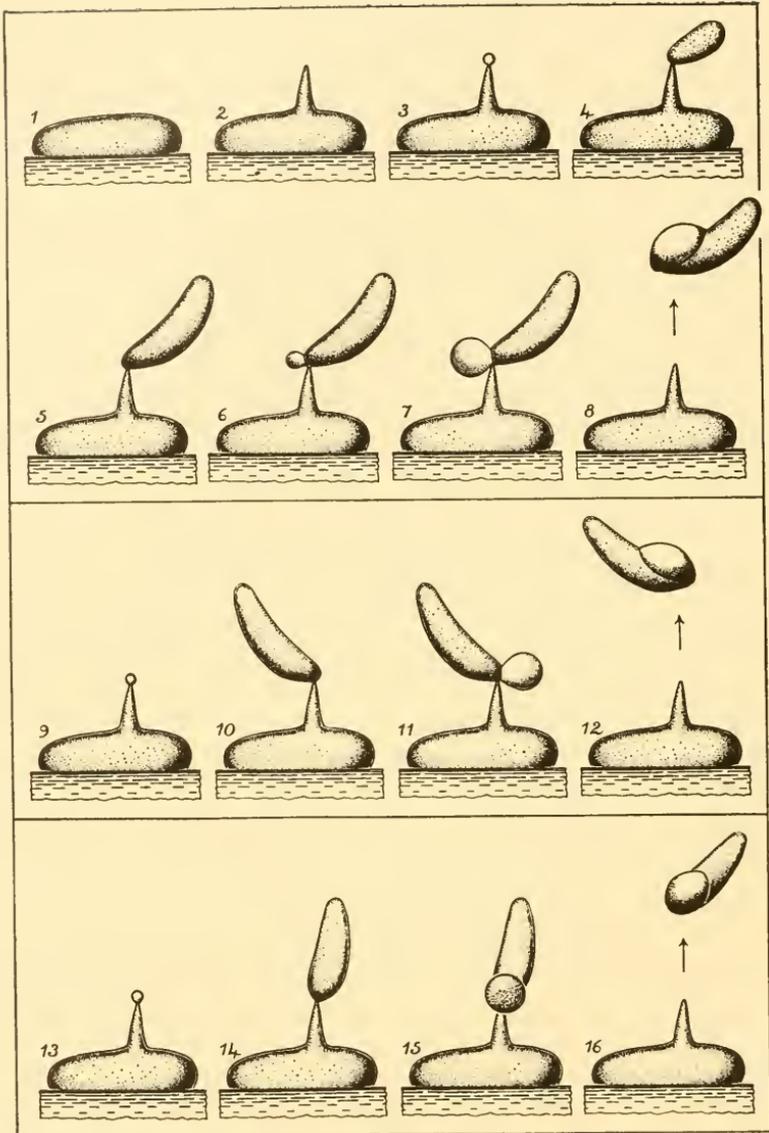


FIG. 90.—*Sporobolomyces rosceus*. Production of three spores successively on one and the same sterigma, represented in lateral view semi-diagrammatically. The yeast cell is on malt-agar. Nos. 1-8, stages in the development of the sterigma and the first spore; nos. 9-12, stages in the development of the second spore; and nos. 13-16, stages in the development of the third spore. Drop-excretion at the spore-hilum prior to spore-discharge is shown: for the first spore at nos. 6 and 7; for the second spore at no. 11; and for the third spore at no. 15. Nos. 8, 12, and 16 show the discharge of the first, second, and third spores respectively. The drop and the spore are shot away together. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 1564.

yeast cell; and other observations go to show that the spores are discharged from their sterigmata to a vertical or horizontal distance of about 0.1 mm., *i.e.* the same distance as observed by Kluver and van Niel<sup>1</sup> for *S. salmonicolor* and the same distance as observed by myself<sup>2</sup> for *Psalliota campestris* and many other Hymenomyces.

There can be no doubt that the production and liberation of a spore in *Sporobolomyces roseus*, as so far described, in all essentials exactly resembles the production and liberation of a basidiospore on one of the sterigmata of a basidium in the Hymenomyces, the Uredineae, and the genus *Tilletia*. In this connexion the reader should compare with Fig. 90, nos. 1-8, the illustrations for the production and liberation of spores given for *Calocera cornea*<sup>3</sup> and *Psalliota campestris*<sup>4</sup> in Volume II, for *Puccinia graminis*<sup>5</sup> and *Endophyllum Euphorbiae-sylvaticae*<sup>6</sup> in Volume III, and for *Tilletia tritici*<sup>7</sup> in this Volume V.

**Abnormal Drop-excretion.**—Normally, the drop which is excreted from the hilum of a spore of *Sporobolomyces roseus* just before the spore is discharged attains a diameter about equal to that of the spore (Fig. 90, nos. 7, 11, and 15). However, under unfavourable cultural conditions it sometimes happens that the drop, after attaining the usual maximum size (Fig. 91, *b*), continues to grow, runs up the spore (*c*), and finally envelops the spore completely (*d*). This abnormally large drop within a minute or two enlarges so much that its volume much exceeds that of the plant which has excreted it. Its maximum size is indicated in Fig. 91, *d*. Whenever abnormal drop-excretion like that just described takes place, the spore is not discharged. Similar observations were made by Kluver and van Niel<sup>8</sup> in the course of their investigations on *Sporobolomyces salmonicolor*.

<sup>1</sup> A. J. Kluver and C. B. van Niel, *loc. cit.*, p. 13.

<sup>2</sup> These *Researches*, Vol. I, 1909, p. 142. Cf. Fig. 65, p. 186.

<sup>3</sup> These *Researches*, Vol. II, 1922, p. 7.

<sup>4</sup> *Ibid.*, p. 12.

<sup>5</sup> These *Researches*, Vol. III, 1924, pp. 503, 505.

<sup>6</sup> *Ibid.*, p. 511.

<sup>7</sup> *Vide infra*, Fig. 108, p. 222, and Fig. 114, p. 232.

<sup>8</sup> A. J. Kluver and C. B. van Niel, *loc. cit.*, p. 16, Taf. I, Fig. 12.

Abnormal drop-excretion like that shown in Fig. 91 occurs in the Hymenomycetes,<sup>1</sup> the Uredineae,<sup>2</sup> and *Tilletia* (Fig. 118, p. 243), and this fact goes far to strengthen the view that the mechanism of spore-discharge in *Sporobolomyces* is identical with the mechanism of basidiospore-discharge in the Basidiomycetes in general.

**The Successive Production of Spores on One and the Same Sterigma.**—A yeast cell of *Sporobolomyces roseus*, which was filled

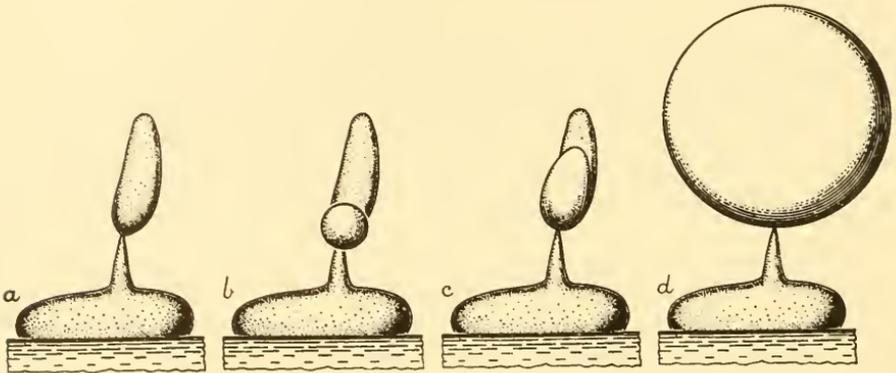


FIG. 91.—*Sporobolomyces roseus*. Abnormal excretion of a very large drop of fluid from the spore-hilum and non-discharge of the spore. At *a*, lateral view of a yeast cell on agar, which has produced a sterigma and a spore; the spore is mature. At *b*, a drop of normal size has just been excreted from the spore-hilum; the spore and drop should now be discharged, but are not. At *c*, the drop has grown beyond the usual size and has ascended on to the spore. At *d*, the drop has increased in size to such a degree that it now forms a spherical globule which encloses and hides the spore and is larger than the whole yeast plant. The spore was not discharged. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 1866.

with protoplasm, was watched as it developed its sterigma and spore (*cf.* Fig. 90, nos. 1–8). During this process it became somewhat vacuolated; but, at the moment when the spore was violently discharged, a considerable amount of protoplasm was still left in the parent cell. The parent cell, after the discharge of the spore, was kept under continuous observation. Its vacuoles were seen to decrease in size, doubtless in consequence of new protoplasm being

<sup>1</sup> *Vide* these *Researches*: for *Psalliota campestris*, Vol. II, Fig. 8 (p. 18); for *Panaeolus campanulatus*, Vol. II, Fig. 104 (p. 308); and for *Coprinus sterquilinus*, Vol. III, Fig. 106 (p. 250).

<sup>2</sup> *Vide* these *Researches*: for *Puccinia graminis*, Vol. III, Fig. 205, A (p. 508).

formed at the expense of the culture medium. About two hours after the first spore had been discharged, a *second spore began to be developed on the end of the sterigma* (Fig. 90, no. 9), and this grew in the normal manner to full size (Fig. 90, no. 10). Then, after the usual interval of time, this second spore was discharged in its turn (Fig. 90, nos. 11 and 12). About an hour and forty minutes after the second spore had been discharged, a *third spore began to be developed on the end of the sterigma*. This third spore developed in the usual manner to full size and its hilum excreted the usual drop preparatory to spore-discharge (Fig. 90, nos. 13–15). However, owing to some abnormality, doubtless due to the culture conditions, discharge did not take place.

A number of other yeast cells were observed in which one and the same sterigma produced and discharged two spores in succession; but, owing to the difficulty of providing suitable conditions for the yeast cells when they are being observed under the high power of the microscope for many hours in succession, the maximum number of spores which a single sterigma may produce was not precisely determined; but, from the observations just recorded, we may conclude that a healthy *S. roseus* yeast cell growing under good conditions normally produces on a single sterigma not one spore only as Kluyver and van Niel<sup>1</sup> supposed, but two, three, or possibly four or more spores.

In the Hymenomyces and the Uredineae my own observations have convinced me that only one spore is produced on each sterigma and that, in the Hymenomyces, a basidium-body, along with the four sterigmata, collapses within 20–30 minutes after the moment of discharge of the last of the four spores<sup>2</sup> Sporobolomyces, in producing two, three, or possibly four or more spores on the end of one and the same sterigma, provides us with a phenomenon which does not find a parallel in any of the typical Basidiomycetes.

**The Successive Production of Spores on Two or More Sterigmata.**—While the production of a single sterigma only seems to be the rule for healthy Sporobolomyces yeast cells growing on a fresh culture medium, yeast cells growing on a more or less exhausted medium often develop two sterigmata and sometimes more.

<sup>1</sup> A. J. Kluyver and C. B. van Niel, *loc. cit.*, p. 18.

<sup>2</sup> These *Researches*, Vol. II, 1922, pp. 271–273, 355.

A yeast cell of *S. roseus*, which was observed continuously for several hours, developed three sterigmata and three spores. The

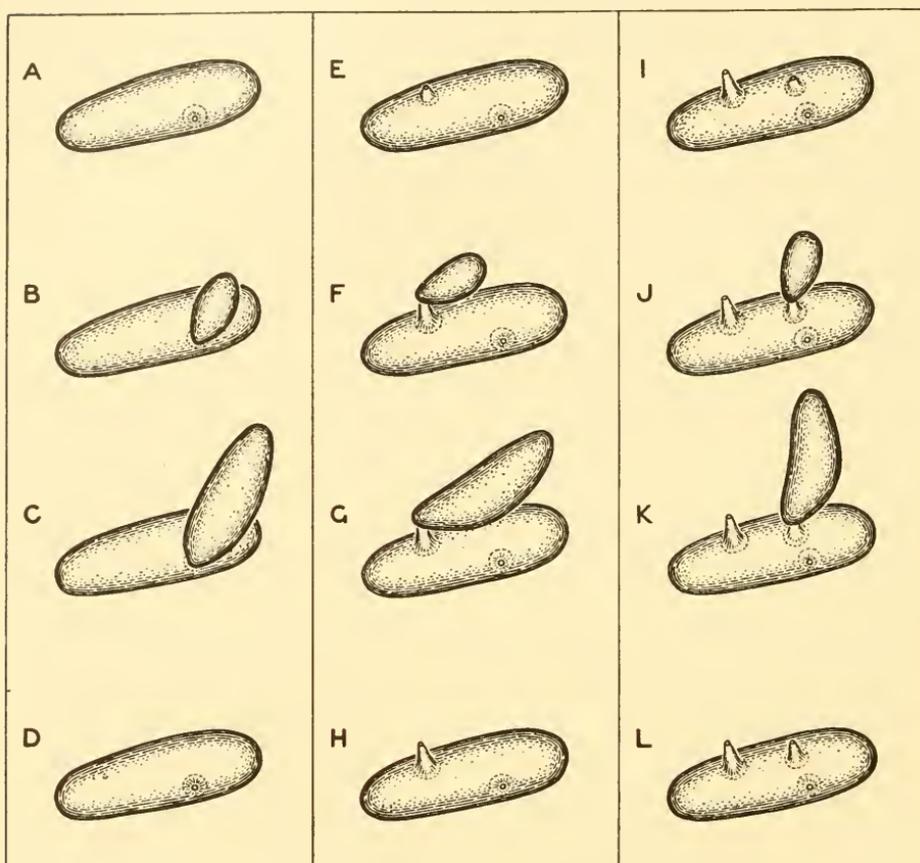


FIG. 92.—*Sporobolomyces roseus*. A yeast cell on malt-agar, seen from above, which produced three spores in succession, each spore on a different sterigma. A, the yeast cell which has just developed its first sterigma; B, the sterigma now bears a half-grown spore; C, the first spore is fully grown; D, the first spore has now been discharged and the sterigma can be seen again; E, a second sterigma is developing; F, the second spore is half-grown; G, the second spore is fully grown; H, the second spore has now been discharged and the first and second sterigmata can both be clearly seen; I, a third sterigma is developing; J, the third spore is half-grown; K, the third spore is fully grown; L, the third spore has now been discharged and the first, second, and third sterigmata are left behind *in situ*. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2100.

first sterigma produced and shot away a spore (Fig. 92, A-D); then, after an interval of about an hour, a second sterigma began to develop

which in due course produced and discharged a second spore (E-H); and then, after another interval of 2 hours 45 minutes, a third

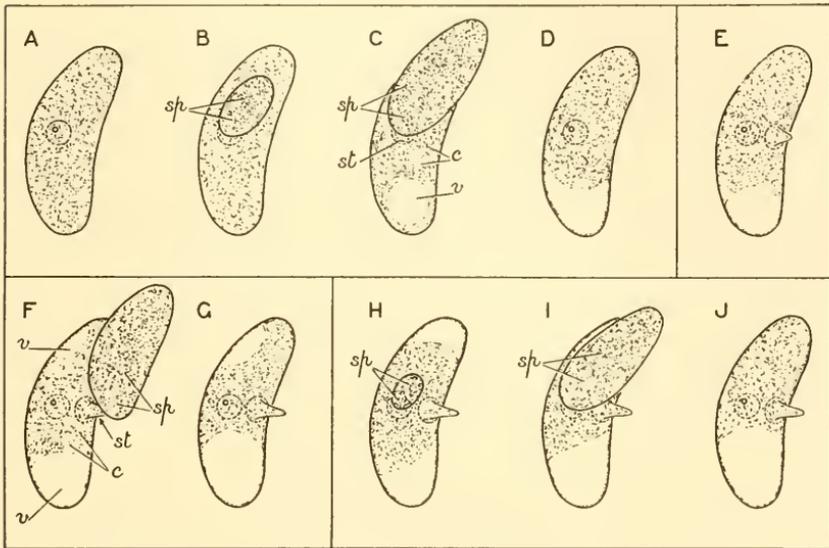


FIG. 93.—*Sporobolomyces roseus*. A spore which, after being shot away from a colony of yeast cells, fell on to an almost exhausted medium and which, instead of producing yeast cells in the usual way, gave rise in the course of about 7 hours to three new spores in succession. A–D, E–G, and H–J, the production and discharge of the first, second, and third spores respectively. A, the original spore, of large size, lying on the surface of the medium and seen from above: it has produced a central sterigma on its upper surface: the shading represents the cytoplasmic contents. B (40 minutes after A): the first new spore *sp* is developing at the top of the sterigma. C (70 minutes after A): the first new spore *sp* is now fully grown; *st*, the sterigma; *c*, the cytoplasm; *v*, a vacuole. D (90 minutes after A): the first new spore has been discharged and the sterigma is again in full view; the vacuole has altered its shape. E (2 hours and 10 minutes after A): a second sterigma has been formed on the right of the first; the vacuole has diminished in size and is rounder. F (four hours after A): the second new spore *sp* has been developed on the second sterigma *st*; *v v*, two vacuoles one at each end of the original spore; *c*, the cytoplasm. G (4 hours and 5 minutes after A): the second spore has just been discharged; the vacuoles have increased in size. H (5 hours and 50 minutes after A): the third new spore *sp* is developing on the first sterigma. I (6 hours and 25 minutes after A): the third new spore *sp* is now fully grown; the lower vacuole has increased in size again. J (7 hours after A): the third new spore has been discharged; the lower vacuole has increased in size. Three hours after the stage J, no other spore had been produced. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2100.

sterigma began to develop, which in its turn produced and discharged a third spore (I–L). Whether or not another sterigma or another spore was developed was not determined.

A spore of *S. roseus* on an almost exhausted culture medium behaved like a yeast cell in that it produced first one sterigma and

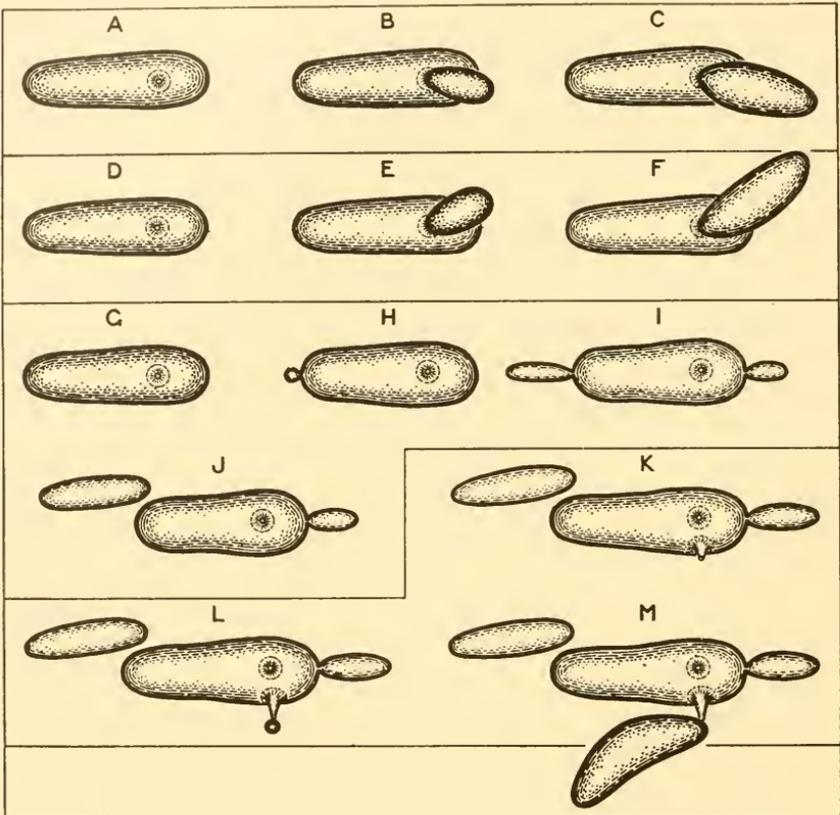


FIG. 94.—*Sporobolomyces roseus*. A yeast cell on malt-agar, seen from above, which produced in succession: (1) a sterigma and spore, as shown at A-C; (2) a second spore on the same sterigma as the first spore, as shown at D-F; (3) two buds, *i.e.* two new yeast cells, as shown at G-J; and, finally, (4) a second sterigma and a third spore, as shown at K-M. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2100.

spore (Fig. 93, A-D), then a second sterigma and spore (E-G) and, finally, a third spore on the first sterigma (H-J).

Another *S. roseus* yeast cell produced first of all a sterigma which developed and discharged two spores in succession (Fig. 94, A-G), then two buds one at each of its ends (H-J) and, finally, a second sterigma which developed a spore (K-M). In this instance the

parent cell budded in the interval between the production of its second and of its third spore.

Sometimes a sterigma becomes branched, and yeast cells with bifurcated sterigmata are shown in Fig. 89 (p. 181). The process of branching was not observed. Presumably a spore is produced at the tip of each sterigmatic branch, and it is therefore probable that of the yeast cells shown in Fig. 89 the one with four sterigmatic points produced four spores and the one with three sterigmatic points produced three spores. Guilliermond,<sup>1</sup> in an illustration, shows a cell of *S. salmonicolor* with two bifurcated sterigmata and four spores, a spore being situated on each of the four sterigmatic tips. It seems to me probable that the four spores were formed not simultaneously but in succession and that they were all found *in situ* by Guilliermond<sup>1</sup> because the unfavourable atmospheric conditions of his culture prevented any of the spores being discharged violently into the air.

I have never seen two spores developing on either a single sterigma or on two sterigmata of a yeast cell at one and the same time and have no reason to suppose that such a simultaneous development ever occurs in *Sporobolomyces*. We may therefore accept the view that, in *Sporobolomyces*, *whenever a cell produces several spores, the spores are always produced in succession.*

**Nuclear Phenomena.**—It seemed possible that the behaviour of the nuclei in the yeast cells when budding and producing spores might throw some light on the taxonomic position of the genus *Sporobolomyces*. On this account Miss Macrae and I, in 1927, without any knowledge of Guilliermond's observations and before his paper appeared in January, 1928, undertook a cytological investigation of *S. roseus*. An account of this investigation, the results of which confirm those of Guilliermond, will now be recorded.

Slides were dipped in malt-agar and placed in a Petri dish on a sheet of moist filter-paper, and then the base of another Petri dish containing a colony of *S. roseus* growing on malt-agar was inverted over the first Petri dish. After 15–20 minutes a sufficient number of spores had been discharged and had fallen on to the agar slides. The inverted base of the second Petri dish was then removed and replaced

<sup>1</sup> A. Guilliermond, *loc. cit.*, p. 251, Fig. 4.

by an ordinary Petri-dish cover. The spores soon germinated and gave rise to small yeast colonies which were suitable for investigation.

A slide bearing some colonies in the desired state of development was immersed in formalin acetic alcohol<sup>1</sup> for about eight hours and then, to remove the acetic acid and formalin, it was thoroughly washed in several changes of 50 per cent. alcohol. The alcohol was then removed by passing the slide through 35, 20, 15, 10 and 5 per cent. solutions of alcohol to pure water.

The staining of the yeast cells was accomplished as follows. The slides bearing the colonies growing on the agar film were placed in a 4 per cent. solution of iron-alum for 20 hours, washed in running water for an hour and a half, and transferred to 0·5 per cent. aqueous solution of haematoxylin. After lying in the haematoxylin for 20–24 hours, they were washed in running water for 10 minutes and then placed in a Petri dish in a 2 per cent. solution of iron-alum where they were watched under the microscope until the de-staining of the colonies had progressed sufficiently far. The slides were then washed in running water for an hour, after which they were placed in an open Petri dish containing 10 per cent. glycerine. The dish was covered by a sheet of filter-paper to protect it from dust. In the course of three days, the water in the glycerine gradually evaporated. As soon as evaporation was complete, the slides were removed from the glycerine and the stained colonies were mounted in Kayser's glycerine-jelly.

In successfully made preparations the nuclei could be readily observed as rounded bodies more deeply stained than the cytoplasm, each of them possessing a nuclear membrane and a conspicuous nucleolus.

Each yeast cell contains only a single nucleus, as may be seen in Fig. 95, A and B. When budding takes place, the young bud is at first devoid of a nucleus (C, *a*). Then the nucleus of the parent cell undergoes division and one of the daughter nuclei passes into the bud, the other remaining in the parent cell (C, *b–e*). In the rare instances in which a dividing nucleus was actually observed, the division appeared to be taking place at the neck joining the parent cell with its bud (C, *b, c*).

<sup>1</sup> Formalin 5 cc., acetic acid 5 cc., and alcohol 90 cc. of a 50 per cent. solution.

When a yeast cell is forming a spore on a sterigma, the young spore, like a young bud, is devoid of a nucleus (Fig. 96, *a-d*) ; but,

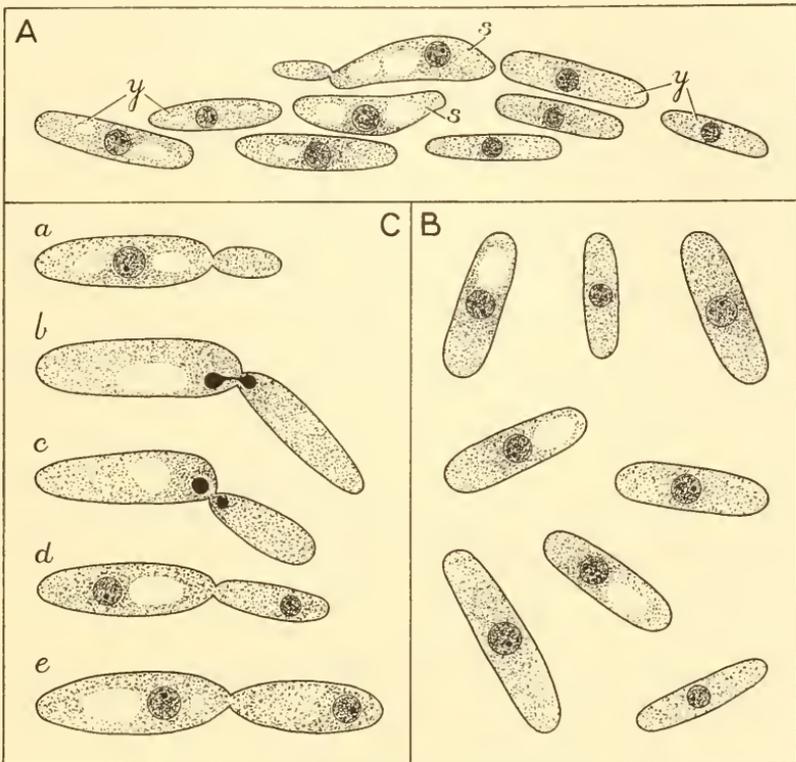


FIG. 95.—*Sporobolomyces roseus*. To show nuclei and the division of nuclei in the yeast cells. Cultures made on malt-agar. Cells fixed with formalin-acetic-alcohol and stained with iron-alum haematoxylin. A, a colony of yeast cells *y y* which have been produced from two spores *s s*. With the exception of a young bud attached to the uppermost spore, all the yeast cells, as well as the two spores, each contain a single nucleus. B, isolated yeast cells from various colonies. Each cell contains a single nucleus and some of the cells are vacuolated. C, the division of the nucleus in a budding yeast cell: *a*, the bud is as yet without a nucleus; *b*, the nucleus of the mother-cell has passed to the neck between itself and its bud and is there dividing; *c*, the nucleus has just divided into two daughter nuclei; *d*, the daughter nuclei have separated and now show their nuclear membranes and nucleoli; *e*, the bud is about to separate from its parent cell; its nucleus has grown in size. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2234.

when the spore has attained almost full size, the nucleus of the parent cell passes into the base of the sterigma and there divides (*c-h*). Of the two daughter nuclei one passes through the neck of the sterigma

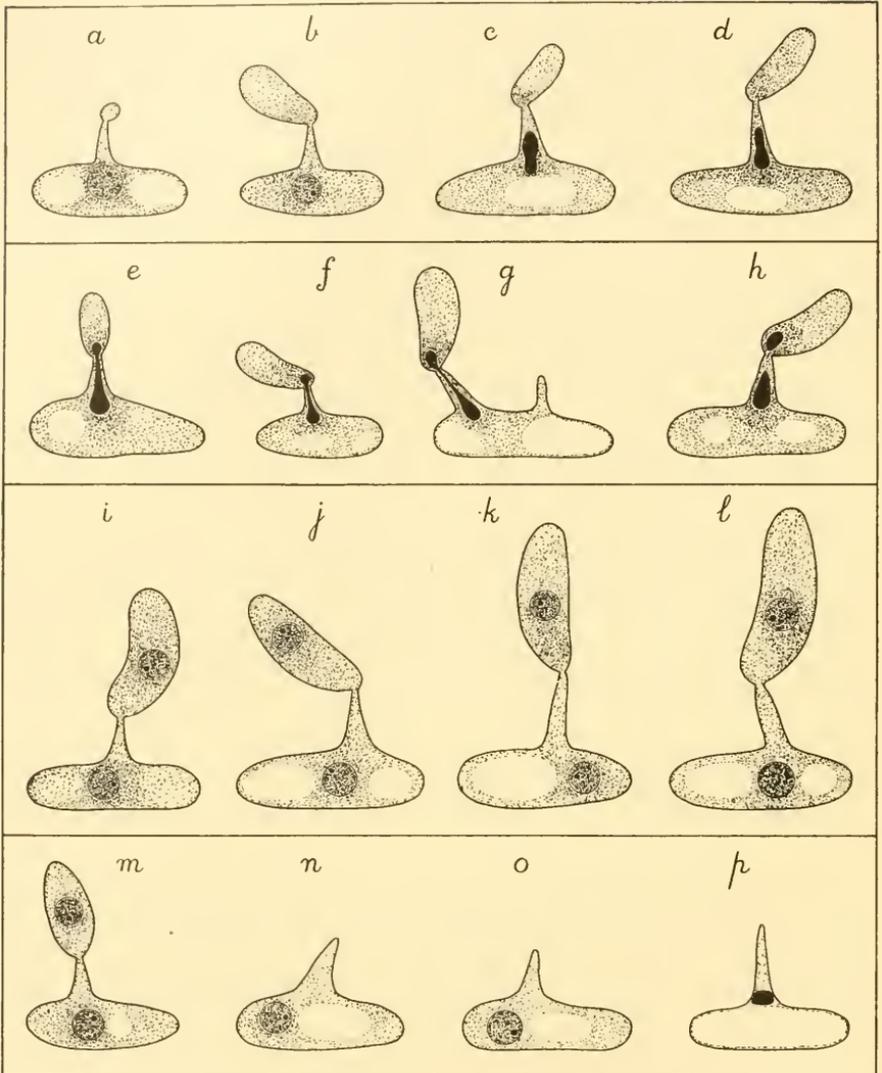


FIG. 96.—*Sporobolomyces roseus*. The nucleus during spore-production by a yeast cell. Cultures made on malt-agar. Cells fixed with formalin-acetic-alcohol and stained with iron-alum haematoxylin. *a*, a yeast cell with a sterigma on which a rudimentary spore has begun to develop; *b*, a similar yeast cell with an older spore; in both *a* and *b* the yeast cell contains a single nucleus and the spore no nucleus; *c-h*, a series of yeast cells bearing young spores, showing stages in the division of the nucleus which passes up into the sterigma and there becomes constricted into two daughter nuclei; *i-m*, a series of yeast cells bearing mature spores, the spore and the yeast cell each contain a single nucleus; *n* and *o*, two yeast cells after the discharge of the spore, each contains a nucleus; *p*, an old and exhausted yeast cell which may have discharged 2-4 spores, it now contains a large vacuole and a degenerate nucleus. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2234.

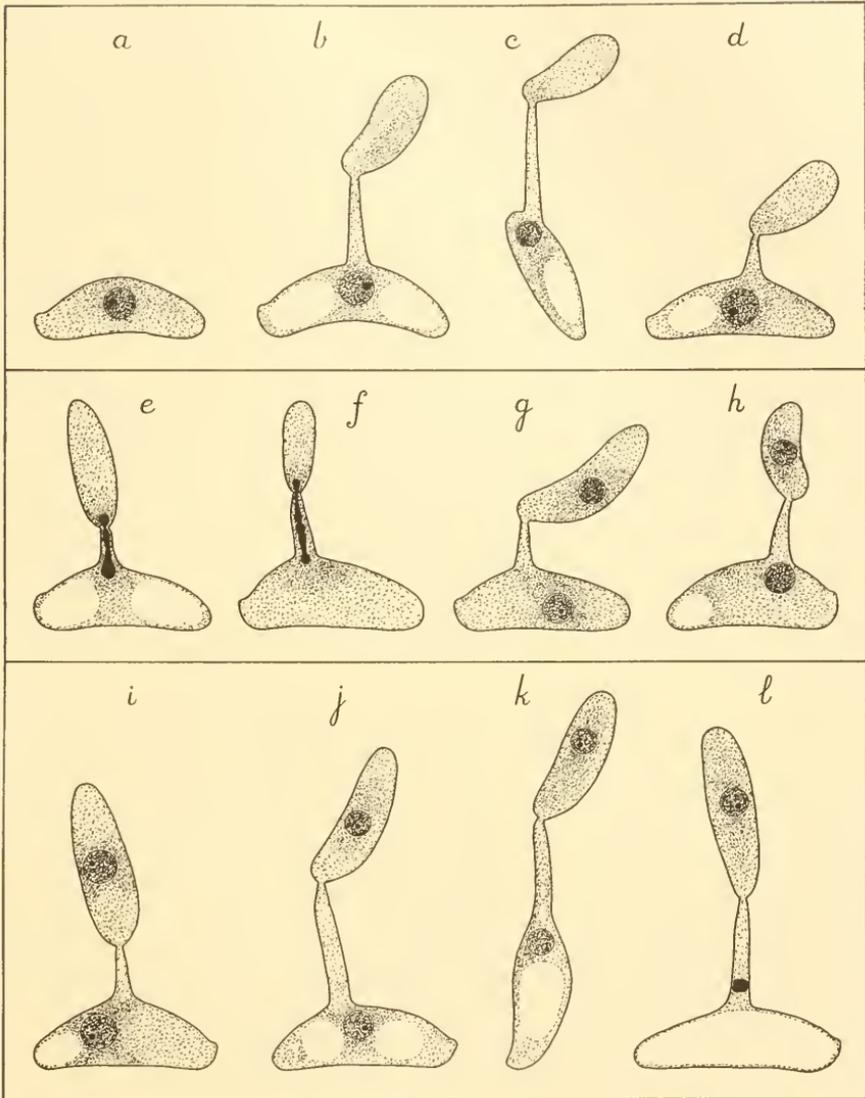


FIG. 97.—*Sporobolomyces roseus*. The nucleus during spore-production by a spore. Cultures made on malt-agar. Cells fixed with formalin-acetic-alcohol and stained with iron-alum haematoxylin. *a*, a spore, with its basal end to the left, containing a single nucleus; *b*, *c*, and *d*, three spores each of which has produced a conical sterigma on the end of which a new spore has developed, as yet the new spore is without a nucleus; *e* and *f*, the nucleus is dividing in the neck between the sterigma and the new spore; *g*, *h*, *i*, *j*, and *k*, the nucleus has divided so that there is now one nucleus in the old spore and another in the new spore; *l*, the old spore is highly vacuolated and its nucleus appears to be degenerate. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2234.

into the spore, while the other passes downwards into the parent cell (*h-o*). A spore, therefore, contains but a single nucleus.

When an old spore is forming a new spore on a sterigma, just as when a yeast cell is forming a spore, the young spore is devoid of a nucleus (Fig. 97, *b-d*) and, as soon as the new spore has attained almost full size, the nucleus of the parent spore passes into the sterigma and there divides (*e-f*). Of the two daughter nuclei one passes through the neck of the sterigma into the new spore, while the other passes downwards into the old spore (*g-l*). Again the newly formed spore contains but a single nucleus.

A number of recently discharged spores, each containing a single nucleus, are shown in Fig. 98, A. The nuclear phenomena attending the germination of a spore and the production of a bud are similar to those already described for the development of a bud from an ordinary yeast cell, *i.e.* the young bud is at first devoid of a nucleus (Fig. 98, B, *a*), the nucleus of the parent spore then passes to the junction of the spore and the bud and there divides (B, *b, c*) and, finally, one of the daughter nuclei passes into the centre of the bud while the other passes back into the main body of the parent spore (B, *d-f*).

The nuclear condition of certain abnormally developed yeast cells and spores is shown in Fig. 99. The cells *b, c*, and *d* in A have very long sterigmata but, as usual, there is one nucleus in the spore and one in the parent yeast cell. The cell *e* has a sterigma with three teeth or branches. Probably a spore was produced on each of the two lower teeth. The spore now on the third tooth, like the parent cell, has the normal single nucleus. In Fig. 99, B, are some spores producing spores. The old spore *b* has two sterigmata. From one sterigma a spore has doubtless been discharged. The spore borne on the other sterigma, like the parent spore, as usual contains but a single nucleus. The old spore *c* bears three sterigmata and contains a single nucleus. Probably the sterigmata were developed in succession and three spores were produced and discharged in succession. If so, the nucleus of the old spore underwent three successive divisions. At *d* is shown an old spore with a branched sterigma. Probably the sterigma was at first simple and produced and discharged a spore from the tooth now sporeless, and the

sterigma then branched. The branch, supposed to have originated

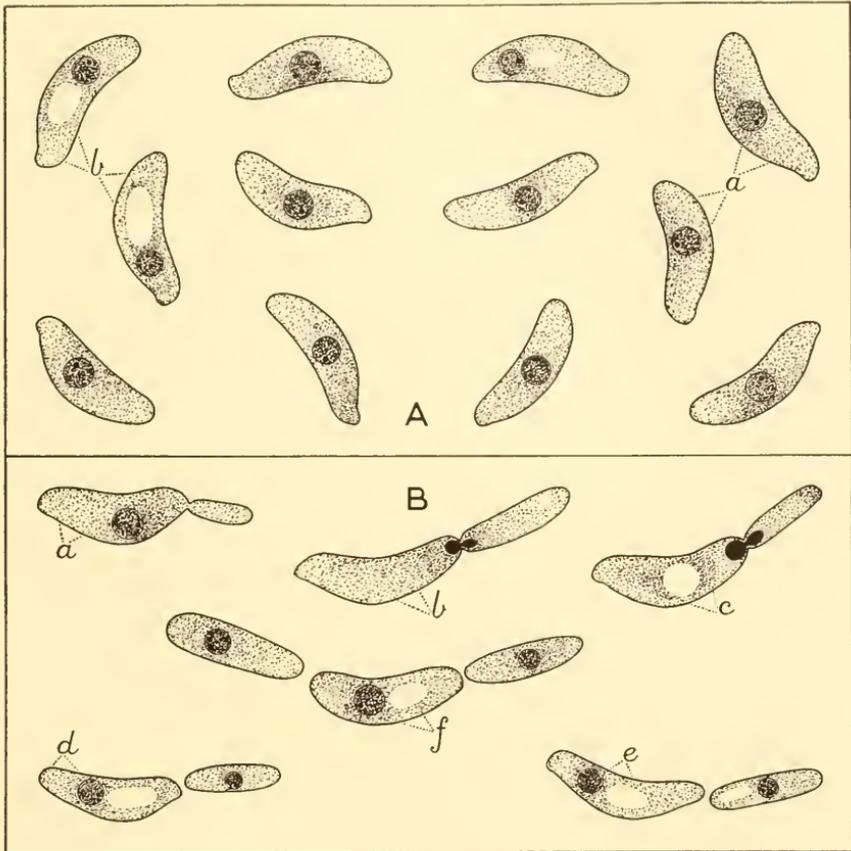


FIG. 98.—*Sporobolomyces roseus*. Spores, and nuclear phenomena during their germination. Cultures made on malt-agar. Cells fixed with formalin-acetic-alcohol and stained with iron-alum haematoxylin. A, spores which were shot from their sterigmata on to malt-agar and were fixed prior to germination; each contains a single nucleus with a nucleolus; *a*, two spores without a vacuole; *b*, two spores with a vacuole; in *a* and *b* the base of each spore looks downwards. B, spores germinating and producing yeast cells: *a*, a spore with a young and, as yet, un-nucleated bud; *b* and *c*, two spores each with an older bud, the nucleus has passed to the junction of the spore and the bud and is there dividing; *d* and *e*, two spores each of which has produced a bud which has recently separated from it, the nucleus of the bud is smaller than that of the spore; *f*, a spore which has produced two buds in succession, each of the three cells contains a single nucleus. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2234.

in this way, is now producing a spore, and normal nuclear division between the spore and the sterigma is in progress. Finally, the old

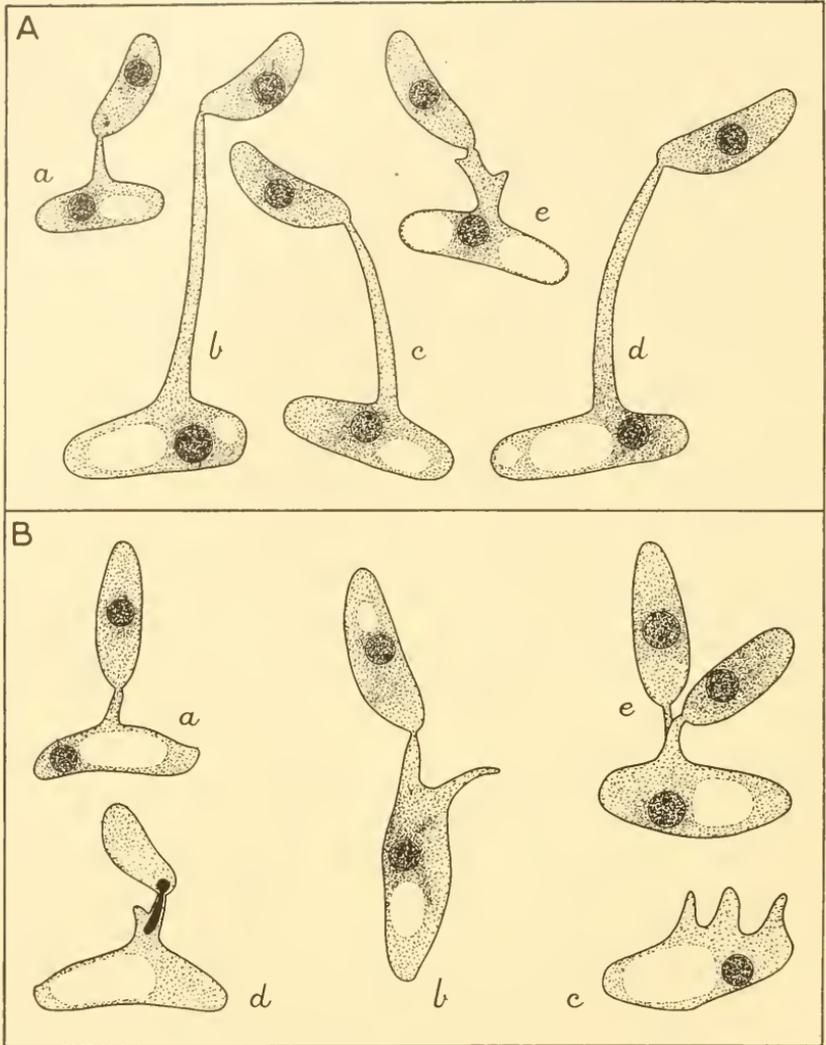


FIG. 99.—*Sporobolomyces roseus*. Abnormalities in development. Cultures made on malt-agar. Cells fixed with formalin-acetic-alcohol and stained with iron-alum haematoxylin. A, yeast cells producing spores; *a*, normal; *b*, *c*, and *d*, the sterigmata are unusually long; *e*, the sterigma is branched and has probably produced two other spores before the one here shown. B, spores producing spores: *a*, normal; *b*, the parent spore has two sterigmata at one end, the right-hand sterigma probably produced and shot away a spore before the left-hand one began to develop; *c*, a spore with three sterigmata, each of which probably produced a spore, but in succession; *d*, the parent spore with a branched sterigma, probably a spore was first produced on the now vacant point and then the sterigma branched and produced a second spore as now shown, the nucleus is dividing; *e*, a parent spore which has a branched sterigma each point of which bears a spore, probably one of the spores was produced first but failed to be discharged and then the sterigma branched and produced the second spore. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 2234.

spore *e* bears a branched sterigma and the top of each branch is crowned with a spore. The two younger spores and the parent spore all contain, as usual, a single nucleus. It is probable that, owing to abnormal culture conditions, one of the two younger spores failed to be discharged as soon as it became mature, with the result that it was left on the sterigma whilst the sterigma branched and produced a second spore. From this discussion it seems probable that the development of the abnormal morphological structures illustrated in Fig. 99 was not accompanied by any abnormalities in nuclear division.

We may conclude from the various observations recorded in this Section that the yeast cells and spores of *Sporobolomyces roseus* each contain only a single nucleus, and that at no stage in the life-history as observed up to the present do the nuclei ever come together to form conjugate pairs or fuse with one another.

**The Taxonomic Position of Sporobolomyces.**—The taxonomic position of Sporobolomyces, as we have seen in the Introduction, has been discussed: (1) by Kluver and van Niel who suggested that the genus belongs to the Basidiomycetes; (2) by Lohwag who opposed this view, and (3) by Guilliermond who asserted that Sporobolomyces ought to be given a special place in the classification of the fungi and that its affinities are as yet unknown. I am of opinion that Sporobolomyces is of basidiomycetous origin for reasons that will be adduced in what follows.

The chief facts which must be taken into account in any attempt to determine the origin and affinities of Sporobolomyces are the following:

(1) Sporobolomyces is yeast-like in form and in its mode of vegetative multiplication.

(2) Sporobolomyces produces conidia on sterigmata aerially.

(3) The mode of development of a conidium on its sterigma and the drop-excretion mode of discharge of the conidium in Sporobolomyces are exactly similar to the mode of development of a basidiospore on its sterigma and the drop-excretion mode of discharge of the basidiospore in the Hymenomycetes, the Uredineae, and Tilletia.

(4) Sporobolomyces, as so far investigated, never possesses

conjugate nuclei and never exhibits karyogamy or any other signs of sexuality.

The vegetative cells of *Sporobolomyces*, in their form and mode of vegetative multiplication, resemble those of the *Saccharomycetes*; but, whereas in *Sporobolomyces* the spores are produced as conidia on aerial sterigmata, in the *Saccharomycetes* the spores are produced inside the mother-cells as ascospores. This striking difference in the mode of spore-production clearly indicates that *Sporobolomyces* is not closely related to the *Saccharomycetes*.

Yeast-like budding occurs not only in the *Saccharomycetes*, but also in *Mucor*, *Exoascus*, *Ustilago*, etc., and by itself is of little or no value in determining the systematic position of the fungus concerned.

As a result of a long series of investigations made on living fungi I have become convinced that, in the *Basidiomycetes*, in every species in which basidiospores are discharged (all *Hymenomycetes*, all *Uredineae*, and all species of the genus *Tilletia*), normally every spore without exception is developed asymmetrically on the end of a conical sterigma, has a tiny excretory spore-hilum on one side of the top of the sterigma and, as soon as it is ripe, is violently discharged by the drop-excretion mechanism.<sup>1</sup>

Since the mode of development and discharge of the basidiospore in all *Hymenomycetes*, all *Uredineae*, and all species of *Tilletia* is identical, one is justified in drawing the conclusion that it is a fundamental character of the basidiospore which has been inherited from the common ancestor of the *Hymenomycetes*, the *Uredineae*, and the *Tilletiaceae*.

In the *Gastromycetes*<sup>2</sup> and the *Ustilaginaceae*, the basidiospores

<sup>1</sup> The data from which this conclusion has been drawn are specially treated of: for the *Hymenomycetes* in Vol. II, Chapter I; for the *Hymenomycetes* and *Uredineae* together in Vol. III, Part II, Chapter I (p. 497 *et seq.*); and for *Tilletia* in this Vol. V. Illustrations showing the structure of the basidiospores, the drop excreted at the spore-hilum, and the discharge of the basidiospores are given: for the *Hymenomycetes* in Vol. II, on pp. 7 (Fig. 2), 10 (Fig. 3), 12 (Fig. 4), 13 (Fig. 5), 14 (Fig. 6), 16 (Fig. 7), 18 (Fig. 8), 19 (Fig. 9), 290 (Fig. 97), 338 (Fig. 121), 429 (Fig. 147), and in Vol. III on pp. 220 (Fig. 93), 227 (Fig. 95), 252 (Fig. 107); for the *Uredineae* in Vol. III, on pp. 503 (Fig. 203), 505 (Fig. 204), 508 (Fig. 205), 511 (Fig. 206), 537 (Fig. 216); and for *Tilletia* in this Vol. V, on pp. 222 (Fig. 108) and 232 (Fig. 114).

<sup>2</sup> For a discussion of basidial degeneracy in the *Gastromycetes* *vide* these *Researches*, Vol. II, 1922, pp. 29-33.

do not develop a spore-hilum on one side of the top of the sterigma, do not excrete a drop, and are not violently discharged. It is probable, however, that the Gastromycetes and the Ustilaginaceae have been derived from the same primitive Basidiomycete as the Hymenomyces, the Uredineae, and the Tilletiaceae. We may suppose that the loss of the power of discharging the basidiospore and the change in the morphological characteristics of the primitive sterigma and basidiospore took place: (1) in the Gastromycetes when the hymenium began to set free its basidiospores in closed chambers instead of into the external air, and (2) in the Ustilaginaceae when the process of budding as a means of vegetative multiplication was initiated.

We may now ask the question: apart from *Sporobolomyces*, are there any fungi other than undoubted Basidiomycetes which develop and discharge their conidia in the same way as the Hymenomyces, the Uredineae, and *Tilletia* develop and discharge their basidiospores? The answer to this question is that, up to the present, no such fungi have ever been found either by myself or by anybody else. In the literature of mycology there are numerous published illustrations of the conidia of Phycomycetes, Ascomycetes, and Fungi Imperfecti. I have examined a considerable number of them, but I have not been able to find in any of them conidia shaped like the basidiospores of the Hymenomyces and Uredineae and having a little spore-hilum projecting out on one side of the top of a sterigma. I have, therefore, no reason to suppose that any of the conidia under discussion are discharged by the drop-excretion mechanism.

The general conclusion to which my study of the production and liberation of fungus spores has led me is as follows: the drop-excretion mechanism of spore-discharge is characteristic of the Basidiomycetes and is lacking in the Phycomycetes, the Ascomycetes and, in general, in the Fungi Imperfecti.

Since the drop-excretion mechanism of spore-discharge found in *Sporobolomyces* is characteristic of Basidiomycetes and is not known to occur in any Phycomycetes or Ascomycetes, we have strong grounds for including *Sporobolomyces* in the Basidiomycetes.

It is possible that *Sporobolomyces* is a degenerate genus of

hymenomycetous origin in which (1) the mycelium has become adapted to a watery habitat by budding, and in which (2) the original fruit-body along with its symmetrical four-spored basidia has been lost ; but in which (3) the original mode of development and discharge of the basidiospore into the air has survived.

In addition to basidiospores the Uredineae and the Hymenomycetes produce conidia. We may therefore enquire whether or not any of these conidia resemble basidiospores in being violently discharged by the drop-excretion mechanism.

The Rust Fungi, in addition to basidiospores (sporidia), produce aecidiospores, pycnidiospores, uredospores, and teleutospores. The aecidiospores, as set forth in Volume III,<sup>1</sup> are violently discharged from the aecidia, but not by the drop-excretion mechanism. The pycnidiospores are not shot into the air, but are merely abstricted from their pedicels into a drop of nectar. The uredospores are abstricted from their pedicels into the air, but without violence, in consequence of which they form a loose powder on the outside of each uredospore pustule. The teleutospores, as a rule, are not dispersed at all, but remain attached to their pedicels until they germinate. In the Rust Fungi, therefore, the drop-excretion mechanism is characteristic of only one of the possible spore forms, namely, the basidiospores.

The Hymenomycetes, in addition to basidiospores are known to produce oidia, conidia, and so-called secondary spores. The oidia which are formed by the breaking up of haploid mycelial hyphae into smaller units, are often produced at the surface of the culture medium in little projecting chains or balls. Each ball of oidia in *Coprinus lagopus* (cf. Vol. IV, Fig. 114, p. 198), etc., is held together above the surface of an agar or other culture medium by a drop of fluid. My observations on the oidia of various Hymenomycetes, made whilst studying heterothallism experimentally, have convinced me that oidia are never violently discharged into the air. Tiny conidia, which we may refer to as *microconidia*, as Brefeld<sup>2</sup> has shown, are produced on germinating basidiospores or on branches of the mycelium derived from a germinating basidiospore in Auricu-

<sup>1</sup> These *Researches*, Vol. III, 1924, pp. 552-559.

<sup>2</sup> O. Brefeld, *Untersuchungen über Pilze*, Heft VII, 1888, Taf. IV, Figs. 4-8, 11.

laria and allied genera, but Brefeld<sup>1</sup> expressly states that these conidia, along with their conidiophores, are produced only under the surface of liquids. In *Craterellus cerasi*, *Tremella lutescens*, etc., the production of the basidial fruit-body is preceded by the production of a conidial fruit-body on which the conidiophores are branched and bear numerous clustered conidia projecting into the air. These conidia, as illustrated by Brefeld,<sup>2</sup> have not the form of basidiospores and are not borne singly on conical sterigmata; and, from Brefeld's account of their development and fate, there is no reason to suppose that they are violently discharged into the air.

The so-called *secondary spores* of the Hymenomycetes (observed in *Auricularia mesenterica*,<sup>3</sup> *Platyglœa nigricans*,<sup>4</sup> etc.) and of the Uredineae (observed in *Puccinia Malvacearum*,<sup>5</sup> *Gymnosporangium Sabinae*,<sup>6</sup> *Cronartium ribicola*,<sup>7</sup> etc.) arise in the following manner. A basidiospore, which has fallen on to the surface of a moist substratum that is defective in nutrient substances, instead of putting out a germ-tube, develops a sterigma which projects into the air and forms at its apex a spore which exactly resembles in form the original basidiospore. It is this new spore which is known as a secondary spore. During secondary-spore formation, the protoplasm of the original basidiospore is transferred to the secondary spore, so that, in the end, the basidiospore becomes completely exhausted of its contents. Presumably, secondary spores, when well formed, are shot away from their sterigmata by the drop-excretion mechanism.

<sup>1</sup> O. Brefeld, *Untersuchungen über Pilze*, Heft VII, 1888, p. 75.

<sup>2</sup> *Ibid.*, Taf. VI, Figs. 13-17; Taf. VII, Figs. 1 and 4.

<sup>3</sup> *Ibid.*, Taf. IV, Fig. 10; Sappin-Trouffy (*Le Botaniste*, Sér. 5, 1896, p. 56, Fig. 5) gives an illustration of a secondary spore of *Auricularia auricula-Judae*.

<sup>4</sup> *Ibid.*, Taf. IV, Fig. 14. Brefeld's *Tachaphantium tiliae* = *Platyglœa nigricans*. In addition to the Hymenomycetes mentioned in the text above, Brefeld observed and illustrated the formation of secondary spores in *Eridia guttata* (Heft VII, Taf. V, Fig. 13), *Sebacina incrustans* (Heft VII, Taf. VI, Fig. 26), *Pachysterigma* (= *Tulasnella incarnatum* (Heft VIII, Taf. I, Fig. 1), *P. fugax* (same plate, Fig. 4) and *Radulum lactum* (Heft VIII, Taf. II, Fig. 2).

<sup>5</sup> Sappin-Trouffy, "Recherches mycologiques," *Le Botaniste*, Sér. 5, 1896, p. 118, Fig. 30, g, h.

<sup>6</sup> *Ibid.*, p. 125, Fig. 33, k.

<sup>7</sup> R. H. Colley, "Parasitism, Morphology, and Cytology of *Cronartium ribicola*," *Journal of Agricultural Research*, Washington, Vol. XV, 1918, p. 638, also Plate LVII, CC and DD (Reproduction of DD in Gäumann's *Vergl. Morph. der Pilze* on p. 439).

Lohwag<sup>1</sup> has argued that, if secondary spores are discharged by the drop-excretion mechanism, this proves that the drop-excretion mechanism occurs not only in basidiospores but also in ordinary conidia. This argument I am unable to accept. I regard a secondary spore as a basidiospore and not as a special form of conidium.<sup>2</sup> Secondary spores, as Brefeld rightly says, are "exceptional structures or in reality only a *Zwischenstation* on the way to true germination." Sappin-Trouffy and Colley both refer to secondary spores produced from sporidia in the Uredineae as *secondary sporidia*. In future, I propose to call the secondary spores of both Hymenomyces and Uredineae *secondary basidiospores*.

Summing up the above discussion of the fate of the various kinds of spores produced by the Hymenomyces and the Uredineae, it is clear that the drop-excretion mode of spore-discharge is restricted to the basidiospores and does not occur in connexion with any other kind of spore. This conclusion tends to strengthen the view that in *Sporobolomyces* the conidium is phylogenetically a basidiospore and not an ordinary conidium.

Miss Macrae and I observed that, when a conidium of *Sporobolomyces roseus* falls on to exhausted malt-agar, it frequently produces an aerial sterigma and a secondary conidium which is just like the parent conidium in its form and mode of discharge (Figs. 93 and 97, pp. 187 and 193). This process of secondary-conidium formation resembles the process of secondary-basidiospore formation in the Hymenomyces, the Uredineae, and *Tilletia*; and this fact may be taken as further evidence that the conidia of *Sporobolomyces* phylogenetically are true basidiospores.

<sup>1</sup> H. Lohwag, "Sporobolomyces—kein Basidiomycet," *Annales Mycologici*, Vol. XXIV, 1926, p. 201.

<sup>2</sup> Lohwag (*loc. cit.*) says that, assuming that secondary spores are discharged by the drop-excretion mechanism: "Denn, Basidiosporen, die an einem Sterigma wieder Basidiosporen abschnüren, wurden in analoger Schlussweise dazu führen, dass die jetzigen Basidiosporen die Basidien bzw. die Sterigmen der Sekundärkonidien sind." If one assumes, as Lohwag evidently does, that only a basidium can produce a basidiospore, then, of course, it is impossible to regard a secondary spore as a basidiospore without confusion in ideas and terminology; but, if one does not make that assumption, then one has no difficulty in regarding a secondary spore, which exactly resembles in outer form, nuclear content, and mode of discharge the basidiospore from which it has been formed, as exactly what it looks like, namely, a basidiospore.

As we have seen, *Sporobolomyces* yeast cells sometimes produce two, three, or four sterigmata instead of one only. Such yeast cells are curiously reminiscent of disterigmatic, tristerigmatic, and tetra-sterigmatic basidia of the Hymenomycetes.<sup>1</sup> Guilliermond's<sup>2</sup> illustration of a cell of *S. salmonicolor* in which there are four sterigmatic tips each crowned by a conidium has the appearance of a grotesque hymenomycetous basidium. If *Sporobolomyces* is of hymenomycetous origin, the production of 2-4 sterigmata by some of the yeast cells may be due to an inherited tendency to develop a typical unicellular basidium.

Monosterigmatic basidia are by no means unknown in the Hymenomycetes.<sup>3</sup> They have been observed, for instance, by others in species of *Pistillaria* and by myself in *Coprinus bisporus* and the cultivated form of *Psalliota campestris*. In the hymenium of the last-mentioned fungus monosterigmatic basidia are commonly found mixed with the more numerous disterigmatic basidia (*vide* Vol. II, Figs. 105, 143, 146, and 147 on pp. 314, 409, 416, and 429). The fact that, as a rule, *Sporobolomyces* yeast cells only produce a single sterigma need not therefore prevent anyone who wishes to do so from regarding the yeast cells as equivalent to reduced basidia.

Whereas in the Hymenomycetes and the Uredineae, as my own observations on living basidia have shown,<sup>4</sup> each basidium produces only one crop of spores and then collapses, so that each sterigma gives birth to but a single spore, in *Sporobolomyces* each yeast cell normally produces on the end of one and the same sterigma several conidia (2, 3, or 4 or possibly more) in succession (Fig. 90, p. 182). This arrangement, which is unknown in any of the typical Basidiomycetes, saves the yeast cell from the burden of producing several sterigmata and, at the same time, makes it possible for the yeast cell to produce several spores each as large as itself. It is certain that, whilst producing its succession of conidia, a yeast cell is absorbing food materials and building up new protoplasm in its

<sup>1</sup> For variations in the number of sterigmata and spores produced by Hymenomycetes *vide* these *Researches*, Vol. II, 1922, pp. 315-321.

<sup>2</sup> A. Guilliermond, *loc. cit.*, p. 251, Fig. 4.

<sup>3</sup> Monosterigmatic basidia in the Hymenomycetes are treated of in these *Researches*, Vol. II, p. 315.

<sup>4</sup> These *Researches*, Vol. II, pp. 27-29.

interior, so that continued spore-production is correlated with continued constructive metabolism in the mother-cell.<sup>1</sup> If *Sporobolomyces* is phylogenetically a reduced Hymenomycete and if the yeast cells are in some degree equivalent to basidia, we may suppose that the change from the production of several spores on several sterigmata simultaneously to the production of several spores on one and the same sterigma in succession took place when the mycelium became aquatic and developed its habit of budding.

Granted that the facts concerned with the drop-excretion mechanism support the view that *Sporobolomyces* is of basidiomycetous origin, let us now enquire whether or not this conclusion is in harmony with the cytological investigations made by Guilliermond and by Miss Macrae and myself, as already recorded.

It has been ascertained that the yeast cells and also the conidia of *Sporobolomyces* each contain only one nucleus and that, throughout the life-history, there is no trace of either conjugate nuclei or karyogamy. This absence of sexual phenomena of the kind usually found in typical Basidiomycetes, it must be frankly admitted, does not support the idea that *Sporobolomyces* belongs to the Basidiomycetes; but, as Kluver and van Niel<sup>2</sup> have pointed out, it does not prove that *Sporobolomyces* does not belong to the Basidiomycetes, but rather leaves the question open.

If *Sporobolomyces* is of basidiomycetous origin, how comes it that the yeast cells show no signs of conjugate nuclei or of karyogamy? There are two possible answers to this question. One is concerned with the phenomenon of heterothallism and the other with a possible loss of sexuality.

*Sporobolomyces* may be heterothallic, and it may be that only one sexual strain of each species, *i.e.* a (+) or a (-) strain, has so far been isolated and grown. If this is so, the cytological investigations have been made on haploid unisexual strains of *Sporobolomyces* only, and the absence of conjugate nuclei and karyogamy can readily be understood. Attempts should be made to isolate new wild strains of *Sporobolomyces* and to mate them with our present strains in the hope that, when strains of opposite sex have been brought together, conjugation may be observed.

<sup>1</sup> *Vide supra*, p. 184.

<sup>2</sup> A. J. Kluver and C. B. van Niel, *loc. cit.*, p. 393.

One of the most remarkable discoveries in connexion with the Hymenomycetes is that, in certain species of Agaricineae, there are haploid fruit-bodies as well as diploid. Bauch<sup>1</sup> has investigated two races of *Hygrophorus* (*Camarophyllus*) *virgineus*, one with 2-spored basidia and the other with 4-spored basidia, and he has shown that the fruit-body of the 2-spored race is *haploid* throughout, while that of the 4-spored race is *diploid* throughout. In the 2-spored race, the walls of the cells of the fruit-body are without clamp-connexions, there is only one nucleus in each cell, each young basidium contains but a single nucleus so that a union of two nuclei in the basidium does not take place, the basidial nucleus divides once only, and a single nucleus passes up into each of the two spores. On the other hand, in the 4-spored race, the walls of the cells of the fruit-body bear clamp-connexions, there are two conjugate nuclei in each cell, each young basidium contains two nuclei which fuse together, the fusion nucleus divides twice and thus produces four nuclei, and these four nuclei pass upwards through the sterigmata into the four spores. Hanna<sup>2</sup> has succeeded in growing *Coprinus lagopus*, which is a heterothallic species, in the haploid condition for ten successive generations. His ten mycelia and ten fruit-bodies with all their spores were all unisexual and of the same sex as the original spore sown at the beginning of the experiment. The investigations on haploid fruit-bodies show that uninucleate haploid cells can develop into perfect basidia, thus proving that the development of a basidium is not dependent on the presence of two nuclei of opposite sex or on the occurrence of karyogamy. A Sporobolomyces yeast cell, before spore-production, contains but a single nucleus, so that karyogamy cannot take place in its interior. In this respect it exactly resembles a haploid basidium of the 2-spored race of *Hygrophorus virgineus* investigated by Bauch. The non-occurrence of conjugate nuclei and of karyogamy in Sporobolomyces cannot therefore be accepted as proving that this genus does not belong to the Basidiomycetes.

<sup>1</sup> R. Bauch, "Untersuchungen über zweisporige Hymenomyceten. I. Haploide Parthenogenesis bei *Camarophyllus virgineus*," *Zeitschrift für Botanik*, Bd. XVIII, 1926, pp. 337-387.

<sup>2</sup> W. F. Hanna, "Sexual Stability in Monosporous Mycelia of *Coprinus lagopus*," *Annals of Botany*, Vol. XLII, 1928, pp. 379-389.

Another possible explanation of the absence of conjugate nuclei and of the non-occurrence of karyogamy in *Sporobolomyces* is that in this genus all traces of a sexual process have disappeared. As Kluyver and van Niel<sup>1</sup> have pointed out, in this connexion facts concerning the *Saccharomycetes* may be cited for comparison. Guilliermond<sup>2</sup> has shown that in this group of Yeasts, in which as defined by him all the species form ascospores, there is a gradual transition between species in which conjugation takes place between two neighbouring cells with resultant karyogamy (*Schizosaccharomyces*, *Zygosaccharomyces*, *Debaryomyces*) to species in which all traces of conjugation and of karyogamy have completely disappeared (*Saccharomyces*). If such yeasts as *Saccharomyces cerevisiae* have ceased to show any signs of sex and yet are able to form ascospores non-sexually, there may be *Basidiomycetes* which have lost all trace of sex and yet are able to form basidiospores non-sexually; and, further, the species of *Sporobolomyces*, which resemble the species of *Saccharomyces* in their vegetative budding, may be just such *Basidiomycetes*.

For the reasons set forth in the above discussion I have come to the following conclusions :

(1) From the point of view of phylogeny, the most important characteristics of *Sporobolomyces* are : (a) the peculiar shape of the conidium which is due to its possessing an excretory hilum that is developed on one side of the top of the sterigma, (b) the presence of a sterigma of typical conical shape beneath each conidium, and (c) the discharge of the conidium by the drop-excretion mechanism.

(2) The possession of the characteristics just enumerated, which are identical with those concerned with the production and liberation of individual basidiospores in all *Hymenomycetes* and *Uredineae*, clearly indicates that *Sporobolomyces* belongs to the *Basidiomycetes*.

(3) The behaviour of the nuclei in *Sporobolomyces*, as ascertained up to the present, cannot be used as an argument either for supporting or for rejecting the conclusion that *Sporobolomyces* belongs to the *Basidiomycetes*.

<sup>1</sup> A. J. Kluyver and C. B. van Niel, *loc. cit.*, pp. 393-394.

<sup>2</sup> A. Guilliermond, *The Yeasts* (translation by F. W. Tanner, New York), 1920, pp. 48-49, 193-232.

## CHAPTER II

### THE VIOLENT DISCHARGE OF THE BASIDIOSPORES (SECONDARY CONIDIA) OF *TILLETIA TRITICI*

BY A. H. R. BULLER AND T. C. VANTERPOOL<sup>1</sup>

Introduction—Bunted Ears and Grains of Wheat—The Germination of the Chlamydo-spores on Agar—Variations in the Development of the Primary and Secondary Conidia—External Conditions and the Germination of the Chlamydo-spores—The Development and Discharge of Secondary Conidia—Discussion of the Significance of Violent Spore-discharge in *Tilletia tritici*—Terminology, New and Old—Nuclei and Sex—Abnormal Basidiospore-discharge—A Basidiospore-discharge Method of making Pure Cultures—Basidiospore-deposits—The Development and Discharge of Basidiospores in Dry Air—The Germination of Basidiospores—The Development of Chlamydo-spores in Culture Media—Spore-fall observed by the Beam-of-light Method—The Distance of Basidiospore-discharge—The Spore-fall Method of Inoculating Wheat Seedlings—The Infection of Wheat Seedlings by Secondary Basidiospores of *Tilletia tritici* and *T. laevis*—The Swelling of Bunted Wheat Grains in Water—The Reactions of the Promycelium to External Stimuli—The Phenomenon of Protoplasmic Migration—Conclusion

**Introduction.**—The Stinking Smut disease or Bunt of wheat (Fig. 100), which occasions enormous losses in our wheat crops, has always been a serious factor in wheat production. Doubtless, it surprised prehistoric man when he first brought wheat under cultivation, and it is referred to in the writings of the ancient Greeks and Romans. The earlier speculations as to the etiology of Bunt were of a superstitious nature ; but, as the centuries went by, the opinion gained ground that the disease is produced within the plant itself by a fermentation of the sap brought about by adverse

<sup>1</sup> My co-operation with Professor Buller in carrying out the investigations recorded in this Chapter was made possible by my election to the Hudson's Bay Company Fellowship at the University of Manitoba for the year 1925-1926.—*T. C. Vanterpool.*



environmental conditions. This view was held up to about the middle of the eighteenth century; but, by that time, it had already been suggested that the cause of the disease might be of external origin.

With the discovery of the infective nature of bunt by the Abbé Tillet<sup>1</sup> in 1755, a new field of experimentation, which might serve as a step toward a clearer conception of the causal factor, was opened up. In adjacent rows in a field, Tillet sowed (1) wheat grains artificially dusted over with bunt spores and (2) clean wheat grains free from spores, and he found that the wheat plants derived from the dusted grains developed bunted heads, whereas those derived from the clean grains remained perfectly healthy. These experiments were soon repeated by other workers and, in the main, with confirmatory results.

In 1807, Bénédict Prevost,<sup>2</sup> with a scientific outlook far in advance of his time, succeeded in germinating the minute black bodies obtained from the

FIG. 100.—An ear of wheat in which the mycelium of *Tilletia tritici* has invaded all the spikelets and has produced chlamydo-spores in every grain. The grains have thus been converted into smut-balls, *s.s.* Drawing prepared by photographing, re-shading, and slightly modifying part of a coloured wall-diagram issued by H. Tuboëuf. Slightly larger than natural size.

<sup>1</sup> H. M. Woolman and H. B. Humphrey, *Summary of Literature on Bunt or Stinking Smut of Wheat*, U.S. Department of Agriculture, Bull. No. 1210, 1924, p. 4.

<sup>2</sup> B. Prevost, *Mémoire sur la Cause Immédiate de la Carie ou Charbon des Blés, et de Plusieurs Autres Maladies des Plantes, et sur les Préservatifs de la Carie*, Paris, 1807.

interior of a stinking smut-ball and thus, for the first time, demonstrated their fungous nature. He observed and illustrated what afterwards came to be known as the chlamydo-spores, the promycelium, the primary conidium, and the secondary conidium.

Prevost held the view that the Stinking Smut disease is caused by the Stinking Smut fungus, which he believed entered healthy wheat seedlings from without; but his attempt to perceive the fungus forcing its way into the host-plant failed. However, the correctness of Prevost's ideas about infection was subsequently demonstrated by Kühn and Wolff who saw the hyphae of the parasite penetrating through the epidermis of the coleophyllum or first leaf-sheath.<sup>1</sup>

In 1847, Tulasne<sup>2</sup> reinvestigated the germinations of the chlamydo-spores of the Stinking Smut fungus and confirmed Prevost's observations. Tulasne's further work upon Rust fungi, Tremellineae, and other Basidiomycetes led to the view, adopted by Brefeld and others, that the promycelium and primary sporidia of the Smut and Rust fungi are homologous with the basidia and basidiospores of the Tremellineae, the Hymenomycetes, and the Gastromycetes.

In recent years, investigations upon the Uredineae,<sup>3</sup> the Hymenomycetes<sup>4</sup> and the basidiomycetous yeast-genus *Sporobolomyces*<sup>5</sup> have shown, in all these fungi, that: (1) a basidiospore is always developed asymmetrically on the end of a conical sterigma; (2) the spore-hilum is situated at the top of the sterigma; (3) a few seconds before spore-discharge, a drop of liquid is excreted at the spore-hilum; (4) violent spore-discharge takes place; and (5), on

<sup>1</sup> Cited from Woolman and Humphrey, *loc. cit.*, p. 7.

<sup>2</sup> L. R. and C. Tulasne, "Mémoire sur les Ustilaginées comparées aux Urédinées," *Ann. Sci. Nat. Bot.*, 3 sér., T. VII, 1847, pp. 12-127; Seconde Mémoire, 4 sér., T. II, 1854, pp. 77-196; L. R. Tulasne, "Observations sur l'organisation des Trémellinées," *ibid.*, 3 sér., T. XIX, 1853, pp. 193-231.

<sup>3</sup> P. Dietel, "Über die Abschleuderung der Sporidien bei den Uredineen," *Mycologisches Centralblatt*, Bd. I, 1912, pp. 355-359; and A. H. R. Buller, *Researches on Fungi*, Vol. II, 1922, pp. 168-169; Vol. III, 1924, pp. 498-519, 533-537.

<sup>4</sup> A. H. R. Buller, *Researches on Fungi*, London, Vol. II, 1922, pp. 4-20.

<sup>5</sup> A. J. Kluyver and C. B. van Niel, "Über Spiegelbilder erzeugende Hefenarten und die neue Hefengattung *Sporobolomyces*," *Centrabl. f. Bakteriologie*, Abt. 2, Bd. LXIII, 1924-1925, pp. 1-20, Taf. I and II. Also A. H. R. Buller, in this volume of *Researches*, pp. 181-183.

discharge, the drop and the spore cling together and are shot to a distance, varying with the species, from about 0·1 mm. to 1·0 mm.

Buller was struck by the great resemblance of the sterigma and sickle-shaped secondary conidium of *Tilletia tritici*, as illustrated by Brefeld,<sup>1</sup> to the sterigma and basidiospore of the Hymenomycetes, the Uredineae, and the species of *Sporobolomyces*, and he therefore thought it probable that a secondary conidium of *T. tritici* is shot violently away from its sterigma by the drop-excretion mechanism. Working in conjunction with Buller, Vanterpool undertook to test this supposition, with the result that violent discharge of the secondary conidia with drop-excretion was actually observed. As a result of this discovery, Buller and Vanterpool came to the following important theoretical conclusions: (1) new and weighty evidence confirming the view that the Tilletiaceae belong to the great group of the Basidiomycetes has been obtained; (2) the so-called secondary conidia of *Tilletia tritici* are true basidiospores; and (3) the so-called primary conidia are morphologically equivalent to sterigmata. A brief communication embodying these conclusions was published in *Nature* in December, 1925.<sup>2</sup>

Normally, under proper conditions of ventilation in cultures and doubtless under natural conditions in the open, the secondary conidia of *Tilletia tritici* and of other species of *Tilletia* are shot away from their sterigmata as soon as they become mature, and they germinate only after they have been discharged. Brefeld, in his illustrations,<sup>3</sup> shows secondary conidia of *T. tritici*, *T. decipiens*, *T. controversa*, and *T. zonata* germinating *in situ*, *i.e.* whilst still attached to the sterigmata on which they developed. This kind of germination, which will be treated of later (*vide* Figs. 109, A, p. 223, and 118, *e, f,* and *g,* p. 243), is abnormal—a fact which Brefeld did not realise. The failure of Brefeld and of others to observe the violent discharge of the secondary conidia of *T. tritici*,

<sup>1</sup> O. Brefeld, *Untersuchungen über Pilze*, Heft V, Leipzig, 1883, Plate XII, Figs. 26, 31–34; Plate XIII, Figs. 35–45. *Vide* also Brefeld's figures of secondary conidia reproduced by F. von Tavel in his *Vergleichende Morphologie der Pilze*, Jena, 1892, Fig. 53, p. 117.

<sup>2</sup> A. H. R. Buller and T. C. Vanterpool, "Violent Spore-discharge in *Tilletia tritici*," *Nature*, Vol. CXVI, 1925, pp. 934–935.

<sup>3</sup> O. Brefeld, *loc. cit.*: Heft V, 1883, Plate XII, Figs. 31a, 32, and 33, Plate XIII, Fig. 43; Heft XII, 1895, Plate X, Figs. 1, 2, 4, and 6.

etc., may well have been due to these investigators not having sufficiently ventilated their cultures.

Since 1925, the violent discharge of the secondary conidia with

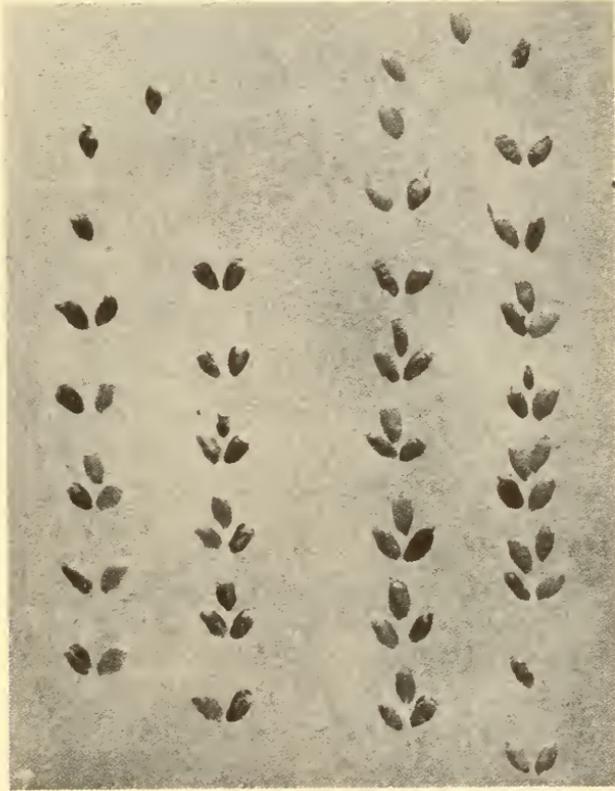


FIG. 101.—The grains of two ears of Common Wheat plants which were parasitised by *Tilletia tritici*. Usually, in an infected plant, all the grains are invaded by the mycelium; but, here, some of the grains (the darker ones) are bunted, *i.e.* converted into smut-balls, whilst other grains (the lighter ones) are quite sound. Material prepared and photographed by I. L. Connors at the Dominion Rust Research Laboratory at Winnipeg. Slightly less than natural size.

drop-excretion in Tilletiaceae, other than *Tilletia tritici*, has been observed: by Buller and Vanterpool in *T. laevis*: by Vanterpool in *T. horrida*, *T. holci*, and *T. asperifolia*: and by Hanna<sup>1</sup> in

<sup>1</sup> W. F. Hanna, personal communication. Observations made at the Dominion Rust Research Laboratory, Winnipeg.

*Entyloma menispermi*, *E. lobeliae*, and *E. linariae*. In all probability further investigation will show that violent discharge of the secondary conidia, coupled with drop-excretion, occurs in many other Tilletiaceae, and, in general, is characteristic of the group.

This chapter contains a detailed account of investigations on the production and liberation of the so-called secondary conidia of *Tilletia tritici* made by T. C. Vanterpool working in conjunction with A. H. R. Buller in the Winnipeg laboratory.

**Bunted Ears and Grains of Wheat.**—In an infected wheat plant the mycelium of *Tilletia tritici*, on entering an ear, usually invades all the spikelets and ultimately produces chlamydo-spores in every grain, so that all the grains become converted into smut-balls (Fig. 100). However, it sometimes happens that the mycelium, on entering an ear, infects some grains and not others. Thus in the same ear there may be present both smutted and sound grains of wheat. The grains of two such partially bunted ears of Common Wheat were removed and set out in their proper order and then photographed. The photograph is reproduced in Fig. 101. The first and second rows of grains shown were taken from the two rows of spikelets on one ear, while the third and fourth rows of grains were taken from the two rows of spikelets on the other ear. The darker grains were all bunted, while the lighter grains were perfectly sound and free from the parasite. Dr. W. F. Hanna has informed me that several times in bunted ears of wheat he has observed individual grains which were in part sound and in part bunted.

Figs. 102 and 103 show healthy and bunted grains of Common Wheat (Marquis) respectively. Fig. 104 shows some smutted grains of Durum Wheat: the two grains to the left are seen in surface view, while the other grains to the right are seen in section. Each smut-ball consists of a very thin light envelope derived from the pericarp and testa of the grain and of a black core consisting of a dense mass of dark-brown chlamydo-spores. As a result of ten trials with a counting apparatus (a haemocytometer), the number of spores contained within a single large smut-ball on wheat was estimated to be 12,125,000.<sup>1</sup>

<sup>1</sup> These *Researches*, Vol. 1, 1909, p. 95.



FIG. 102.—Normal healthy grains of Common Wheat (Marquis). Photographed by I. L. Connors at the Dominion Rust Research Laboratory at Winnipeg. Much enlarged.



FIG. 103.—Bunted grains (bunt-balls) of Common Wheat (cf. Fig. 102). Each grain contains upwards of a million chlamydospores of *Tilletia tritici*. Photographed by I. L. Connors at the Dominion Rust Research Laboratory at Winnipeg. Much enlarged.

There are two species of *Tilletia*<sup>1</sup> which cause bunt of wheat in Western Canada and the United States of America: *T. tritici* and *T. laevis*. *T. tritici* is distinguished from *T. laevis* by having the walls of the chlamydo-spores reticulated instead of smooth.

There is a considerable amount of variation in different strains



FIG. 104.—Bunted grains (bunt-balls) of Durum Wheat parasitised by *Tilletia tritici*. Those on the left are intact, while the others are shown in median-longitudinal or in transverse section. The dark chlamydo-spores, which have an unpleasant odour, fill the whole of each grain's interior. Photographed by I. L. Connors at the Dominion Rust Research Laboratory at Winnipeg. Much enlarged.

of both *Tilletia tritici* and *T. laevis* in respect to the amount of stunting of the host plant, the shape of the bunt balls, the production of trimethylamine, and pathogenicity for different host plants.

In 1931, Rodenheiser,<sup>2</sup> from experimental data, concluded that the difference in the relative degree of stunting of the host plant varies with different strains of the same species and cannot be generally accepted as a criterion for separating *T. tritici* and *T. laevis*.<sup>3</sup>

Potter and Coons,<sup>4</sup> in 1918, found that the bunt balls of *Tilletia tritici* are rounded-oval, whereas those of *T. laevis* are elongated. Hanna<sup>5</sup> grew a strain of *T. tritici* and a strain of *T. laevis* side by

<sup>1</sup> The genus *Tilletia* was so named by Tulasne in 1847 in honour of the Abbé Tillet.

<sup>2</sup> H. A. Rodenheiser, "Stunting of Wheat caused by *Tilletia laevis* and *T. tritici*," *Journal of Agricultural Research*, Vol. XLIII, No. 5, 1931, pp. 465-468.

<sup>3</sup> Dr. O. S. Aamodt of the University of Alberta has informed me *in litt.* that he has obtained results from greenhouse and field experiments which confirm those of Rodenheiser.

<sup>4</sup> A. A. Potter and G. H. Coons, "Differences between the Species of *Tilletia* on Wheat," *Phytopathology*, Vol. VIII, 1918, pp. 106-113.

<sup>5</sup> W. F. Hanna, personal communication.

side in a greenhouse on Kota wheat (Fig. 105) and also on Reward wheat (Fig. 106). The first of the strains caused the grains to become rounded-oval and the second elongated. However, the



FIG. 105.—A difference in the effect of strains of two species of *Tilletia* on Kota Wheat. The elongated smut-balls on the left were produced by a strain of *Tilletia laevis* and the more rounded ones on the right by a strain of *T. tritici*. From greenhouse cultures made by W. F. Hanna. Natural size.

observations of Rodenheiser<sup>1</sup> and, more recently, Aamodt<sup>2</sup> have led these workers to the conclusion that the shape of the bunt balls varies with different physiological forms and cannot be generally accepted as a criterion for separating *T. tritici* and *T. laevis*.



FIG. 106.—A difference in the effect of strains of two species of *Tilletia* on Reward Wheat. The elongated smut-balls on the left were produced by a strain of *Tilletia laevis* and the more rounded ones on the right by a strain of *T. tritici*. From greenhouse cultures made by W. F. Hanna. Natural size.

Further comparisons of the differences between the bunt balls of *T. tritici* and *T. laevis* based on experiments made with inocula derived from monosporidial cultures seem desirable.

<sup>1</sup> H. A. Rodenheiser, *loc. cit.*

<sup>2</sup> O. S. Aamodt, *in litt.*

Hanna, Vickery and Pucher<sup>1</sup> isolated trimethylamine from the spores of *Tilletia laevis* and have thus shown that the odour of the spores is due to a definite chemical substance. Hanna<sup>2</sup> has observed that trimethylamine is present in some strains of *T. tritici* and absent in others, so that it is impossible to differentiate *T. tritici* and *T. laevis* from one another on the basis of odour.

Both Flor<sup>3</sup> and Hanna have succeeded in crossing *Tilletia tritici* and *T. laevis*, thus obtaining hybrid bunt balls. Using pure cultures made from single secondary conidia, which are apparently haploid, Hanna<sup>4</sup> made crosses between *T. laevis* which had trimethylamine and a strain of *T. tritici* which lacked trimethylamine. The first-generation hybrid spores resulting from this cross were smooth, as was to be expected from the work of Flor, and they emitted an odour of trimethylamine. In this cross, therefore, the factors for smooth spore-wall and odour were dominant.

**The Germination of the Chlamydospores on Agar.**—Brefeld germinated the chlamydospores of *Tilletia tritici* and cultivated the fungus successfully in nutrient solutions but less successfully on solid media, as the latter became contaminated with moulds in the course of about ten days. Sartoris,<sup>5</sup> in 1924, appears to have been the first to germinate the chlamydospores and to keep the fungus in pure culture on a solid medium. His solid medium consisted of agar containing oatmeal, malt extract, or the solution recommended by Leonian.<sup>6</sup>

<sup>1</sup> W. F. Hanna, H. B. Vickery, and G. W. Pucher, "The Isolation of Trimethylamine from Spores of *Tilletia laevis*, the Stinking Smut of Wheat," *Journ. Biol. Chem.*, Vol. XCVII, 1932, pp. 351-358.

<sup>2</sup> W. F. Hanna, "The Odor of Bunt Spores," *Phytopathology*, Vol. XXII, 1932, pp. 978-979.

<sup>3</sup> H. H. Flor, "Heterothallism and Hybridisation in *Tilletia tritici* and *T. laevis*," *Journal of Agricultural Research*, Vol. XLIV, 1932, pp. 49-58.

<sup>4</sup> W. F. Hanna, "The Odor of Bunt Spores," *loc. cit.*, p. 979.

<sup>5</sup> G. B. Sartoris, "Studies in the Life History and the Physiology of Certain Smuts," *Amer. Journ. Bot.*, Vol. XI, 1924, pp. 617-647.

<sup>6</sup> Leonian agar has the following composition :

Dihydrogen potassium phosphate . . . . .	1.25 grams
Magnesium sulphate . . . . .	0.60 "
Peptone . . . . .	0.60 "
Maltose . . . . .	6.00 "
Malt extract . . . . .	6.00 "
Agar . . . . .	15-20 "
Water . . . . .	1000 cc.

In the present investigation, the following was the method employed for germinating the chlamydo-spores under sterile conditions.

With a pair of sterile forceps having somewhat flattened arms a mature bunt-ball is carefully removed from between the glumes of an intact bunted head of wheat. The cover of a sterile Petri dish is then raised and the bunt-ball is crushed with the forceps, so that the spores fall in a heap in the centre of the dish while the seed-coat is still held with the forceps. The seed-coat, which of course may be contaminated on its exterior surface by the spores of other fungi, is then thrown away. Altogether four to six bunt-balls taken from as many bunted heads are treated in the manner just described, and the spores from all the crushed bunt-balls are caused to accumulate in a single heap in the Petri dish. The mixing of the spores from several bunted heads goes far to ensure that at least some of the spores will germinate readily. With a sharply-pointed sterile scalpel some spores are now removed from the heap in the Petri dish and distributed sparsely over the surface of a plate containing freshly-poured, non-nutrient, 1.5-2.0 per cent. agar. Instead of plain agar, 2-5 per cent. malt-extract agar may be used, but plain agar has the advantage in that it keeps moulds and bacteria in check. To keep the air above the spores moist, the plate (with its cover on) is then set on a low stand (an inverted Petri-dish cover) in a large crystallising dish (22 × 9 cm.) containing a thin layer of water. The crystallising dish is then almost, but not quite, covered with a sheet of glass, an aperture being left for ventilation. The whole is set on a table and incubation allowed to proceed at room temperature (18°-20° C.).

In from three to six days, under the conditions just described, the sculptured outer wall of the chlamydo-spores ruptures, while the inner wall begins to protrude as a germ-tube or promycelium (Fig. 107, B). The average width of the promycelium is about 8  $\mu$ , while the length is very variable. Sometimes the promycelium becomes only 20  $\mu$  in length, *i.e.* scarcely longer than the diameter of the chlamydo-spore; while, at other times, specially when there is a surface water-film, it may attain an extreme length of about 0.5 mm. Growth in length is apical. At first the promycelium is

filled with dense protoplasm, but sooner or later its basal end becomes vacuolated (Fig. 107, C and E). As a promycelium increases in length, the protoplasm creeps along it acropetally, always filling the apical end. As the protoplasm migrates along the tube, septa are formed at intervals in such a way as to cut off those parts of the promycelium which have become devoid of protoplasm (Fig. 107, C, E, F). In very short promycelia one can usually observe at least one septum, while in long ones there may be a dozen or more. Not infrequently one may observe branched promycelia (Fig. 107, D), but these appear to be abnormalities brought into existence by unsuitable cultural conditions.

From the fourth to the eighth day, when germination is proceeding normally, the end of the promycelium grows away from the substratum as if it were negatively hydrotropic, enlarges at its apex, and produces peripherally a number of very short protuberances some of which may fork once (Fig. 107, E, just below *p*). From the ends of these protuberances there then grow upwards from four to sixteen primary conidia (Fig. 107, E and F). These conidia take four hours or somewhat longer for their full development from tiny rudiments. When fully grown, a conidium is 70–80  $\mu$  long, about 4  $\mu$  wide in the middle, about 2–3  $\mu$  wide at its base, and pointed at its apex. Several observers have incorrectly represented the primary conidium as tapering to a fine point at the base as well as at the apex.

The work of Rawitscher<sup>1</sup> has shown that two nuclei, presumably of opposite sex, fuse together in the young chlamydospore; that the reduction divisions take place in the chlamydospore; that, as a rule, three nuclear divisions succeed one another, so that eight daughter-nuclei come to be present in the chlamydospore; that some of these nuclei may divide again, so that often a total of 8–16 nuclei may be produced; that these nuclei wander out of the chlamydospore into the promycelium; that the number of primary conidia formed at the apex of the promycelium is equal to the number of nuclei which the promycelium contains; and that a single nucleus passes into each primary conidium.

During the development of the primary conidia, as may be seen

<sup>1</sup> F. Rawitscher, "Beiträge zur Kenntnis der Ustilagineen," *Zeitschr. f. Bot.*, Bd. II, 1922, pp. 273–296.

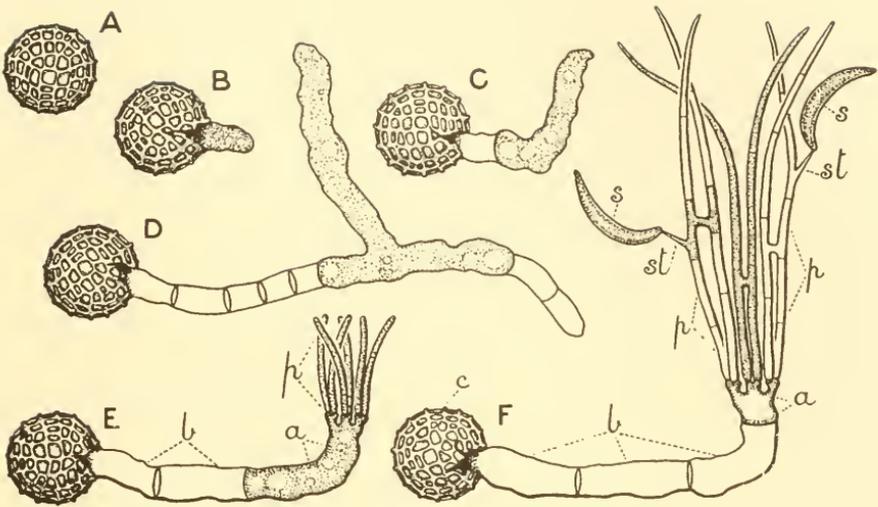


FIG. 107.—*Tilletia tritici*. The germination of the chlamydospore, the formation of the promycelium (later interpreted as the basidium-body), and the production of primary conidia (later interpreted as primary sterigmata) and secondary conidia (later interpreted as basidiospores) on malt-agar. A, a mature chlamydospore showing its sculptured outer wall. B, a chlamydospore that has just emitted a germ-tube. C, a chlamydospore with a germ-tube which has become divided into two cells, a dead basal cell and an apical cell filled with protoplasm. D, a chlamydospore with an abnormal germ-tube; the germ-tube has put out a lateral branch into which the protoplasm is passing; the empty parts of the germ-tube have become septate and consist of dead cells.

E, a chlamydospore with a normal germ-tube; the germ-tube has become the promycelium (basidium-body) and it consists of two empty basal cells *b* and an apical cell *a* filled with protoplasm; the apical cell *a* has developed twelve primary conidia (primary sterigmata) *p*, of which, for the sake of simplicity, only the front six are shown; the primary conidia, as yet, have attained only about one-third of their ultimate length.

F, a mature basidium. The chlamydospore *c* has germinated on malt-agar and has produced a promycelium (basidium-body) consisting of three basal cells *b* which have lost their protoplasmic contents and are dead, and of an apical cell *a* which is lined with cytoplasm and is living. The promycelium may be supposed to have been crowned by twelve long and slender primary conidia (primary sterigmata) *p* which have conjugated in pairs; but, for the sake of clarity, only the front three pairs have been represented. The protoplasm migrated from the promycelium into the primary conidia and, during the migration, the promycelium developed three septa in succession from its base to its apex. The right and left pairs of primary conidia have each produced a short sterigma (secondary sterigma) *st* and a secondary conidium (primary basidiospore) *s*. The protoplasm in the pair of primary conidia on the right migrated into the secondary conidium *s* and, during the migration, the primary conidia became exhausted and septate. In the pair of primary conidia on the left the migration of the protoplasm into the secondary conidium is nearly but not quite completed. The middle pair of primary conidia has only recently conjugated and has not yet produced a sterigma and a secondary conidium. Hence it is full of protoplasm and not yet septate. Within an hour the secondary conidium *s* on the right would be shot violently away from its sterigma. Magnification, 660.

by continuous observation with the microscope, the apices of the conidia do not keep the same relative distances from one another as they have when they can first be seen, but often alter them considerably. This is correlated with the fact that the conidia during their growth in length become curved, so that they intermingle irregularly. Thus, in the end, the mature conidia, collectively, may be tortuous and somewhat spreading or, as frequently happens, they may be compact and intertwining so that they resemble a besom (*cf.* Fig. 107, F).

As soon as they have attained their full length and whilst they are still seated on the promycelium, the primary conidia conjugate in pairs. This is accomplished by a *bridging hypha* which is formed across an air-gap which may be 1-3 times the diameter of each conidium. Exactly how this bridging hypha comes into existence awaits further investigation; but, in all probability, it is by means of a *peg-to-peg* fusion,<sup>1</sup> *i.e.* by two very short lateral branch-hyphae (pegs), one produced by one conidium and the other by the other conidium, coming into existence opposite to one another, growing toward one another through the air, meeting, and fusing at their ends (*cf.* Fig. 16, p. 32). If the bridging hypha is formed in this way, we have exhibited during its formation a zygotropic phenomenon comparable with that observed by Blakeslee<sup>2</sup> during the aerial conjugation of the zygophores in some species of the Mucorineae. The bridging hypha is usually situated near the middle of the two conidia involved (*cf.* Fig. 107, F), but it may be formed at their very base or at any distance upwards to within about 15  $\mu$  of their apices. Thus conjugation has been observed to occur over four-fifths of the length of the conidia measured from their bases. Conjugation has never been seen to occur at the very apices of two conidia, and this may be due to the fact that the apices taper to a point and, in general, are far apart relatively to other portions of the two shafts.

Rawitscher<sup>3</sup> and Boss<sup>4</sup> have shown that, during conjugation of

<sup>1</sup> *Vide supra*, pp. 30-33.

<sup>2</sup> A. F. Blakeslee, "Sexual Reproduction in the Mucorineae," *Proc. Amer. Acad. of Science*, Vol. XL, 1904, p. 274.

<sup>3</sup> F. Rawitscher, "Zur Sexualität der Brandpilze: *Tilletia tritici*," *Ber. d. Deutsch. Bot. Ges.*, Bd. XXXII, 1914, p. 310.

<sup>4</sup> G. Boss, "Beiträge zur Zytologie der Ustilagineen," *Planta*, Bd. III, 1927, pp. 619-622.

two primary conidia, the nucleus present in one of the conidia passes *via* the bridging hypha into the other conidium, so that this becomes binucleate. Thus two nuclei of opposite sex become associated with one another. The long slender curving form of the primary conidia must favour the chance of two conidia of opposite sex coming sufficiently near one another to make conjugation possible.

**Variations in the Development of the Primary and Secondary Conidia.**—After conjugation has been completed, further development of the H-shaped pairs of primary conidia may take place in any one of five ways. These will now be considered *seriatim*.

(1) The pairs of primary conidia remain seated on the promycelium, and each pair puts out laterally a short pointed hypha or sterigma (*st* in Fig. 107, F) upon which there develops a single secondary conidium (*s*) which is soon violently discharged. In Fig. 108 is shown the whole of a basidium with six pairs of primary conidia producing and discharging secondary conidia. Whilst each pair of primary conidia is producing a secondary conidium, the protoplasm of the pair of primary conidia passes *en masse* into the secondary conidium (Fig. 107, F); and, after a secondary conidium has been shot away, the H-shaped pair of primary conidia which produced it is a dead structure, devoid of protoplasm, and quite incapable of developing any further. The mode of development just described and represented in Figs. 107, F, and 108, must be considered as fully *normal*. It takes place regularly when chlamydospores are sown on a solid medium such as dung-agar, under suitable conditions of temperature and ventilation, and are not mechanically disturbed; and no doubt it takes place under natural conditions.

When one looks down on a chlamydospore culture with the low power of the microscope, one can often observe tufts of primary conidia which bear two secondary conidia (Fig. 107, F) and, occasionally, a tuft which bears as many as three or four secondary conidia (Fig. 108). In any tuft, the H-shaped pairs of primary conidia develop and discharge their secondary conidia not of necessity simultaneously, but often in succession. Hence the full number of secondary conidia produced by a tuft of primary conidia

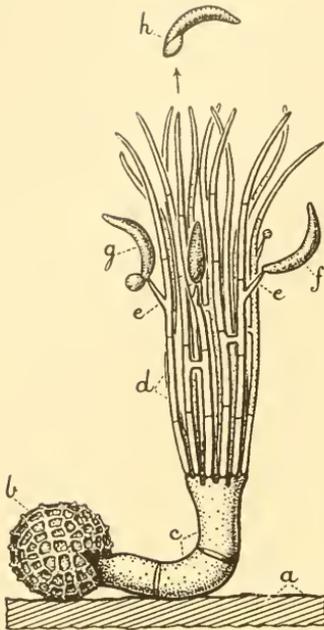


FIG. 108.—*Tilletia tritici*. Diagram of a basidium showing several primary basidiospores (Brefeld's secondary conidia) developing simultaneously and their mode of discharge. In the description the authors' terminology will be employed and the Brefeldian terminology added in brackets: *a*, the substratum; *b*, a *chlamydospore* which has germinated; *c*, the *basidium-body* (promycelium); *d*, twelve *primary sterigmata* (primary conidia) which have conjugated in pairs. Each pair of conjugated primary sterigmata has produced a short tapering *secondary sterigma* (sterigma) *ee*, bearing a sickle-shaped *primary basidiospore* (secondary conidium) *f, g*. Above *e-f* is a very rudimentary *primary basidiospore* (secondary conidium) which is globular and seated symmetrically on its sterigma; *f*, a ripe *primary basidiospore* (secondary conidium), asymmetrically seated on its sterigma; *g*, a *primary basidiospore* (secondary conidium) which has just excreted a drop of liquid at its hilum and is about to be discharged; *h*, a *primary basidiospore* (secondary conidium) which, along with its drop, has just been shot away from a sterigma at the back of the basidium. Magnification, 666.

is rarely, if ever, to be seen on the tuft at any particular moment.

(2) Just as in (1), the pairs of primary conidia remain seated on the promycelium and each pair puts out laterally a short sterigma (*f* in Fig. 109, A) upon the end of which there develops a single secondary conidium (*g* and *h*); but, unlike what happens in (1), the secondary conidia are not discharged and each of them puts out a germ-tube into the air (*i*). As a germ-tube grows in length, the H-shaped pair of primary conidia, together with its sterigma, the secondary conidium, and the germ-tube, may fall away from the promycelium. If the germ-tube then happens to come into contact with a nutrient medium, such as malt-agar, it may develop into a mycelium which later may produce numerous secondary conidia. The mode of development just described and illustrated in Fig. 109, A, must be considered as *abnormal* for it occurs under unfavourable conditions (closed chamber, excess moisture). It is doubtless correlated with a failure in the drop-excretion discharge mechanism: each secondary

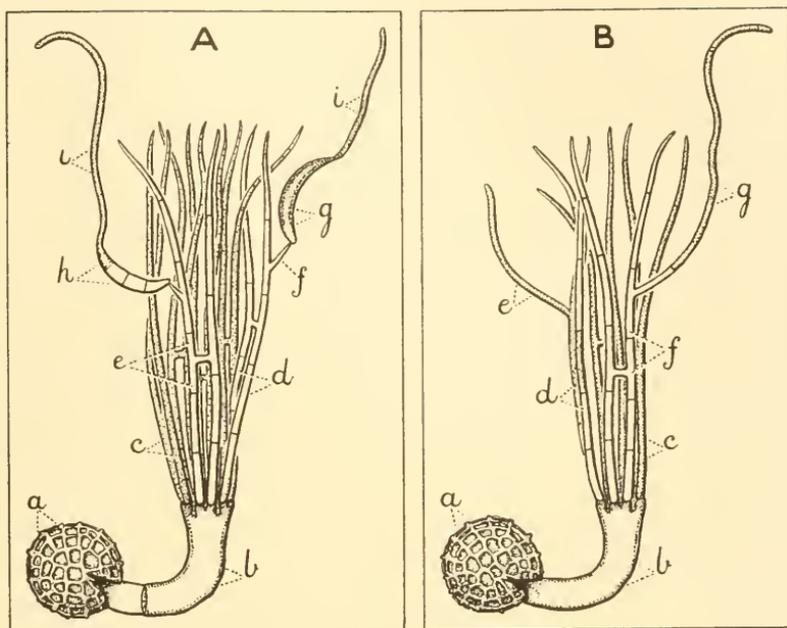


FIG. 109 —*Tilletia tritici*. Diagrams illustrating abnormalities in the development of the basidium : in A two secondary conidia (the authors' basidio-spores) instead of being discharged have germinated *in situ* ; and in B two H-shaped pairs of primary conidia (the authors' primary sterigmata) instead of producing secondary conidia have germinated directly. A : the chlamydospore *a* has developed a promycelium (basidium-body) *b* consisting of one dead basal cell and a living apical cell ; the latter bears 12 primary conidia which have conjugated in pairs ; four of the H-shaped pairs, as at *c*, have not yet produced secondary conidia and are full of protoplasm ; two of the pairs, *d* and *e*, have each produced a short lateral sterigma *f* bearing a secondary conidium, *g* and *h* ; the conidia *g* and *h*, owing to a failure in the drop-discharge mechanism, have not been shot away, and they have germinated *in situ* ; the protoplasm is passing from the conidia into the germ-tubes *i i* ; the septation of the H-shaped pairs of primary conidia *d* and *e* and of the secondary conidium *h* took place during the evacuation of the protoplasm. B : the chlamydospore *a* has developed a promycelium *b* consisting of one curved living cell which bears 8 primary conidia that have conjugated in pairs ; two of the H-shaped pairs, as at *c*, have not produced either secondary conidia or germ-tubes and are full of protoplasm, while two of the pairs *d* and *f* instead of giving rise to secondary conidia have germinated directly ; protoplasm is still creeping out of the H-shaped pair of primary conidia *d* into the germ-tube *e*, but it has entirely vacated the pair *f* and is now in the germ-tube *g* ; septation is accompanying or has accompanied the evacuation of the protoplasm from the conidia-pairs *d* and *f*, and already there is one septum in the germ-tube *g*. Such H-shaped pairs as *d* and *e* in A and *d* and *f* in B readily become detached from the promycelium *b* and fall on to the substratum where, if nutriment is present, the germ-tubes may develop into mycelia. Magnification, 660.

conidium, having failed to be discharged, has germinated *in situ* instead of after discharge. Similar abnormalities have been observed by me in the Rust Fungi, and for *Puccinia graminis* they are illustrated in Volume III, Fig. 203, *h-l* (p. 503).

Brefeld<sup>1</sup> described and illustrated the germination of secondary conidia whilst still attached to H-shaped pairs of primary conidia in *Tilletia tritici*, *T. decipiens*, *T. controversa*, and *T. zonata*. He did not know that, under favourable conditions, the secondary conidia of *Tilletia* species are violently discharged from their sterigmata and he was therefore unaware that the germination of these secondary conidia while still attached to sterigmata is abnormal.

(3) The conjugated pairs of primary conidia, just as in (1) and (2), remain seated on the promycelium. However, when they germinate they give rise not to a sterigma and secondary conidium, but to a slender germ-tube (Fig. 109, B, *e* and *g*). The germ-tube grows out into the air and, sooner or later as it elongates, the H-shaped pair of primary conidia becomes detached from the promycelium and, bearing the germ-tube with it, falls on to the substratum. If the germ-tube then comes into contact with nutriment, it may develop into a branched mycelium which may give rise to numerous secondary conidia. The mode of development just described and illustrated in Fig. 109, B, is certainly *abnormal* and, as the primary conidia do not produce any secondary conidia whatever, it may be regarded as still more abnormal than that described under (2) and illustrated in Fig. 109, A. As in (2), it occurs under unfavourable conditions (closed chamber, excess of moisture).

(4) The pairs of conjugated primary conidia do not remain seated on the promycelium but, shortly after conjugation has taken place, become detached from the promycelium and settle on the substratum. Here, as shown in Fig. 110, each H-shaped pair of primary conidia develops a sterigma and secondary conidium, and the conidium is shot away into the air by the drop-discharge mechanism. As the secondary conidium is being produced, the protoplasm of the pair of primary conidia passes into it *en masse*, and after the secondary conidium has been discharged, the H-shaped

<sup>1</sup> O. Brefeld, *Untersuchungen über Pilze* : Heft V, 1883, pp. 149-150, Plate XII, Figs. 31a and 32 ; Heft XII, 1895, pp. 161-164, Plate X, Figs. 1, 2, 4, and 6.

pair of primary conidia is left behind as an exhausted and dead structure incapable of further development. The falling away of the H-shaped pairs of primary conidia does not appear to take place if the culture is undisturbed, but it is easily effected by a

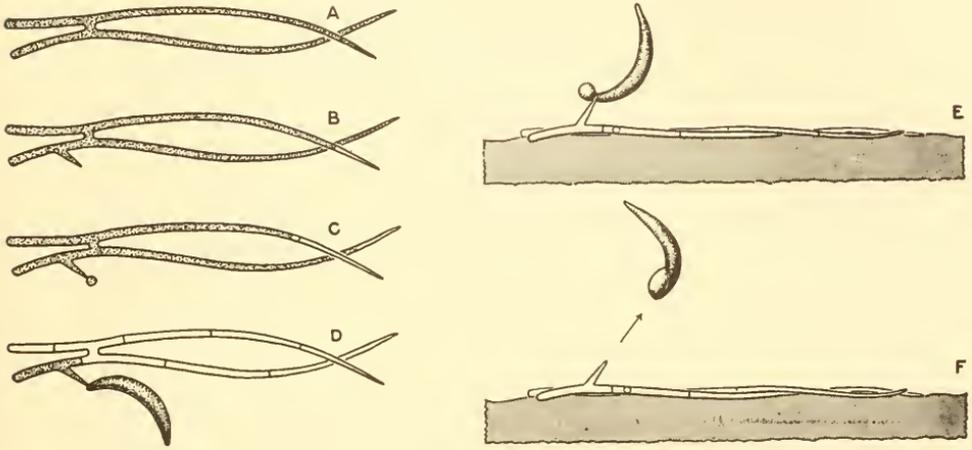


FIG. 110.—*Tilletia tritici*. A semi-diagrammatic representation of a pair of conjugated primary conidia (the authors' primary sterigmata) which was jarred off the promycelium (basidium-body) and fell on to a moist substratum where it produced and shot away a secondary conidium (the authors' primary basidiospore). A, the primary conidia just after falling; B, a few hours later, they are producing a sterigma (the authors' secondary sterigma); C, still later, a secondary conidium (primary basidiospore) is developing at the end of the sterigma; in B and C the protoplasm is creeping out of the ends of the primary conidia and in C a septum has already been formed; D, about 1 hour and 30 minutes after C, the secondary conidium is fully grown and the protoplasm has almost entirely crept into it, the septa were formed during the protoplasmic migration; E, lateral view of the pair of primary conidia lying on its moist substratum, showing the sterigma and the secondary conidium projecting into the air, the protoplasm has now completely migrated into the secondary conidium and the secondary conidium is about to be discharged, as is indicated by the drop of liquid which is being excreted at the spore-hilum; F, about 2 seconds later than E, the secondary conidium and the drop have just been violently discharged from the sterigma and are travelling together. Magnification, 560.

slight mechanical shock. Possibly, under natural conditions, the dislodgment of the primary conidia may sometimes be brought about by wind pressure. The mode of development just described and illustrated in Fig. 110 may be regarded as normal or, if not, as only slightly abnormal; for, in the end, a secondary conidium is produced and discharged into the air and thus a normal climax of development is attained.

(5) As in (4), the pairs of conjugated primary conidia do not remain seated on the promycelium but, shortly after conjugation

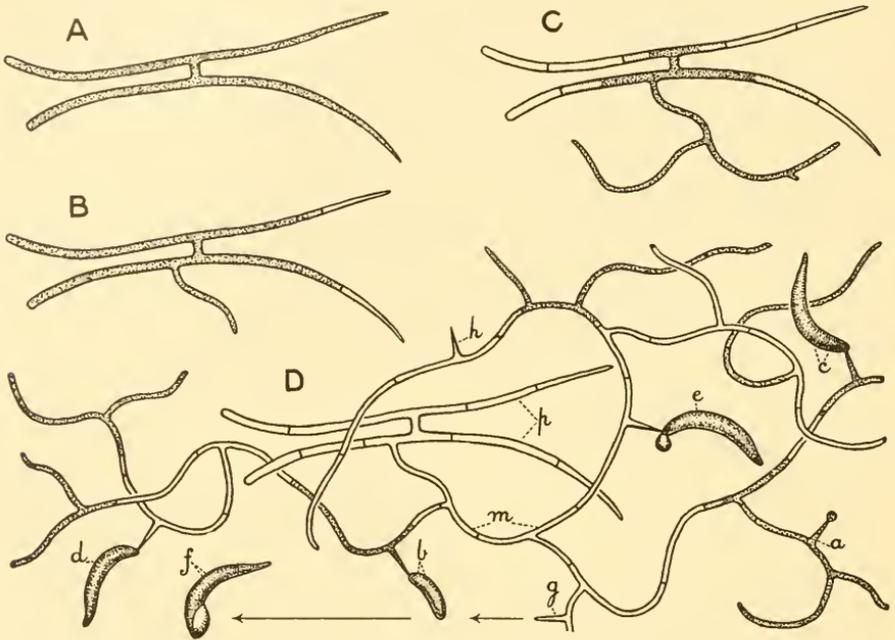


FIG. 111.—*Tilletia tritici*. A semi-diagrammatic representation of a pair of conjugated primary conidia (the authors' primary sterigmata) which was shaken off the promycelium (basidium-body) and fell on to a nutrient substratum of malt-agar and there produced a mycelium which gave rise to secondary conidia (the authors' secondary basidiospores). A, the primary conidia just after falling; B, a few hours later, one of them has sent out a lateral hypha, the protoplasm is creeping out of the ends of the primary conidia and two septa have already been formed; C, still later, the lateral hypha has become branched, the migration of the protoplasm from the primary conidia into the mycelium and the formation of septa in the primary conidia are further advanced; D, some days later, the primary conidia *p*, which have now lost all their protoplasm, have given rise to an extensive mycelium *m* characterised by the winding course of its hyphae and by the formation and discharge of secondary conidia (the authors' secondary basidiospores) developed on short sterigmata: *a*, a very rudimentary secondary conidium symmetrically placed on the end of its sterigma; *b*, a half-grown secondary conidium asymmetrically situated on the end of its sterigma; *c* and *d*, two full-grown secondary conidia, protoplasm is still flowing into *c* but no longer into *d*; *e*, a secondary conidium which is excreting a drop of liquid from its hilum and is about to be discharged; *f*, a secondary conidium and its drop which have just been discharged from the sterigma *g*; *h*, a sterigma from which a secondary conidium has been discharged; the mycelium exhibits septa in its empty parts from which the protoplasm has migrated away into terminal hyphae and secondary conidia. Magnification, 660.

has taken place, become detached from the promycelium and settle on the substratum. Here, as shown in Fig. 111, each H-shaped

pair of primary conidia, instead of producing a secondary conidium as in (4), sends out a slender germ-tube which, if nutriment is available, may develop into an extensive mycelium producing numerous secondary conidia (Fig. 111, D). The falling away of the

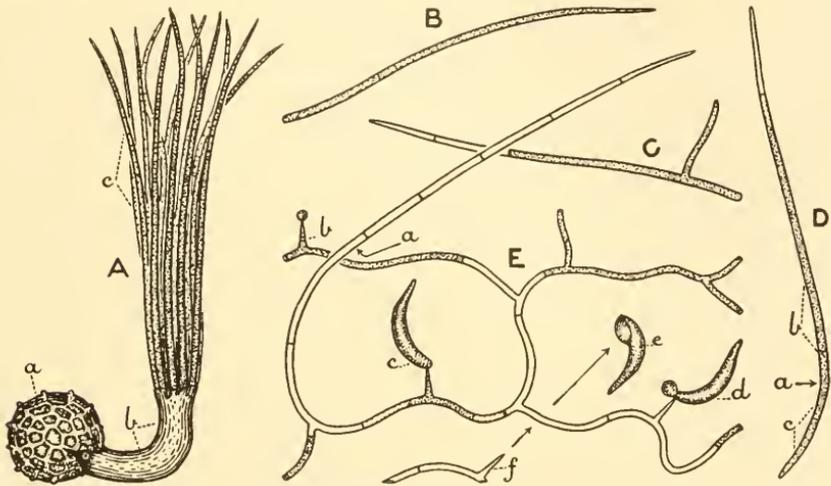


FIG. 112.—*Tilletia tritici*. Germination of a primary conidium (the authors' primary sterigma) when sown by itself. A, a chlamyospore *a* has produced a promycelium (basidium-body) *b* which in turn has produced twelve primary conidia *c*; the conidia are full-grown but have not yet conjugated in pairs. B–D, primary conidia which have been isolated from a basidium like A and have been placed on malt-agar. B, before germination. C, a germ-tube has been put out laterally. D, the conidium *b* at its base *a* has emitted the germ-tube *c*. E, a primary conidium which has germinated at its base *a*, has become septate and empty of protoplasm, and has given rise to a mycelium which has produced secondary conidia (secondary basidiospores) *c*–*e*; the protoplasm of the mycelium is creeping into the secondary conidia and the mycelium is becoming septate; *b*, a spherical rudiment of a secondary conidium on the end of its sterigma; *c*, a full-grown secondary conidium into which protoplasm is still passing; *d*, a secondary conidium about to be discharged, a drop of liquid has been excreted from its hilum within the last six seconds; *e*, a secondary conidium which along with its drop has just been shot away from the sterigma *f*. Drawings based on experiments carried out by W. F. Hanna at the Dominion Rust Research Laboratory. Magnification, 660.

pairs of primary conidia from the promycelium, as in (4), is not spontaneous but is effected by a mechanical shock and possibly by wind pressure. It may be that, under natural conditions, the mode of development just described and illustrated in Fig. 111 actually takes place, but as yet it has not been observed.

We may now consider the development of *unconjugated* primary conidia, and here we encounter a sixth possible mode of development.

(6) As long as primary conidia are growing in length, they remain firmly attached to the top of the promycelium ; but, as soon as they have attained full length and before conjugation has taken place, they can be removed from the promycelium without difficulty. Hanna has removed and sown large numbers of individual unconjugated primary conidia ; many of these conidia have germinated, and a few of them have developed a mycelium bearing secondary conidia, as shown in Fig. 112.

Brefeld <sup>1</sup> observed in *Tilletia zonata* that, if primary conidia, before they have fused with one another, are pressed down beneath the surface of the culture medium, they germinate individually and that the germ-tubes or mycelia to which they give rise may develop secondary conidia. He <sup>2</sup> also observed in *T. controversa* that any unpaired primary conidium may germinate in a nutrient solution and develop a mycelium bearing secondary conidia.

Under natural conditions, doubtless, some primary conidia are left unconjugated and it is possible that some of them succeed in germinating. However, one may regard this mode of development as abnormal and as but little likely to assist the fungus concerned in propagating itself from one generation to another.

**External Conditions and the Germination of the Chlamydospores.**—As is well known, the chlamydospores of *Tilletia tritici* will not germinate unless they are supplied with a sufficient amount of moisture and are kept at a suitable temperature. According to Woolman and Humphrey,<sup>3</sup> the minimum temperature for germination is 0°–1° C., the optimum 18°–20° C., and the maximum 20°–29·1° C.

Some chlamydospores were placed upon agar in a Petri dish and then a thin layer of sterile water was poured over them, so that they were submerged. Some of the spores germinated, and their promycelia grew upwards to the surface of the water. This experiment affords conclusive evidence that chlamydospores are able to germinate even although they are not in direct contact with the air.

<sup>1</sup> O. Brefeld, *loc. cit.*, Heft XII, 1895, p. 161, Plate X, Fig. 6.

<sup>2</sup> *Ibid.*, p. 163.

<sup>3</sup> H. M. Woolman and H. B. Humphrey, *Studies in the Physiology and Control of Bunt or Stinking Smut of Wheat*, U.S. Department of Agriculture, Bull. No. 1239, 1924, pp. 15–18.

However, numerous experiments go to show that chlamydospores germinate much better in direct contact with the air than when submerged in water.

It was found that, other things being equal, chlamydospores germinate just as well in diffuse light as in total darkness.

One of the most important external conditions affecting germination is what, for the present, may be called *ventilation*. Chlamydospores were sown at the surface of a layer of water in a number of Syracuse watch-glasses, and it was found that germination took place far better when the watch-glasses were exposed to the moving air of the laboratory than when they were covered over with large or small bell-jars.

Syracuse watch-glasses (*cf.* Fig. 113, A), when placed one above the other, do not fit exactly, so that crevices are left between them and the air can pass in and out of any one of the chambers. Chlamydospores were sown on water in twelve watch-glasses, and of these two stacks of six each were made. One stack was exposed to the air of the laboratory and the other was covered by a large bell-jar set upon a glass plate. After 4–6 days it was found that the spores in the first stack had germinated, whereas those in the second showed no sign of germination. This experiment was repeated several times, always with the same result. After 8–20 days, in some of the experiments the spores under the bell-jar had still failed to germinate, whilst in other experiments a few of the spores under the bell-jar had germinated. The germ-tubes of these tardily germinating spores did not grow well.

From the results of the experiments just described we may conclude that ventilation favours the germination of the chlamydospores. However, an analysis of the factors included under the term ventilation still remains to be accomplished.

Woolman and Humphrey<sup>1</sup> have supposed that the failure of chlamydospores to germinate in small closed vessels is due to the lack of sufficient free oxygen; but, in the experiment recorded above where the stack of watch-glasses was kept under a bell-jar,

<sup>1</sup> H. M. Woolman and H. B. Humphrey, *Studies in the Physiology and Control of Bunt or Stinking Smut of Wheat*, U.S. Department of Agriculture, Bull. No. 1239, 1924, p. 16.

the chlamydospores had access to all the oxygen contained within the bell-jar. The failure of the spores to germinate in 4-6 days, therefore, can scarcely have been due to lack of oxygen. Obviously insufficient ventilation and insufficient oxygen are not identical conditions. Ventilation includes movement of the air over the surface of the culture medium, and it is possible that this movement is a factor of some importance in germination. Another factor which may be concerned in ventilation is the saturation of the air with moisture. The spores in the stack of watch-glasses exposed to the air of the laboratory must have been subjected to air which was not saturated with water-vapour; whereas, within a few hours of the commencement of the experiment, the spores in the stack exposed to the air contained within a closed bell-jar must have been subjected to air saturated with water-vapour. Perhaps, therefore, saturation of the air with water-vapour is unfavourable to the germination of the chlamydospores.

**The Development and Discharge of Secondary Conidia.**—As we have seen, each H-shaped pair of primary conidia, while still attached to the promycelium or after its fall, may put out a short lateral hypha or sterigma at the end of which a secondary conidium is developed, or it may produce a germ-tube which in a nutrient medium may give rise to a mycelium. This mycelium grows in a peculiar coiling manner, branches and rebranches for a long time, and produces singly, at intervals along its hyphae, many scores or even hundreds of secondary conidia (Fig. 111, D). Normally, every secondary conidium, wherever produced, is violently discharged from its sterigma. The means by which this was discovered will now be described.

Some chlamydospores of *Tilletia tritici* were germinated on agar in a Syracuse watch-glass (Fig. 113, A). On the sixth or seventh day, when secondary conidia were being produced both on the H-shaped primary conidia (Fig. 114, A) and on the mycelium described above (Fig. 114, B and C), a suitable field was chosen and a sterile glass ring was placed over it (Fig. 113, B, *g*). A sterile cover-glass (*c*) was set on the ring; and a piece of moistened filter paper (*f*), perforated so as to allow for the free passage of the microscope, was placed over the watch-glass to prevent the culture

from drying too rapidly. The preparation was then watched for several hours under the low power of the microscope (magnification, 160).

Subsequently, it was found better to employ Petri dishes instead

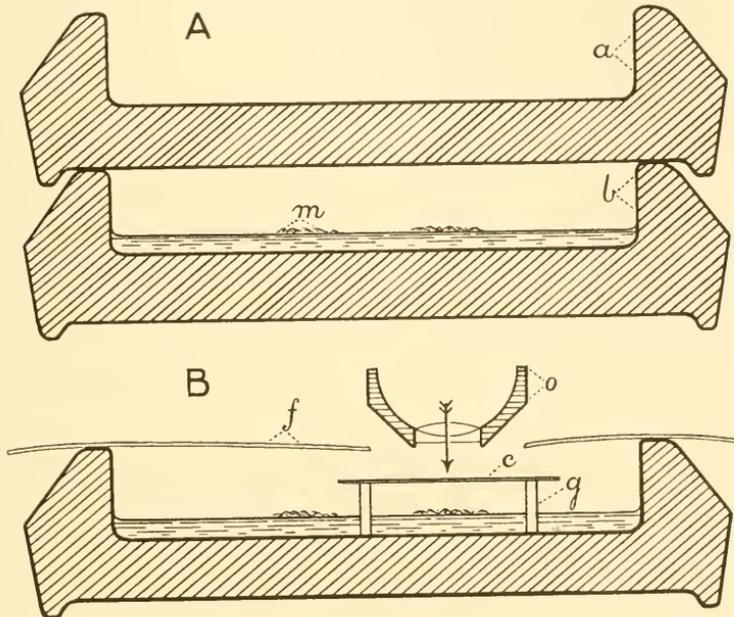


FIG. 113.—The Syracuse watch-glass method for observing the discharge of the secondary conidia (secondary basidiospores) of *Tilletia tritici*. A, two watch-glasses, *a* covering *b*; *m*, a mycelial mat at the surface of the agar seven days after the sowing of the chlamydo-spores. B, arrangement of apparatus while the discharge of secondary conidia is being observed: the upper watch-glass has been removed; a sterile glass ring *g* covered by a sterile cover-glass *c* has been placed around a mycelial mat; *f*, a perforated sheet of moistened filter paper; *o*, the low power of the microscope with the arrow indicating the direction of observation. Natural size.

of Syracuse watch-glasses, as with the former it was easier to keep the cultures pure.

Under the conditions just described, the development and discharge of secondary conidia was observed in detail.

Sterigmata are never developed in the culture medium but always in the air above it (*cf.* Fig. 114, C). Each sterigma is a short slender conical structure. As soon as it has attained its full length, the rudiment of a secondary conidium begins to appear at its apex.

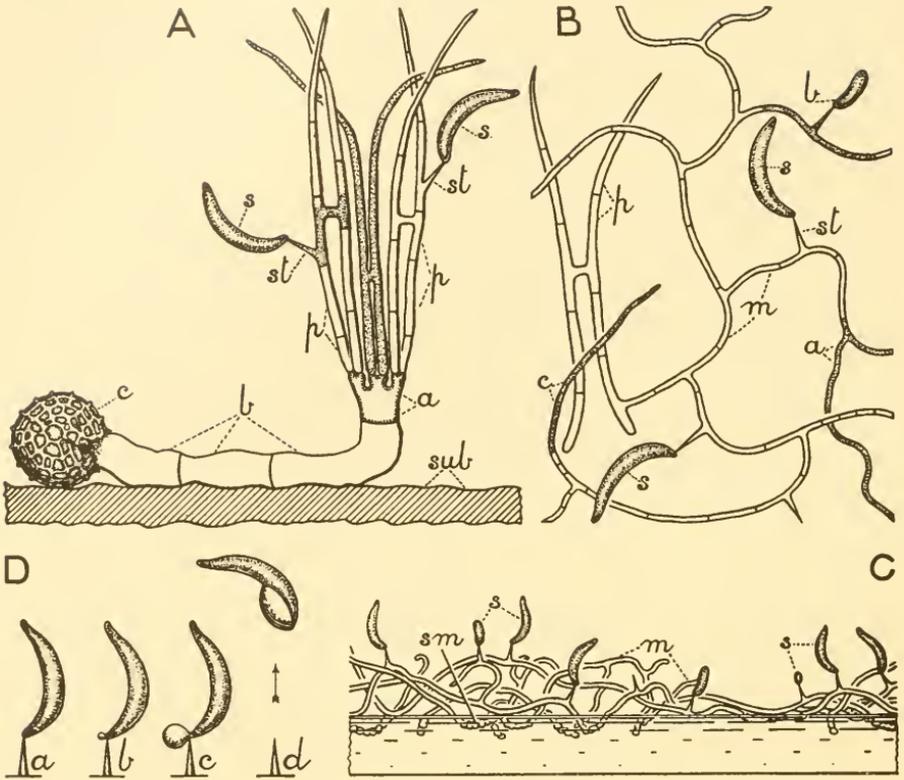


FIG. 114.—*Tilletia tritici*. The development and mode of discharge of so-called secondary conidia (the authors' primary and secondary basidiospores). A : the chlamydospore *c* has germinated on a substratum of malt-agar *sub* and has produced a promycelium (basidium-body) consisting of three basal cells *b*, which have lost their protoplasmic contents and are dead, and of an apical cell *a*, which is lined with cytoplasm and is still living. The promycelium may be supposed to have been crowned by twelve long and slender primary conidia (primary sterigmata) *p p* which have conjugated in pairs ; but, for the sake of clarity, only the front three pairs have been represented. The protoplasm migrated from the promycelium into the primary conidia and, during this migration, the promycelium developed three septa in succession from its base to its apex. The right and left pairs of primary conidia have each produced a short sterigma (secondary sterigma) *st* and a secondary conidium (primary basidiospore) *s*. The protoplasm in the pair of primary conidia on the right migrated into the secondary conidium *s* and, during the migration, the primary conidia became exhausted and septate. In the pair of primary conidia on the left the migration of the protoplasm into the secondary conidium is nearly, but not quite, completed. The middle pair of primary conidia has only recently conjugated and has not yet produced a sterigma and a secondary conidium. Hence it is still full of protoplasm and not yet septate. The secondary conidia (primary basidiospores) *s s*, within about an hour, would be shot from their sterigmata by the drop-excretion method illustrated in D. Magnification, 660.

B : a surface view of a pair of primary conidia (primary sterigmata) *p* which has fallen on to the nutrient substratum (malt-agar) and has developed directly into a branched and somewhat coiled mycelium *m*. The mycelium, at intervals,

This rudiment is at first globular (Figs. 108, p. 222, and 110, C, p. 225), but soon it develops more on one side than on the other, so that it grows upwards from the axis of the sterigma at an angle of about  $45^\circ$ . As growth continues, the conidium becomes curved and, when fully grown, it is sickle-shaped (Fig. 114, A–D, *s*). The time taken for a conidium to grow from a tiny rudiment to full size is from an hour and fifteen minutes to an hour and a half.

During the growth and maturation of a secondary conidium developed on an H-shaped pair of primary conidia, the cytoplasm and the two nuclei which these contain are gradually transferred to the secondary conidium, so that in the end the H-shaped pair of primary conidia becomes emptied of its contents. As emptying proceeds, the two primary conidia become septate, the septa being produced in succession and serving to cut off those parts of the conidia which have become free from protoplasm (Fig. 114, A). The process of septation in the promycelium is of a similar nature and will be described in detail in a Section called *The Phenomenon of Protoplasmic Migration*.

During the growth and maturation of a secondary conidium developed on a mycelium (Fig. 114, B), a mass of protoplasm is transferred from the mycelium *via* the sterigma to the conidium. The hypha concerned thus gradually loses its contents and, like the H-shaped pair of secondary conidia just described, becomes septate.

As soon as a secondary conidium has attained maturity (Fig. 114, D, *a*), a tiny drop of liquid begins to be excreted from the spore

FIG. 114—*cont.*

has produced sterigmata *st* which bear secondary conidia (secondary basidiospores) *s*. The protoplasm migrated out of the pair of primary conidia and out of the mycelium, except at *a*, *c*, and below the immature conidium *b*, and, during the migration, the primary conidia and the mycelium became septate. The secondary conidia (secondary basidiospores) *s s* are shot from their sterigmata by the drop-excretion method illustrated in D. Magnification, 660.

C: a diagrammatic representation of a mycelial mat at the surface of malt-agar. Part of the mycelium *sm* is submerged and somewhat knotty, while the rest of the mycelium *m* is aerial. The aerial part of the mycelium bears sterigmata and secondary conidia (secondary basidiospores) *s s* which in general are directed away from the substratum. Magnification, about 330.

D: stages in the discharge of a secondary conidium (primary or secondary basidiospore) of the kind shown in A, B, and C: *a*, a full-grown conidium (basidiospore) on its sterigma; *b*, about two seconds later, a drop of liquid has just been excreted from the spore-hilum; *c*, about 18 seconds after *b*, the drop has now attained its maximum size; *d*, about one second after *c*, the spore and the drop have been violently shot away from the sterigma. Magnification, 767.

at the spore-hilum, *i.e.* at that part of the spore which bulges outwards just above the top of the sterigma (*b*). The drop grows visibly, protruding laterally, and in about 20 seconds attains a diameter equal to about one and one-half times the thickness of the spore (*c*). Then, suddenly, the spore and the drop are shot away from the sterigma together and disappear from view (*d*). The sterigma left behind is apparently unchanged. The hyphal cell below it, with which it is continuous, can often be seen at this stage to have a thin layer of cytoplasm lining its cell-wall. This layer doubtless extends into the sterigma.

Violent discharge of the secondary conidia of *Tilletia laevis* was found to take place in exactly the same manner as in *T. tritici*.

**Discussion of the Significance of Violent Spore-discharge in *Tilletia tritici*.**—The chief conclusions drawn by Buller and Vanderpool in respect to the theoretical significance of violent spore-discharge were but briefly stated in their original communication.<sup>1</sup> They will now be more fully discussed.

The asymmetrical development of a secondary conidium of *Tilletia tritici* on a conical sterigma and the discharge of the conidium with drop-excretion exactly resemble the processes of development and discharge of a basidiospore as described by Buller<sup>2</sup> for the Hymenomycetes and by Dietel<sup>3</sup> and Buller<sup>4</sup> for the Uredineae. This resemblance can have no other meaning than that *the sickle-shaped secondary conidia of Tilletia tritici are the true basidiospores of this fungus.*

If the sickle-shaped so-called secondary conidia are the true basidiospores of *Tilletia tritici*, what then are the long slender primary conidia which hitherto have always been considered homologous with basidiospores? The answer is: *sterigmata of a highly specialised type.* The evidence supporting this conclusion is as follows: (1) the primary conidia are produced on the end of the promycelium or basidium-body, (2) they are slenderly conical and

<sup>1</sup> A. H. R. Buller and T. C. Vanderpool, "Violent Spore-discharge in *Tilletia tritici*," *Nature*, Vol. CXVI, 1925, pp. 934-935.

<sup>2</sup> A. H. R. Buller, *Researches on Fungi*, Vol. II, 1922, pp. 4-18.

<sup>3</sup> P. Dietel, "Über die Abschleuderung der Sporidien bei den Uredineen," *Mycologisches Centralblatt*, Bd. I, 1912, pp. 355-359.

<sup>4</sup> A. H. R. Buller, *Researches on Fungi*, Vol. III, 1924, pp. 497-519.

taper to a point, (3) whilst still attached to the promycelium they may give rise directly to basidiospores, and (4) they are never shot away and do not serve to disseminate the fungus. In all these characteristics the primary conidia of *Tilletia tritici* resemble the sterigmata of the Hymenomycetes.

Further evidence, and evidence of a weighty kind, supporting the view that the sickle-shaped so-called secondary conidia are the true basidiospores and that the structures which support them are not in reality primary conidia but sterigmata is furnished by the comparative morphology of the basidial apparatus in the Tilletiaceae in general. Let us consider *seriatim* the basidial apparatus of (1) *Urocystis violae*, (2) *Tubercinia trientalis*, (3) *Tilletia tritici*, and (4) *Neovossia moliniae*. (1) In *Urocystis violae* (Figs. 115 and 116) the fact that the primary conidia and the secondary conidia are in reality nothing but sterigmata and spores respectively seems obvious from their shape and appearance<sup>1</sup> and, involuntarily, the basidial apparatus of this species reminds one of the basidia of *Tulasnella*, one of the Hymenomycetes. (2) In *Tubercinia trientalis* the primary conidia look like fat sterigmata, but they conjugate basally in pairs whilst still attached to the basidium-body.<sup>2</sup> As a result of conjugation, a single secondary conidium is produced *terminally* on the end of one of each pair of conjugated elements. Again the whole basidial

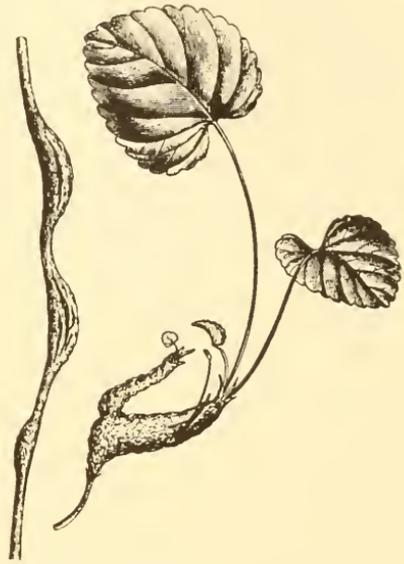


FIG. 115.—Swellings in the tissues of *Viola odorata* caused by *Urocystis violae*. After Dietel. From *Die natürlichen Pflanzenfamilien*. Natural size.

<sup>1</sup> O. Brefeld, *Untersuchungen über Pilze*, Münster, Heft XII, 1895, Taf. XI, Fig. 10.

<sup>2</sup> M. Woronin, "Beiträge zur Kenntniss der Ustilagineen," in de Bary and Woronin's *Beiträge zur Morph. u. Phys. der Pilze*, No. 5, 1882, Taf. III, Figs. 1-12.

apparatus when fully developed looks like a basidium of a Hymenomycete, but one into which conjugation of the sterigmata has been introduced. (3) We now come to *Tilletia tritici*. Here the basidial apparatus, as compared with that of *Tubercinia trientalis*, has undergone a considerable degree of further modification. The primary

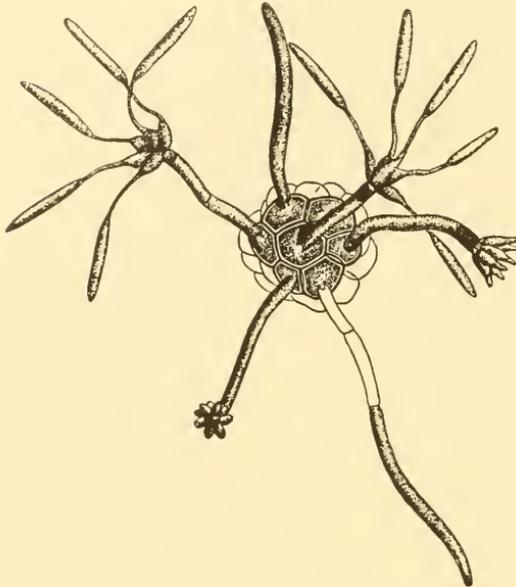


FIG. 116.—*Urocystis violae*. A spore-ball germinating in water. Each spore is producing what the authors interpret as a basidium consisting of (1) a cylindrical basidium-body, (2) a group of sterigmata swollen at their bases, and (3) a single basidiospore terminating each sterigma. After Brefeld. From *Die natürlichen Pflanzenfamilien*. Magnification, 350.

conidia (sterigmata) have become greatly elongated, conjugation takes place usually about the middle of the primary conidia, and each secondary conidium (basidiospore) arises not on the end of a primary conidium (sterigma) as in *Tubercinia trientalis*, but on a lateral branch (secondary sterigma) of one of the two paired primary conidia. (4) Finally, in *Neovossia moliniaie*, the primary conidia (sterigmata), while rod-like like those of *Tilletia tritici*, do not, so far as is known, conjugate with one another, and

they are increased in number to over fifty on each basidium. When these stick-like primary conidia germinate, they each give off a lateral branch (secondary sterigma) on the end of which, as in *Tilletia tritici*, there develops a sickle-shaped secondary conidium (basidiospore) which doubtless is shot into the air by means of the drop-excretion mechanism.<sup>1</sup> In the basidial apparatus of the species of *Urocystis*, *Tubercinia*, *Tilletia*, and *Neovossia* which have been considered we seem to have a progressive series leading

<sup>1</sup> O. Brefeld, *loc. cit.*, Taf. X, Figs. 8-21.

from a type which resembles the basidium of one of the Hymenomycetes to a type which appears to be like the basidium of one of the Hymenomycetes but very greatly modified.

Granted that the primary conidia of *Tilletia tritici* are in reality sterigmata, we must assume that these sterigmata are highly specialised. The evidence for this specialisation is indicated by the following facts : (1) the sterigmata are extremely long and slender ; (2) the sterigmata conjugate in pairs by means of a bridging hypha, thus differing from the sterigmata of the Hymenomycetes and the Uredineae ; (3) not every sterigma but only one of each pair of sterigmata produces a basidiospore ; (4) the basidiospores do not develop at the apices of the main shafts of such sterigmata as bear them ; but (5) each conjugated H-shaped pair of sterigmata sends out laterally a short conical branch on which the basidiospore is developed, so that the sterigmata are branched.

In the heterothallic Hymenomycetes, e.g. *Coprinus lagopus*, the process of conjugation takes place between two mycelia derived from two spores of opposite sex. In *Tilletia tritici* the conjugation process has been put back a stage, so that it takes place not between two haploid mycelia or two haploid spores but between two haploid sterigmata, and it is therefore accomplished before the basidiospores are formed. The sterigmata are specialised for the sexual process : (1) by being very elongated and curved, so that sterigmata of opposite sex often come near enough to one another to allow of conjugation ; and (2) by having the power of sending out bridging hyphae toward one another through the air. The production of one basidiospore only from two conjugated sterigmata is evidently correlated with the sexual process. The nucleus of one sterigma passes *via* the bridging hypha into the other sterigma and thus the two nuclei become associated as a pair. To receive this pair of nuclei only one basidiospore is required. Since the sterigmata are so very long and slender, and since conjugation takes place usually near the middle of each pair, it is perhaps not surprising that the basidiospore is developed not at the tip of one of the main shafts of the two sterigmata but on a short branch or secondary sterigma which has a lateral origin from one of the two conjugated primary sterigmata.

From the foregoing it appears that, contrary to the view held by Brefeld, the basidium of *Tilletia tritici* is not primitive, but rather it is more highly developed than the basidium of the Hymenomycetes and the Uredineae. Its specialisation is doubtless correlated with the peculiar parasitic mode of life of the fungus which produces it.

A recognition of the fact that *Tilletia tritici* produces true basidiospores which resemble in their special form and mode of discharge the basidiospores of the Hymenomycetes and the Uredineae strengthens the view generally held by botanists that the Tilletiaceae belong to the great group of the Basidiomycetes.

It has already been mentioned that violent basidiospore-discharge with drop-excretion takes place not only in *Tilletia tritici* and *T. laevis* but also in other species of *Tilletia* and in *Entylooma menispermii*, *E. lobeliae* and *E. linariae*. Judging by the form of the sterigmata (primary conidia) and of the basidiospores (secondary conidia), as illustrated by Brefeld and others, it is probable that violent basidiospore-discharge takes place in *Neovossia* and possibly in some other genera of the Tilletiaceae; but the discovery of violent spore-discharge in *Ustilago* is scarcely to be expected, as the sporidia in this genus are not asymmetrical in form and are not situated on slender conical sterigmata. According to Buller,<sup>1</sup> when a basidiospore is shot away with drop-excretion, the sterigma acts as an organ to bring about the discharge. The absence of a typical sterigma and the absence of violent basidiospore-discharge seem to be correlated.

A malt-agar plate bearing rapidly multiplying sporidia of *Ustilago zae* was inverted over a freshly-poured second malt-agar plate in the manner shown in Fig. 119 (p. 246), but no sporidia fell from the upper plate, so that no new colonies of sporidia were formed in the lower plate. This experiment clearly indicates that the sporidia of *Ustilago zae* are not violently discharged.

**Terminology, New and Old.**—In subsequent Sections, a new nomenclature based on the discussion in the last Section will be adopted. The whole product of a chlamydospore, such as that shown in Fig. 108 (p. 222) and in Fig. 114, A (p. 232), is conceived

<sup>1</sup> A. H. R. Buller, these *Researches*, Vol. II, 1922, p. 31.

of as a *basidium*. The secondary conidia of Brefeld and others will be called *basidiospores*, and the primary conidia of Brefeld and others *sterigmata*. The shafts of the sterigmata which crown the promycelium will be referred to as *primary sterigmata*, while the short conical branch which an H-shaped pair of primary sterigmata puts out laterally and which bears a basidiospore will be referred to as a *secondary sterigma*. The promycelium is homologous with the *basidium-body* of the Hymenomycetes and the Uredineae. The basidiospores which are produced on the secondary sterigmata may be called *primary basidiospores* and those which are produced on a mycelium *secondary basidiospores*. The sterigmata which bear these secondary basidiospores may be referred to as *mycelial sterigmata* or simply as sterigmata.

The new and the old or Brefeldian terminology, as used for *Tilletia tritici*, may be summarised in tabular form as follows :

*Terminology for Tilletia tritici.*

Now proposed	Brefeldian
Basidium { <ul style="list-style-type: none"> <li>Basidium-body . . . . .</li> <li>Primary sterigma . . . . .</li> <li>Secondary sterigma . . . . .</li> <li>Primary basidiospore . . . . .</li> </ul>	Basidium { <ul style="list-style-type: none"> <li>Promycelium.</li> <li>Primary conidium or basidiospore.</li> </ul> Sterigma. Secondary conidium.
Mycelium { <ul style="list-style-type: none"> <li>produced from a primary basidiospore, or a conjugated pair of primary sterigmata, or a secondary basidiospore.</li> </ul>	Mycelium { <ul style="list-style-type: none"> <li>produced from a secondary conidium or a conjugated pair of primary conidia.</li> </ul>
Mycelial sterigma . . . . .	Sterigma, produced on a mycelium.
Secondary basidiospore { <ul style="list-style-type: none"> <li>produced on a mycelial sterigma.</li> </ul>	Secondary conidium.

**Nuclei and Sex.**—A few remarks will now be made on the nuclear condition and sexual processes of *Tilletia tritici*. The new terminology will be employed.

We have seen<sup>1</sup>: that two nuclei, presumably of opposite sex, fuse together in a young chlamydospore; that the fusion nucleus undergoes division producing a number of haploid nuclei; that these nuclei pass up into the primary sterigmata; and that, when two primary sterigmata have conjugated, two nuclei, presumably of opposite sex, pass through the secondary sterigma into a primary basidiospore.

The conjugation process just described, resulting in primary basidiospores containing two nuclei, indicates that *Tilletia tritici*, in the stage of its life-history under discussion, is *homothallic*.

According to Boss,<sup>2</sup> the two nuclei which enter a primary basidiospore do not fuse and do not long remain together; for, when a primary basidiospore germinates, the two nuclei separate from one another and divide separately. Of the crop of haploid nuclei so produced in the mycelium, a single nucleus enters each secondary basidiospore, so that secondary basidiospores differ from primary basidiospores in being haploid instead of diploid.

Boss also found that, when an H-shaped pair of sterigmata germinates directly (*cf.* Fig. 111, p. 226), the two nuclei which go into the germ-tube separate and that the secondary basidiospores produced on the mycelium, like those described in the preceding paragraph, contain one nucleus only.

From Boss's nuclear studies it is to be concluded that some secondary basidiospores are (+) and others (—) in sex.

A secondary basidiospore, on germinating, gives rise to a unisexual mycelium. When one considers the nuclear condition of the secondary basidiospores and of the mycelia which they produce, we are obliged to regard *Tilletia tritici*, at the stage of its life-history under discussion, as *heterothallic*.

It thus appears that we must regard *Tilletia tritici* as homothallic in one stage of its life-history and heterothallic in another stage.

There is the possibility, even the likelihood, that two haploid mycelia derived from two haploid secondary basidiospores of opposite sex, (+) and (—), may meet in the tissues of the host plant, fuse

<sup>1</sup> *Vide supra*, p. 218.

<sup>2</sup> G. Boss, "Beiträge zur Zytologie der Ustilagineen," *Planta*, Bd. III, 1927, pp. 619-622.

together, and so give rise to a diploid mycelium which may produce chlamydospores.

The fact that a primary basidiospore contains two nuclei, one (+) and one (—), and that a secondary basidiospore contains only one nucleus, (+) or (—), must be of considerable importance in respect to the infection of host plants and the production of bunt balls. One would expect that, if primary basidiospores, which are bisexual, were sown on a host-plant, diploid bunt balls would be produced; and one would also expect that, if one host-plant were inoculated with (+) secondary basidiospores only, a second with (—) secondary basidiospores only, and a third with both (+) and (—) secondary basidiospores (or mycelia derived from them), bunt balls would appear on the third plant but not on the first two.

The ideas, based on cytological facts, which have just been suggested, find a strong support in the phytopathological work of Hanna<sup>1</sup> who has observed: (1) that primary basidiospores allowed to fall on wheat seedlings give subsequently a good crop of bunted heads; (2) that (+) secondary basidiospores, or mycelia derived from them, give no bunted heads; (3) that (—) secondary basidiospores, or mycelia derived from them, give no bunted heads; and (4) that mixed (+) and (—) secondary basidiospores, or mycelia derived from them, give bunted heads.

In a diploid mycelium which has been formed in a host-plant, the nuclei are associated in conjugate pairs and, during conjugate nuclear division, clamp-connexions<sup>2</sup> are formed. Finally, in a young chlamydospore the two nuclei of a conjugate pair fuse together.

The existence of one nucleus only in secondary basidiospores has been observed by one of us (T.C.V.) in *Tilletia tritici*, *T. laevis*, and *T. horrida*; and Hanna<sup>3</sup> has found no more than a single nucleus in the secondary basidiospores of *Entyloma menispermi*. By the spore-fall method, shortly to be described, Vanterpool and Hanna had no difficulty in collecting good spore-deposits of secondary

<sup>1</sup> W. F. Hanna, Dominion Rust Research Laboratory, personal communication.

<sup>2</sup> R. Seyfert, "Über Schnallenbildung im Paarmyzel der Brandpilze," *Zeitschrift f. Botanik*, Vol. XI, 1927, pp. 577-601.

<sup>3</sup> W. F. Hanna, Dominion Rust Research Laboratory, personal communication.

basidiospores derived from mycelial mats like that shown in Fig. 114, C (p. 232). The secondary basidiospores were then fixed and stained with iron-alum haematoxylin. In every secondary basidiospore of the *Tilletiae* and of the *Entylo* investigated there was one nucleus only and never two (Fig. 117).



FIG. 117.—*Tilletia tritici*. Secondary basidiospores (Brefeld's secondary conidia) from a mycelium like that shown at D in Fig. 111 (p. 226) or at C in Fig. 130 (p. 260) stained with iron-alum haematoxylin. There is only one nucleus in each spore. Magnification, about 480.

A separation of nuclei, comparable with that which takes place when a primary basidiospore of *Tilletia tritici* germinates has been found by Bauch<sup>1</sup> in *Ustilago longissima* and its variety *macrospora* and by Dickinson<sup>2</sup> in *Ustilago laevis* and *U. hordei*. They observed that, after two haploid hyphae have conjugated and two nuclei of opposite sex have become associated in the same cell, when this cell produces sporidia the nuclei of opposite sex separate again, one going into one sporidium and the other into another sporidium, so that all the subsequent sporidia are uninucleate and haploid. Gilmore<sup>3</sup> has observed a similar phenomenon in *Psilocybe coprophila*, one of the Hymenomycetes. Here a diploid mycelium with clamp-connexions gives rise to haploid uninucleate oidia. Vandendries and Martens<sup>4</sup> have recently found that, in *Pholiota aurivella*, the diploid mycelium produces both haploid and diploid oidia.

**Abnormal Basidiospore-discharge.**—When basidiospores are developed in closed chambers in artificial cultures, they frequently

<sup>1</sup> R. Bauch, "Über *Ustilago longissima* und ihre Varietät *macrospora*," *Zeitschrift f. Botanik*, Jahrg. XV, 1923, pp. 241-279.

<sup>2</sup> S. Dickinson, "Experiments on the Physiology and Genetics of the Smut Fungi.—Hyphal-Fusion," *Proc. of the Roy. Soc., B*, Vol. CI, 1927, pp. 126-135.

<sup>3</sup> K. A. Gilmore, "Culture Studies in *Psilocybe coprophila*," *Botanical Gazette*, Vol. LXXXI, 1926, pp. 419-433, Plates XXXII and XXXIII.

<sup>4</sup> R. Vandendries and P. Martens, "Oidies haploïdes et diploïdes sur Mycélium diploïde chez *Pholiota aurivella*," *Bull. de l'Acad. roy. de Belgique, classe des Sciences*, T. XVIII, 1932, pp. 468-472.

exhibit abnormalities in connexion with their discharge. These abnormalities are doubtless due to the lack of ventilation of the air of the chambers, and it seems probable that one of the ventilation factors adversely affecting spore-discharge is saturation of the air with water-vapour. The abnormalities about to be described were all observed in closed glass-ring chambers with the low power of the microscope (magnification, 160).

The semi-diagrammatic Fig. 118 shows an H-shaped pair of primary sterigmata, *st*, which has sent out a hypha which on malt-agar has produced a winding and branched mycelium *m*. At intervals along the hyphae of the mycelium have been developed a number of short sterigmata each of which bears a single sickle-shaped secondary basidiospore. At *a* is shown a basidiospore which is just fully

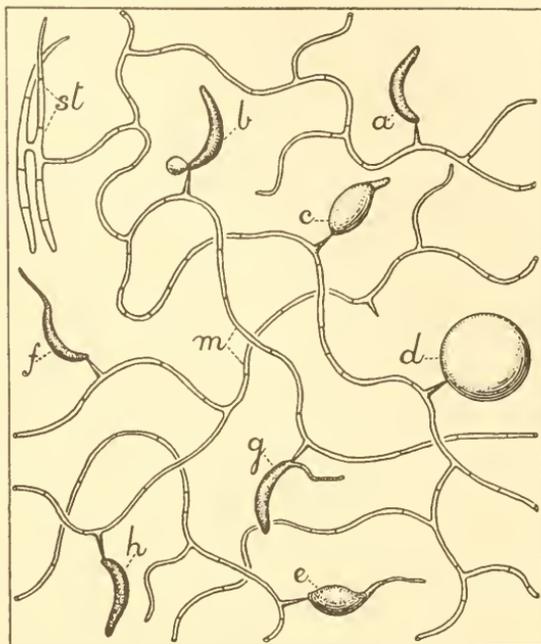


FIG. 118.—*Tilletia tritici*. Abnormalities in the discharge of secondary basidiospores (Brefeld's secondary conidia) represented semi-diagrammatically. An H-shaped pair of primary sterigmata (primary conidia) *st* has germinated on malt-agar and has produced a winding and branched mycelium *m*; *a*, a sickle-shaped basidiospore fully grown, borne on the end of a short sterigma; *b*, another basidiospore which, within the last 20 seconds, has excreted a drop of liquid of normal size at its hilum; *c*, the drop has become abnormally large and has run up on to the basidiospore; *d*, the drop has become so large that it has enveloped and hidden the basidiospore; *e*, a basidiospore, covered by an abnormally large drop, which has not been discharged but has germinated *in situ*, the germ-tube having been emitted from its apex; *f*, a basidiospore which did not excrete a drop of liquid, was not discharged, and has emitted a germ-tube at its apex; *g*, an undischarged basidiospore which has emitted a germ-tube at its base; *h*, a basidiospore which did not excrete a drop of liquid, was not discharged and, even after several hours, has not germinated. Magnification, about 330.

At *a* is shown a basidiospore which is just fully

grown ; while at *b* is shown another basidiospore which, within the last 20 seconds, has excreted a drop of liquid at its hilum. The drop is now of full size and the spore should be shot away within the next two seconds: A series of abnormalities is shown in connexion with the basidiospores *c-h*.

At *c* in Fig. 118, drop-excretion began normally but, instead of the spore and drop being shot away when the drop had attained full size, the drop continued to grow and became larger and larger until it ran up the spore. The drop may cease to grow when it has become as great as that shown on the spore at *c*, or it may continue to enlarge by further excretion from the spore-hilum until it forms a perfect sphere 16–20  $\mu$  in diameter, as represented at *d*. The drop then completely encloses the spore and hides it from view. Basidiospores which bear drops like those shown at *c* and *d* are never violently discharged from their sterigmata ; but, subsequently, either before the drop has evaporated as shown at *e* or after it has evaporated as shown at *f*, they may germinate *in situ*. A spore which has borne an abnormally large drop may germinate either apically as shown at *e* and *f* or basally as shown at *g*. Sometimes a spore, after attaining full size in the normal manner, may fail to excrete a drop. Such a spore, *e.g.* the one shown at *h*, is never discharged violently from its sterigma. It may germinate apically, in which case it resembles the spore shown at *f* or it may remain unchanged on the top of its sterigma for many hours or even as long as three days.

The abnormalities of the basidiospore-discharge mechanism just described for *Tilletia tritici* closely resemble the abnormalities in the same mechanism which are known to occur in the Hymenomycetes, the Uredineae, and the genus *Sporobolomyces*. Thus excessive excretion of drops correlated with an absence of violent spore-discharge has been illustrated : by Buller for the basidiospores of *Psalliota campestris*<sup>1</sup> and *Coprinus sterquilinus*<sup>2</sup> in the Hymenomycetes ; by Buller for *Puccinia graminis*<sup>3</sup> in the Uredineae ; and by Kluver and van Niel<sup>4</sup> for *Sporobolomyces salmonicolor*, and by

<sup>1</sup> *These Researches*, Vol. III, 1924, p. 508, Fig. 205, D–G.

<sup>2</sup> *Ibid.*, p. 250, Fig. 106.

<sup>3</sup> *Ibid.*, p. 508, Fig. 205, A.

<sup>4</sup> A. J. Kluver and C. B. van Niel, "Über Spiegelbilder erzeugende Hefenarten und die neue Hefengattung *Sporobolomyces*," *Centralb. f. Bakteriologie*, Abt. 2, Bd. LXIII, 1924–25, p. 16, Taf. I, Fig. 12.

Buller<sup>1</sup> for *S. roseus*. Buller<sup>2</sup> also observed the germination of the basidiospores of *Puccinia graminis in situ*, i.e. whilst they were still seated on their sterigmata. However, up to the present, in the Hymenomycetes and the Uredineae, no undischarged basidiospores have been seen to germinate basally like the one shown in Fig. 118 at *g*.

**A Basidiospore-discharge Method of making Pure Cultures.**— Previous investigators who have sown chlamydospores from which they have obtained a saprophytic mycelium may always have had in their pure cultures a mixture of mycelia of two different origins : (1) a mycelium derived directly from one or more H-shaped pairs of sterigmata (Fig. 114, B, p. 232), and (2) a mycelium derived from basidiospores which have been borne on sterigmata (Fig. 130, C, p. 260). Taking advantage of the fact that basidiospores normally are always shot away from their sterigmata, the following simple and convenient method of making pure cultures of basidiospore origin only has been developed.

Chlamydospores are germinated on 2 per cent. plain agar in a Petri dish in the manner already described.<sup>3</sup> After six or seven days, basidiospores are being actively produced on sterigmata borne on promycelia (Fig. 114, A, p. 232) and on sterigmata borne on mycelia (Fig. 114, B and D). The Petri-dish culture may now be used for making another Petri-dish culture.

The original Petri dish containing the fungus is inverted, so that the agar looks downwards. The base of the dish is now held in the right hand while the cover is removed with the left hand. The left hand is now used to remove the cover from a freshly-poured malt-agar plate of the same size as the first ; and then, with the right hand, the original inverted plate-base is set directly over the new plate-base, so that a single closed chamber is formed. To keep off air-currents, the combination is now set on a sheet of glass and covered with a small bell-jar (Fig. 119).

Basidiospores continue to develop in large numbers at the surface of the agar in the upper inverted Petri-dish base and, in due

<sup>1</sup> This volume of *Researches*, Fig. 91, p. 184.

<sup>2</sup> These *Researches*, Vol. III, 1924, p. 503, Fig. 203, *h-l*.

<sup>3</sup> *Vide supra*, p. 217.

course, they are shot away successively into the air. They fall through the air, as represented diagrammatically in Fig. 119, and settle on the surface of the malt-agar in the lower upright Petri-dish

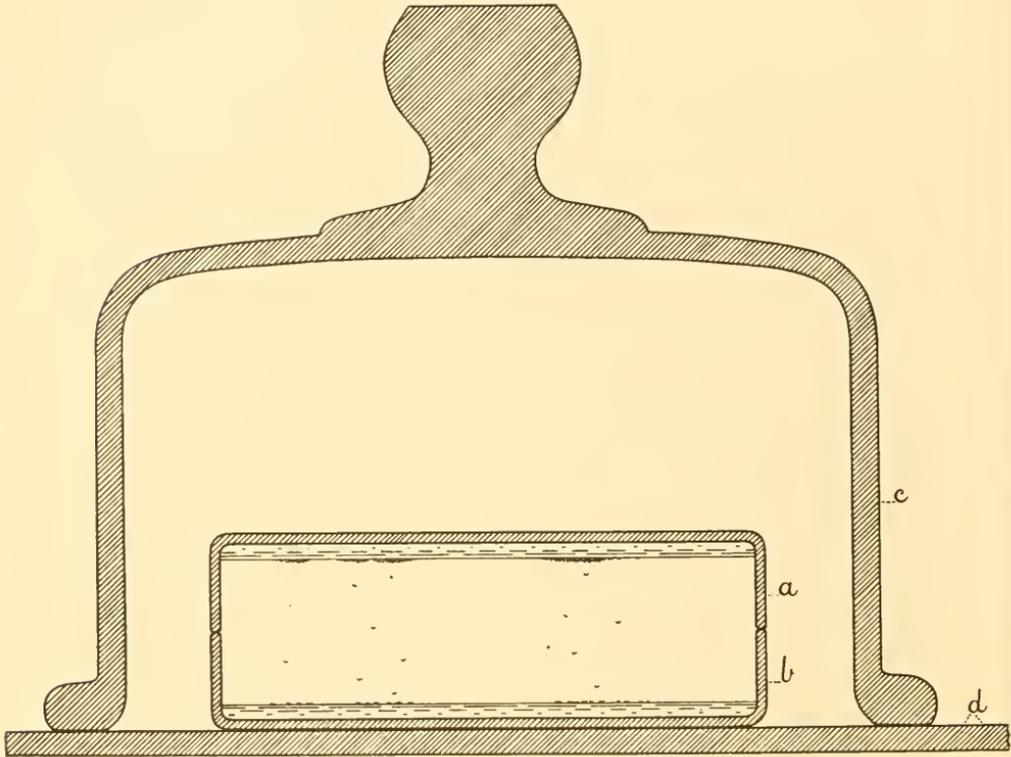


FIG. 119.—*Tilletia tritici*. The making of a new culture by the basidiospore-discharge method. Vertical section through the apparatus employed: *a*, a malt-agar plate (Petri dish) with mycelial mats at the surface of the agar, inverted over a newly poured malt-agar plate *b*; *c*, a bell-jar resting on a sheet of glass *d*. The basidiospores are shot from sterigmata produced by the mycelium and, as shown diagrammatically, fall through the air and settle on the sterilised agar in the lower plate. The plate *b* is now sufficiently inoculated with basidiospores and may be removed and covered with a sterilised Petri-dish cover. Falling spores diagrammatically enlarged; apparatus about three-quarters the natural size.

base. The fall of the basidiospores on to the new dish is allowed to proceed for about three hours. At the end of this time, the old inverted Petri-dish base is removed. The lower Petri-dish base is then covered with its own cover (of which due care has been taken) and set under a bell-jar. The basidiospores which fell on to the

malt-agar in the dish germinate rapidly and in large numbers, so that numerous well-distributed new mycelia, derived entirely from *basidiospores*, come into existence within a few days (Fig. 120). The new culture may now be designated *sub-culture No. 1*.

Oatmeal-agar or Leonian agar may be used instead of malt-agar



FIG. 120.—*Tilletia tritici*. The basidiospore-discharge method of making pure cultures. Basidiospores were allowed to fall from a mycelium in an inverted agar dish on to the surface of the malt-agar in the Petri dish here shown (cf. Fig. 119). The photograph shows the appearance of the mycelium derived from the basidiospores eight days after inoculation. Natural size.

as the nutrient medium in making sub-culture No. 1. On any of these media the young mycelia grow well and, in the course of two to three weeks, form continuous white mats.

The mycelium derived from the basidiospores in sub-culture No. 1 progresses in its development so rapidly that, 24 hours after inoculation, sub-culture No. 1 can be used to make a sub-culture

No. 2 by the spore-fall method already described. However, it is best to leave sub-culture No. 1 undisturbed for at least a week before using it as a source of inoculum. By this time, the sub-culture is well established and is producing basidiospores in great numbers.

Sub-culture No. 1, or any similar sub-culture, may be used for making not merely one new sub-culture but many sub-cultures, as it produces and violently discharges basidiospores continuously for several weeks.

By taking the usual bacteriological precautions, one may make numerous new sub-cultures by the spore-fall method without the culture medium becoming contaminated by extraneous organisms such as moulds and bacteria.

It is to be emphasised that the sterigmata (Brefeld's *primary conidia*) produced on a basidium-body (promycelium) are never violently discharged. If an agar culture which was sown with chlamydo-spores and in which there are developing numerous sterigmata and basidiospores is inverted over a glass slide for some hours without being disturbed, and if the spore-deposit is then carefully examined, one observes that the deposit consists *entirely of basidiospores* (cf. Fig. 124, p. 251) among which one seeks for fallen sterigmata in vain. Hence one may safely conclude that sub-culture No. 1, which was made from a chlamydo-spore culture by the spore-fall method, originated solely from fallen basidiospores.

By employing the spore-fall method of inoculation, and by using a sub-culture as soon as it is established as a source of inoculum for a succeeding sub-culture, *Tilletia tritici* may be kept in culture in an active state indefinitely. Twelve successive sub-cultures were made by the spore-fall method and, although the twelfth sub-culture was not obtained until about three months after the first, yet sub-culture No. 12, when from one to two weeks old, was producing and discharging its basidiospores just as freely as sub-culture No. 1 when this was of the same age.

A vigorous test-tube culture of *Tilletia tritici*, such as that shown in Fig. 121, can be obtained within two weeks as follows. The upper end of an agar slope is inoculated with a mycelium-bearing piece of agar about 1 cm. square cut from a Petri-dish culture. One now sets the test-tube in such a position that the surface of the agar

is almost vertical, an angle of only about  $1^{\circ}$  being made between it and a vertical line. The agar surface thus comes to look upwards very slightly. Under these conditions, as the basidiospores are shot away from their sterigmata on the piece of inoculum, they settle on the surface of the agar all down the slope. After the slope has been thus seeded with basidiospores for about two days, it is placed horizontally with the surface of the medium facing upwards. The new mycelia derived from the basidiospores which settled on the slope soon begin to discharge a new crop of basidiospores which fall on to the agar and there germinate. Thus, in the course of about two weeks, a white mat of mycelium comes to cover almost the whole of the surface of the slope (Fig. 121).

The spore-fall method has been applied with success to making cultures on agar media in 150 cc. conical flasks. A test-tube culture, like that just described, is held over the mouth of the flask for 2-3 hours until a sufficient number of basidiospores have fallen into the flask. The test-tube is then removed and a sterile cotton-wool plug is placed in the mouth of the flask in the usual way.

**Basidiospore-deposits.**—As soon as it had been discovered that the basidiospores of *Tilletia tritici* are violently discharged, the

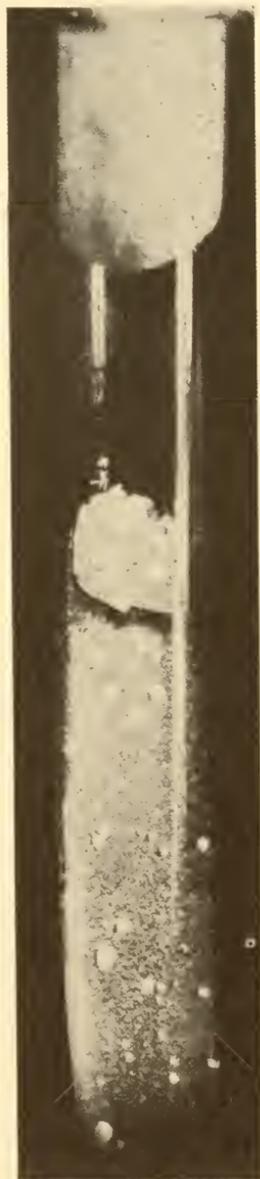


FIG. 121.—*Tilletia tritici*. Large test-tube culture. The piece of inoculum (agar with a mycelial mat) is seen at the top of the malt-agar slope. After inoculation the test-tube was maintained in an upright position. The basidiospores shot from the inoculum settled below all over the agar slope and there germinated so that there is now a mycelial mat covering the surface of the agar. Culture about two weeks old. Natural size.

possibility of obtaining spore-deposits of this fungus, resembling those of *Hymenomyces*, at once presented itself. Accordingly,

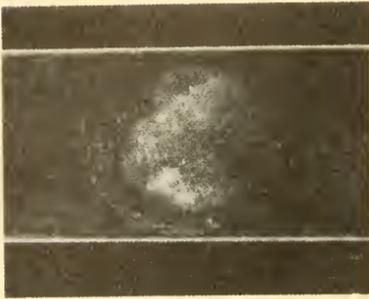


FIG. 122.—*Tilletia tritici*. A white basidiospore-deposit on a dry copper-sulphated glass slide, formed in three days underneath an inverted malt-agar-slope test-tube culture like that shown in Fig. 121, photographed against a black background. The spores fell through comparatively moist air. Natural size.

some Petri-dish cultures were inverted so that the basidiospores fell on to the Petri-dish covers. After 24 hours inversion of a dish, the spore-deposit was visible to the naked eye as a thin white film. Subsequently, spore-deposits were collected by inverting agar cultures over microscope slides.

When a spore-deposit made on the cover of a closed inverted Petri dish during a period of 6–12 hours is examined microscopically, it is found to consist of thousands of sickle-shaped basidiospores all of which, except those most recently discharged, have germinated on

the film of moisture on the surface of the glass. In fact, under these conditions, it is impossible to obtain a spore-deposit in which some of the spores have not germinated.

In order to obtain an ungerminated spore-deposit suitable for photomicrographing and for making measurements of spores, it was found necessary to prevent the spores from germinating by allowing them to fall on to a slide or Petri-dish cover which had been treated with a dilute solution of copper sulphate or some other toxic substance. Usually, a few drops of the copper sulphate solution were put on a slide, were spread over it with a glass rod,



FIG. 123.—*Tilletia tritici*. A white basidiospore-deposit obtained by inverting a vigorous, Petri-dish, potato-dextrose-agar culture over a dry copper-sulphated glass slide for several days. The copper sulphate prevented the spores from germinating. Photographed against a black background. Natural size.

and were then allowed to dry. When the spores fell upon such a slide, they never germinated.

The spore-deposit shown macroscopically in Fig. 122—the first Smut spore-deposit ever illustrated—was obtained as follows. A



FIG. 124.—*Tilletia tritici*. A photomicrograph of a dry basidiospore-deposit like that shown in Fig. 122. The spores fell in *moist* air and did not double up in falling. They vary considerably in size. Magnification, 110.

vigorously-growing test-tube culture (Fig. 121), made in the manner already described, was inverted over a copper-sulphated glass slide for three days, the combination being covered with a bell-jar to prevent desiccation of the agar and of the mycelium growing upon it. There were tens of thousands of hyaline sickle-shaped basidiospores in the deposit. A denser basidiospore-deposit is illustrated

in Fig. 123. It was obtained by inverting a vigorous, Petri-dish, potato-dextrose-agar culture over a dry copper-sulphated glass slide for several days, and it was photographed against a black background. Two photomicrographs showing basidiospores from a



FIG. 125.—*Tilletia tritici*. A photomicrograph of a basidiospore-deposit collected during one night on a dry glass slide from an inverted Petri-dish culture. The slide was not copper-sulphated, so that before it was removed from the Petri dish some of the spores had begun to germinate. The basidiospores vary considerably in size. Stained with iodine. Magnification, 230.

less crowded spore-deposit are reproduced in Figs. 124 and 125.

When the basidiospores fall through air which is saturated with moisture or nearly so, the spore-deposit produced always has the appearance shown in Figs. 124 and 125; but, when they fall through relatively dry air, the spore-deposit has the appearance shown in Fig. 126. Spore-deposits like that shown in Fig. 126 can be obtained by inverting either a Petri-dish or a test-tube culture over a glass slide in a room containing dry air, with a space of 0.5–1.0 cm. left between the base of the glass vessel and the slide (*cf.* Fig. 128, p. 256). The air in the laboratory at Winnipeg

during the winter is extremely dry and its relative humidity was found to be 30–35.

The spores of a spore-deposit collected in dry air, when seen from above with the microscope (Fig. 126), appear as oval particles, but careful focussing shows that each one exhibits two more or less rounded parts closely adherent to one another. These rounded parts in reality are the ends of the spores. This can easily be proved by taking the glass slide to which the spores are attached, by turning

it so that its spore-deposit surface is in a vertical position, and by then looking at the surface with the high power of the microscope. The spores which then come into view resemble those shown in Fig. 127.

What explanation can be offered for the peculiar form of the basidiospores of *Tilletia tritici* shown in Figs. 126 and 127 ?

It has been shown by Buller and Hanna<sup>1</sup> that, while falling through the air, the spores of *Coprinus sterquilinus* rapidly dry and become boat-shaped, and that every spore falls and settles with its



FIG. 126.—*Tilletia tritici*. Photomicrograph of a basidiospore-deposit collected in relatively *dry* air (relative-humidity 30–35) by the method illustrated in Fig. 128. The sickle-shaped basidiospores, whilst falling 1 cm. through the dry air, doubled up and became pyriform with their two ends (seen in the photograph as glistening points) directed upwards (cf. Fig. 127).

convex side downwards and its concave side upwards. Doubtless, therefore, when a sickle-shaped basidiospore of *Tilletia tritici* is shot into the air, it falls with its convex side downwards and its concave side upwards, as represented diagrammatically in Fig. 119 (p. 246). If the spore falls through very moist air, it retains its shape, strikes the substratum with the middle of its convex side, and falls over ; so that, when seen from above in a spore-deposit, it appears sickle-shaped as shown in Figs. 124 and 125. If, on the other hand, the spore falls through very dry air, it dries up in about one second and, in so doing, it buckles up in the middle and its two arms become appressed together, so that the spore as a whole becomes

<sup>1</sup> A. H. R. Buller, *Researches on Fungi*, Vol. III, 1924, p. 277, Fig. 95.

pyriform. For mechanical reasons connected with the resistance of the air, when the spore, after becoming pyriform, is falling, its rounded end is directed downwards and its pointed end upwards; so that, finally, it settles in the manner shown in Figs. 126 and 127. One question, however, still remains to be answered: Why, when drying, does the spore buckle up, so that its two arms become closely applied one against the other? The drop excreted from the spore-hilum just before spore-discharge undoubtedly travels with the spore, as in the Hymenomyces<sup>1</sup> and the Uredineae.<sup>2</sup> In all probability, in *Tilletia tritici*, as may actually be seen in abnormal

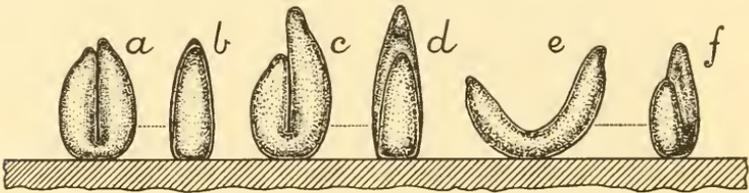


FIG. 127.—Semi-diagrammatic lateral view of part of a spore-deposit of secondary basidiospores of *Tilletia tritici*, collected in *dry air*. The spores fell from an inverted agar plate (*cf.* Fig. 128) through the dry air of the laboratory on to a glass slide (for a surface view of the spore-deposit *vide* Fig. 126). The plate was raised only about 1 cm. above the slide. The air of the laboratory was very dry. *a* and *b*, two views of one spore; *c* and *d*, two views of a second spore; *e* and *f*, two views of a third spore. Whilst falling through the air, the first and second spores completely doubled up and the third spore became much bent. Magnification, 1330.

excretion (*cf.* Fig. 118, *c* and *e*, p. 243), the drop makes its way into the hollow on the concave side of the spore, *i.e.*, on the side which looks upwards as the spore is falling. It may be imagined that, in some way, as the drop is drying up, by means of its adhesive and cohesive properties, it pulls the two halves of the spore together so that, finally, they come to touch one another and to adhere together. It may be remarked that the wall of a basidiospore of *Tilletia tritici* is extremely thin, so that it can offer but little resistance to any force exerted upon it by the drying drop.

If to such a fresh dry spore-deposit as that shown in Fig. 126 one adds a drop of water, the majority of the spores at once spring open, re-assume their sickle-shaped form, and fall over on their sides so

<sup>1</sup> A. H. R. Buller, *Researches on Fungi*, Vol. II, 1922, pp. 5–18.

<sup>2</sup> *Ibid.*, Vol. III, 1924, p. 504.

that, when seen from above, they resemble the spores shown in Figs. 124 and 125.

Basidiospore-deposits collected on copper-sulphated slides are very convenient for studying the size of the spores. One mounts the spores in water under a cover-glass in the usual way. It is then seen that the spores are very variable in size (*cf.* Figs. 124 and 125). The smallest spores are  $14\ \mu$  long and  $3\ \mu$  wide, while the largest ones are about  $42\ \mu$  long and  $6\ \mu$  wide. Thus the largest spores are three times as long as the shortest and twice as wide. For 118 spores the average length was found to be  $21.1\ \mu$  and the average width about  $4.5\ \mu$ .

The dry-needle method of making monosporous cultures from basidiospores, as described by Hanna<sup>1</sup> for species of *Coprinus* and other Hymenomycetes, is impracticable for the basidiospores of *Tilletia tritici*: (1) because the spores do not survive desiccation; and (2) because the spores become so firmly attached to a glass slide that, when one attempts to remove them with a needle, they are torn into pieces.

**The Development and Discharge of Basidiospores in Dry Air.**—During the winter, when the experiments on *Tilletia tritici* were carried out, the air in the laboratory was extremely dry, its relative humidity being often only 30–35. Chlamydospores were sown on plain agar in a Petri dish and, a few days afterwards, sterigmata and basidiospores were developing on the promycelia in the usual way. The lid of the dish was removed and the culture, exposed to the air of the laboratory, was examined with the high power of the microscope. Attention was being directed to the development of the basidiospores on the H-shaped pairs of sterigmata when it was noticed that, although the culture had been open for half an hour, certain young basidiospores under observation continued to increase in size, as though they were not affected by the dry air. In order to determine whether or not basidiospores can develop and be discharged in large numbers in dry air, and, if so, for how long a time, the following experiment was made. A Petri-dish culture containing mycelium which was producing numerous basidio-

<sup>1</sup> W. F. Hanna, "The Dry-needle Method of making Monosporous Cultures of Hymenomycetes and Other Fungi," *Annals of Botany*, Vol. XXXVIII, 1924, pp. 791–794.

spores, after removal of its cover, was inverted and supported on two corks at a height of 4.5 cm. above the surface of a table in the laboratory where the air had a relative humidity of 35 (Fig. 128). A slide was placed at a distance of 1.2 cm. beneath the mycelium of the culture dish, and the slide was changed at intervals as required. Whether or not basidiospores continued to be discharged from the mycelium could be decided by examining the slides with the micro

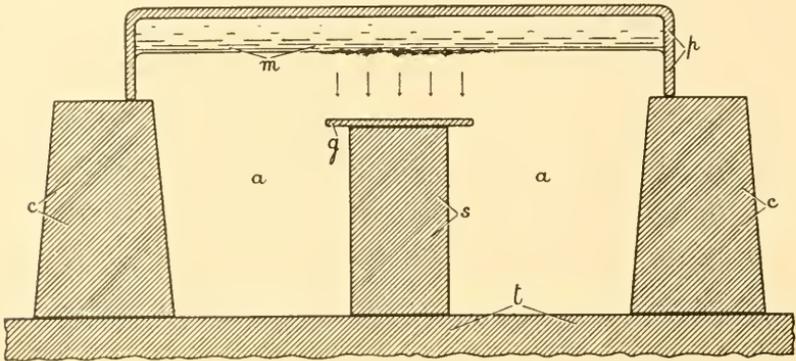


FIG. 128.—*Tilletia tritici*. The discharge of basidiospores in dry air. The Petri dish *p* containing oatmeal-agar *m*, bearing at its surface a mycelial mat, is inverted and supported on two corks *c c* resting on a laboratory table *t*. A glass slide *g* rests on a support *s* about 1 cm. beneath the mycelial mat. The very dry air of the laboratory (relative humidity 30–35) freely circulated under the Petri dish at *a a*. Basidiospores continued to be shot away from the mycelial mat for upwards of twelve hours; and they fell downwards, as indicated by the arrows, so as to form a spore-deposit on the glass slide *g*. Whilst they were falling, the agar shrank considerably and cracked owing to loss of water by evaporation (*vide* Fig. 129). About three-fourths the natural size.

scope and finding out whether or not basidiospores had settled upon them.

The experiment was started at 10 o'clock in the morning and, as shown by an examination of the slides, basidiospores continued to be produced and discharged until 10.30 in the evening. A fresh slide was then placed under the mycelium. At 10.30 next morning, *i.e.* 24 hours after the beginning of the experiment, the slide was found to bear a thin spore-deposit. That the mycelium had been exposed to very dry air was shown by the fact that, at the end of the 24-hour period, the agar in the plate had cracked across in the middle, the breach between the two parts measuring 8 mm. in width (Fig. 129),

while the thickness of the agar had become reduced from 6 mm. to 4 mm.

From the result of the experiment just described it is evident



FIG. 129.—*Tilletia tritici*. The production and discharge of basidiospores in relatively dry air (relative humidity 30–35). The oatmeal-agar plate here shown was inoculated by falling basidiospores and, after about seven days, it bore vigorously-growing masses of mycelium. It was then uncovered, inverted, and suspended in the dry air of the laboratory in the manner shown in Fig. 128. Basidiospores continued to be produced and to form a spore-deposit on a slide for 12–24 hours. During this period, the agar lost so much water by evaporation that it cracked across and shrank from the side of the Petri dish, as shown in the photograph. Natural size.

that, so long as the mycelium of *Tilletia tritici* can procure sufficient water from its substratum, it continues to develop and discharge basidiospores in a normal manner, even in extremely dry air. Doubtless, this fact has some significance for the production of basidiospores under natural conditions.

Following up the clue given by *Tilletia tritici*, it was observed that the large conidia of the phycomycetous species *Conidiobolus villosus*<sup>1</sup> are actively produced and discharged from an agar medium just as long as the medium contains any available water. A Petri-dish culture of *C. villosus*, exposed to dry air in the same manner as the *Tilletia tritici* culture, continued to produce and discharge conidia for 48 hours, at the end of which time the agar medium had become reduced to a mere parchment. Doubtless the mycelia of many other fungi, when growing on a moist substratum with their surface hyphae exposed to dry air, produce and liberate their spores as freely as *Tilletia tritici* and *Conidiobolus villosus*. In this connexion, as bearing upon the production and liberation of spores in the Uredineae and the Hymenomycetes in dry air, it may be recalled that Zalewski<sup>2</sup> found that aecidiospores are shot out of their aecidia in dry air as well as in moist, although not so freely, and that Buller<sup>3</sup> has formulated the general conclusion that, in the Hymenomycetes: "so long as a fruit-body has sufficient moisture in itself the dryness or dampness of the atmosphere without makes no appreciable difference to the rate of spore-discharge."

**The Germination of Basidiospores.**—Freshly discharged basidiospores, which have not been allowed to dry up during their fall or afterwards, germinate very readily at ordinary room temperatures in a film of moisture or on agar media. Germination, under these conditions, begins within an hour of discharge and, within a few hours, one finds that 100 per cent. of the spores have germinated. The viability of the basidiospores of *Tilletia tritici*, therefore, is very great.

Freshly discharged basidiospores, *which have dried up* during their fall or afterwards, when placed in water or under moist conditions, *never germinate*. With upwards of a dozen dry spore-deposits, made at different times, in which there were tens of thousands of

<sup>1</sup> A pure culture of this fungus, *Conidiobolus villosus* Martin, was kindly sent to one of us (A. H. R. B.) by its discoverer, Professor G. W. Martin, from Iowa State University. In the cultures exposed to dry air the conidiophores were much shorter than in cultures exposed to moist air.

<sup>2</sup> A. Zalewski, "Über Sporenabschnürung und Sporenabfallen bei den Pilzen," *Flora*, Jahrg. LXVI, 1883, pp. 268–270.

<sup>3</sup> A. H. R. Buller, *Researches on Fungi*, Vol. I, 1909, p. 123.

spores, there was not a single exception to this rule. We may therefore conclude that desiccation, for however short a time, is fatal to every basidiospore of *Tilletia tritici*. This conclusion is in harmony with the finding of Appel and Riehm,<sup>1</sup> who state that the basidiospores (their secondary conidia) are destroyed by drying.

The basal end of a basidiospore can usually be distinguished from the apical, either before or after germination, by the presence of a minute highly refringent particle on the convex side of its cell-wall at the place where the basidiospore broke away from its sterigma (Fig. 130, A, *p*).

When basidiospores germinate *in a film of water* which has condensed on a glass slide in a closed chamber, there is no external food available and growth takes place at the expense of the substances contained within the protoplasm of the spore. Each spore on germinating puts out a single germ-tube, usually from its apical end but sometimes from its basal end (Fig. 130, A, *c-f*). This germ-tube is very slender and may grow without branching until it is several times the length of the spore, the growth being accompanied by protoplasmic migration toward the apex of the tube and by the formation of septa, or the germ-tube may branch. Often the original germ-tube, while still very short, may cease to grow, in which case a new germ-tube arises from the other end of the spore and draws all the protoplasm toward its apex, protoplasmic migration and septation taking place as before (Fig. 130, A, *g*). Other variations in germination are shown in Fig. 130, A, *h, i*. The germ-tubes usually exhaust themselves and cease to grow without producing any new basidiospores. Only rarely and on relatively short germ-tubes are basidiospores ever produced. Germination of an essentially similar type takes place on the free surface of sterile water contained in a sterile watch-glass; but, under these conditions, the germ-tubes are somewhat more coiled than they are in moisture films.

When basidiospores germinate *on malt-agar, oatmeal-agar, or*

<sup>1</sup> O. Appel and E. Riehm, "Zur Frage der Überwinterung des Steinbrandes im Boden," *Mitt. K. Biolog. Anst. Land- u. Forstw.*, 1914, Heft 15, p. 6. Cited from Woolman and Humphrey, *Summary of Literature on Bunt or Stinking Smut of Wheat*, U.S. Department of Agric., Bull. No. 1210, 1924, p. 12.

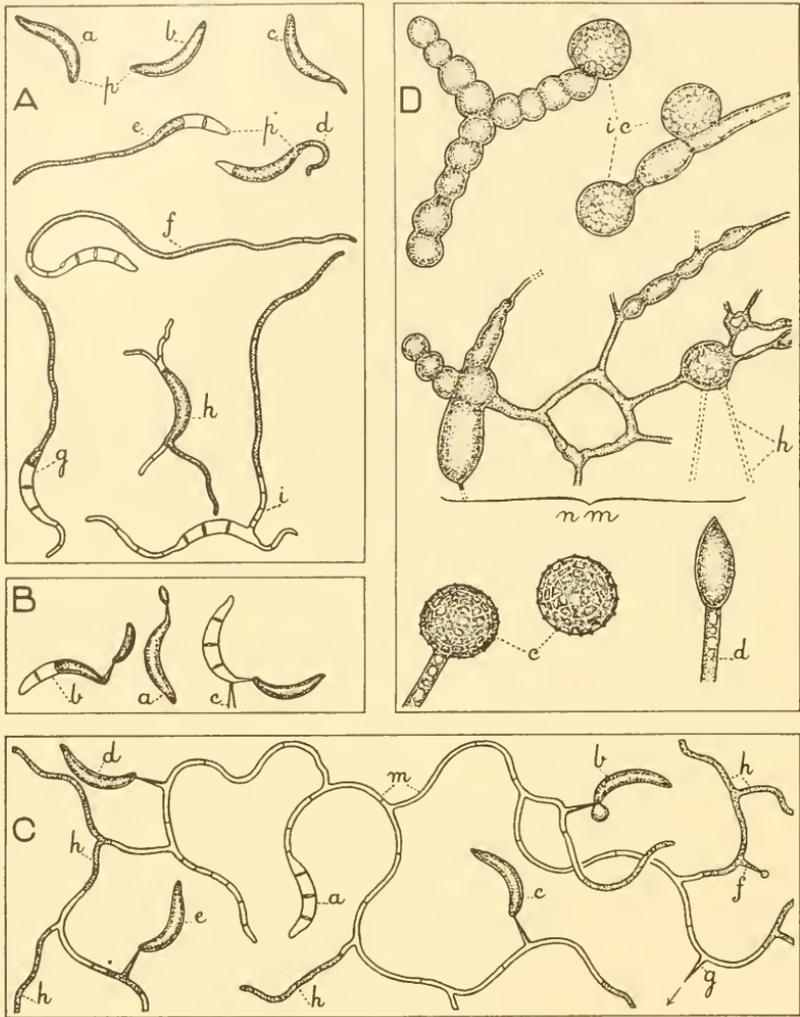


FIG. 130.—*Tilletia tritici*. The germination of basidiospores and the production of chlamydospores in pure cultures made with malt-agar. A, the emission of germ-tubes by basidiospores: *a* and *b*, two basidiospores which have just been discharged, each exhibiting a refringent particle *p* at its basal end where it broke away from its sterigma; *c* and *e*, basidiospores which have emitted germ-tubes from their basal end; *d* and *f*, basidiospores which have emitted germ-tubes from their apical end; *d*, *e*, and *f* show normal germination, including the migration of the protoplasm along the germ-tubes and the formation of septa; *g*, *h*, and *i* show abnormal germination, for new germ-tubes have developed and have withdrawn protoplasm from the old ones. Magnification, 570.

B, a new basidiospore produced directly on an old one: *a* and *b*, two discharged basidiospores each of which, on an exhausted substratum,

*Leonian agar*, each basidiospore, as a rule, puts out a single germ-tube only. The conditions of nutrition permit of the fungus living saprophytically for a long time. The germ-tube develops into a mycelium which branches and rebranches in a coiled manner and eventually gives rise to numerous new basidiospores, as illustrated in Fig. 130, C. Protoplasmic migration and the production of septa eventually take place, and these phenomena are to a large extent correlated with the transference of protoplasm into the new crop of basidiospores. The mycelium developed during the first few days is composed of very thin hyphae; but, later on, thick irregular hyphae are produced in addition. Both types of hyphae give rise to basidiospores. When a basidiospore is shot away and falls on to the agar, it sometimes at once gives rise to another basidiospore which may be shot away (Fig. 130, B, *a, b*). Sometimes a chain of three successive basidiospores is formed in this way; but, in such a case, the second basidiospore cannot have been shot away.

Basidiospores were successfully germinated in a Petri dish *on sterile green-house soil*, and the mycelium gave rise to new generations of basidiospores up to the fifteenth day after the original

FIG. 130—*cont.*

has directly produced a sterigma and is now developing a new basidiospore; *c*, an undischarged basidiospore, still seated on its sterigma, which at its base has produced a new sterigma and a new spore. Magnification, 570.

C, a basidiospore (primary or secondary) *a* which has been shot from its sterigma, has fallen on to fresh malt-agar, has germinated, and has given rise to a fine branched winding mycelium *m* bearing numerous new secondary basidiospores, *b-f*, which in turn will be violently discharged from their sterigmata: the original basidiospore *a* and the older parts of the mycelium are now septate, devoid of protoplasm, and dead; the younger parts of the mycelium *h h* still contain protoplasm which is creeping towards the apices of terminal hyphae and into the developing basidiospores and, basally, is forming new septa; *b*, a mature basidiospore with a drop of maximum size excreted from its hilum and about to be discharged; *c* and *d*, two full-grown and almost mature basidiospores, a few minutes before drop-excretion and discharge; *e*, a full-grown basidiospore into which protoplasm is still flowing from the mycelium; *f*, a rudimentary basidiospore which at present is no more than a tiny spherical body symmetrically seated on the end of its sterigma; *g*, a sterigma, devoid of protoplasm, from which a basidiospore has been violently shot away in the direction of the arrow. Magnification, 570.

D, the production of chlamydo-spores in old malt-agar cultures. The mycelium has become relatively thick, very irregular, and often moniliform: *ic*, immature chlamydo-spores with slight reticulations on their outer walls; *c*, chlamydo-spores which have developed at the ends of branches and whose outer walls are distinctly reticulated; *d*, a swollen ovoid cell terminating a hypha; *nm*, netted mycelium, some of the hyphae *h* have lost their protoplasmic contents and their walls are disintegrating. Magnification, 660.

basidiospores were sown. Attempts to obtain similar results on unsterilised soil always failed.

**The Development of Chlamydospores in Culture Media.**—The development of chlamydospore-like bodies of *Tilletia tritici* on artificial media has been observed by Brefeld,<sup>1</sup> Sartoris,<sup>2</sup> and others. In the present investigation basidiospores were sown by the spore-fall method on the surface of oatmeal-agar and Leonian agar in Petri dishes, and the mycelium resulting therefrom was kept in culture for some three months. Many of the hyphae grew along the surface of the agar, others developed aerially, while others penetrated below the surface of the agar to a depth of about 0·2 mm. A few weeks after the sowing of the basidiospores, the mycelial mat began to turn yellow; and, after 2–3 months, it was very brown and, in some spots, almost black.

A microscopic examination of the darkest patches of the mycelium showed that they consisted of various types of hyphae and of chlamydospore-like bodies (Fig. 130, D). Some of the hyphae were thin and regular. Others were very thick and often moniliform, and they frequently bore short, lateral, empty, septate branches which terminated in dark, thick-walled, oil-containing, oval or rounded, chlamydospore-like bodies. At the end of three months, some of the dark bodies were spherical in shape and exhibited on their dark outer wall the reticulations which are so characteristic of the chlamydospores of *Tilletia tritici* formed in diseased grains of wheat (Fig. 130, D, c). Very old cultures appeared to consist almost entirely of large-celled chlamydospore-like bodies, most of the hyphae having disintegrated and disappeared. Thus the life-history of *Tilletia tritici* when grown on artificial media was followed in detail from chlamydospore to chlamydospore and Brefeld's conclusion that this parasite can be grown saprophytically was once more confirmed. Whether or not the chlamydospores developed on agar will germinate and give rise to typical promycelia bearing sterigmata and basidiospores has not yet been decided by experiment.

<sup>1</sup> O. Brefeld, *Untersuchungen über Pilze*, Heft V, 1883, pp. 158–163, Taf. XIII, Figs. 47–52.

<sup>2</sup> G. B. Sartoris, "Studies in the Life History and Physiology of Certain Smuts," *Amer. Journ. Bot.*, Vol. XI, 1924, pp. 626–627.

Boss,<sup>1</sup> in 1927, as a result of his cytological studies, observed that chlamydospores of *Tilletia tritici* produced in artificial cultures contain one nucleus instead of two. Possibly the non-germination of artificially produced chlamydospores is correlated with their haploid condition.

**Spore-fall Observed by the Beam-of-Light Method.**—In certain Hymenomycetes growing wild, in daytime under favourable conditions of light, clouds of spores may be observed coming away from the under side of the fruit-bodies. Thus Buller,<sup>2</sup> by daylight, has seen such clouds escaping from the pilei of *Polyporus squamosus*, *Fomes applanatus*, *Armillaria mellea*, *Pleurotus ostreatus*, *P. ulmarius*, *Amanita muscaria*, and *Tricholoma personatum*. In all Hymenomycetes, the emission of spore-clouds from the fruit-bodies can be observed in the laboratory by means of a concentrated beam of light. The beam directed under active pilei of *Schizophyllum commune*, *Daedalea unicolor*, or *Psalliota campestris* reveals to the eye millions of escaping spores.<sup>3</sup>

If a strong test-tube culture (an agar slope) of *Tilletia tritici* is inverted, the spores falling in succession slowly downwards through the air in the interior of the tube are invisible in diffuse daylight, but they can readily be seen when illuminated artificially. A beam of light emitted by a strong incandescent lamp in a projection lantern was concentrated by means of a plano-convex lens and passed through the air beneath the agar in an inverted test-tube culture (Fig. 131). In the narrow beam of light the individual spores could be seen with the naked eye falling slowly downwards as white particles. The visibility of the spores was somewhat increased when the naked eye was aided with a magnifying glass.

**The Distance of Basidiospore-discharge.**—The maximum *vertical* distance to which the basidiospores of *Tilletia tritici* are shot from their sterigmata in still air was determined as follows. A sub-

<sup>1</sup> G. Boss, "Beiträge zur Zytologie der Ustilagineen," *Planta*, Bd. III, 1927, pp. 619-622.

<sup>2</sup> A. H. R. Buller, *Researches on Fungi*, Vol. I, pp. 89-93; Vol. II, pp. 100-102, 133; Vol. III, pp. 480, 483-484; and observations hitherto unpublished.

<sup>3</sup> A. H. R. Buller, *ibid.*, Vol. I, pp. 102-104.

culture of the mycelium was made on a stiff 2-per-cent. agar medium

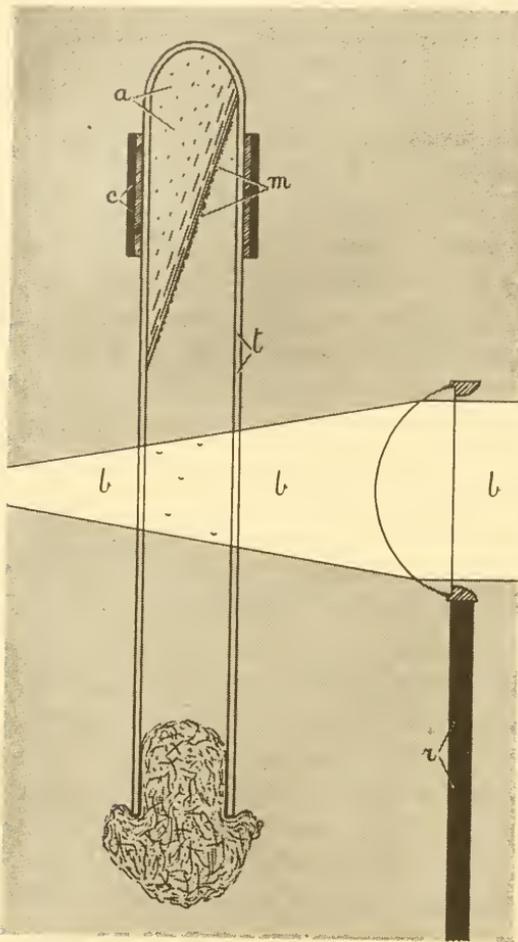


FIG. 131.—*Tilletia tritici*. Observation of the fall of basidiospores by the beam-of-light method. A test-tube *t* containing a malt-agar slope *a* with a mycelial mat *m* at its surface is held inverted by a clamp *c* in front of a concentrated beam of light *b b b* passing from a projection lantern through a bull's-eye condenser held on the brass rod *r*. Spores, after being discharged from their sterigmata, fell down through the air of the test-tube and could be seen individually with the naked eye as they passed through the beam of light. The spores shown in the beam of light are diagrammatically enlarged. Apparatus, two-thirds the natural size.

in a Petri dish. When the culture was about ten days old, the mycelium had formed a thin mat which was only slightly raised above the surface of the agar (Fig. 132). At this stage, two pieces of a sterile glass slide of known thickness were placed one on each side of the mat, and a sterile cover-glass (Fig. 132, *c*) was laid upon the pieces of glass so that it formed a bridge above the mycelium below. The Petri-dish cover was then replaced and the whole covered with a bell-jar. In some of the experiments, to support the cover-glass, sterile portions of sewing needles of known diameter were used instead of pieces of glass slides. By using pieces of glass or needles of different thicknesses, the cover-glass was set in succession at different heights above the mycelium. Whenever a basidiospore is shot

upwards and strikes the under surface of a cover-glass, just as in the *Hymenomyces*,<sup>1</sup> it sticks to the glass where it strikes (Fig. 132, *b*).

In each experiment the cover-glass was placed at a definite distance above the surface of the agar and left there for several hours. At the end of this time, the under surface of the cover-glass was examined with the microscope to find out whether or not any basidiospores had become adherent to it. In a series of experiments the height of the cover-glass above the agar varied from 0.5 to 1.1 mm. To determine the maximum height to which the mycelium projected above the surface of the agar, immediately after the completion of the series of experiments a vertical section was made through the mycelium and agar and the amount of projection was measured with the microscope. In every experiment in estimating the height of basidiospore-discharge, the maximum distance of projection of the mycelium was deducted from the vertical distance between the upper surface of the agar and the under surface of the cover-glass. Altogether, four complete series of experiments were made.

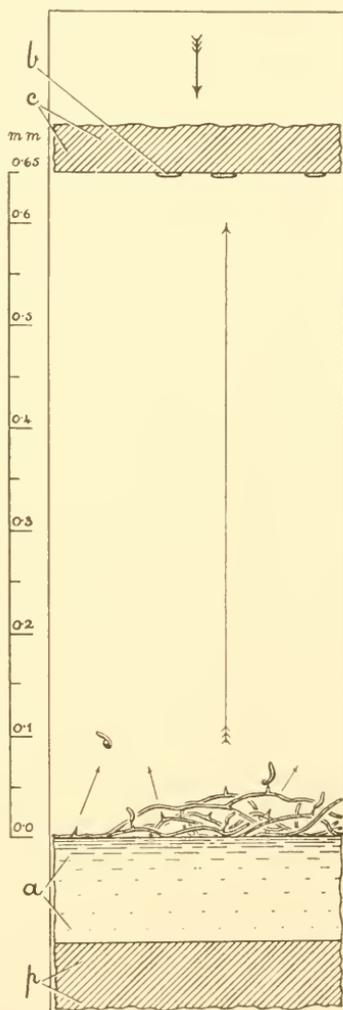


FIG. 132.—*Tilletia tritici*. Height to which the basidiospores are discharged; *p*, a Petri dish containing malt-agar *a* at the surface of which there is a mycelial mat discharging basidiospores; *c*, part of a cover-glass supported in the air above the mycelium. The basidiospores which are shot high enough to touch the under side of the cover-glass stick there, as shown at *b*. The scale on the left enables one to determine the height to which the basidiospores were shot in the particular experiment illustrated. Magnification, about 133.

<sup>1</sup> A. H. R. Buller, *Researches on Fungi*, Vol. II, 1922, p. 16.

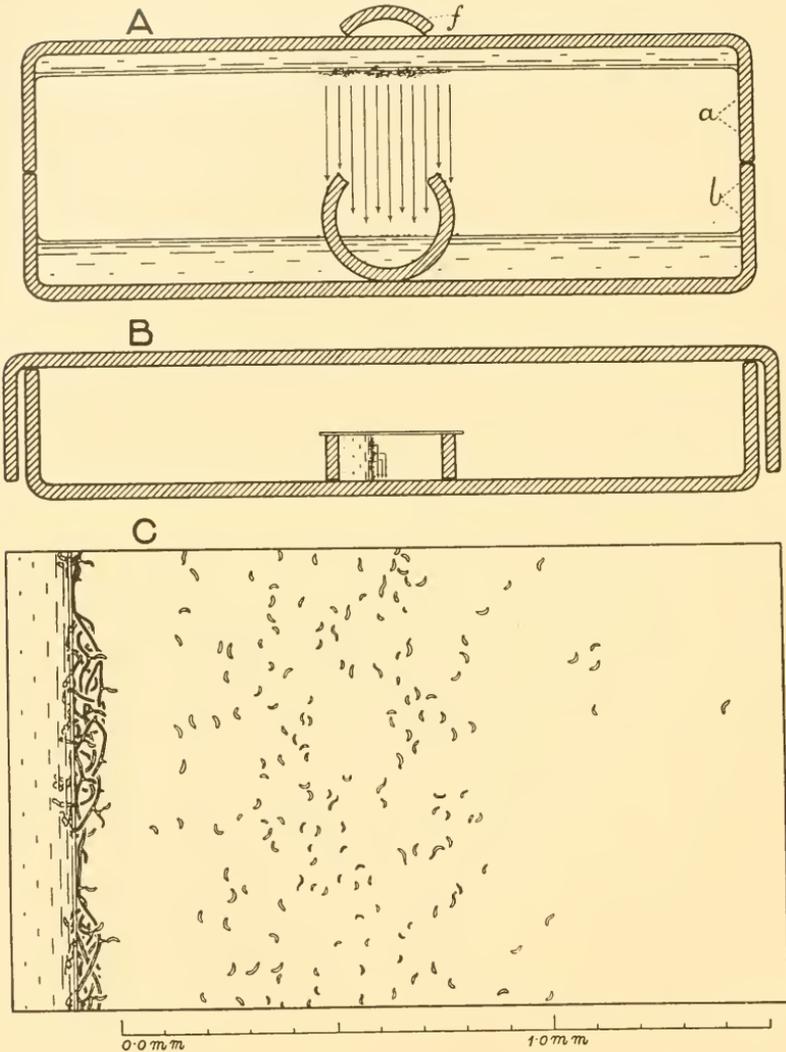


FIG. 133.—*Tilletia tritici*. Diagrams illustrating the mode of measuring the horizontal distance to which the basidiospores are discharged. A: a Petri-dish malt-agar culture *a* has been inverted over a new malt-agar plate *b* containing a glass ring from which a piece of the side *f* has been removed. The arrows indicate the path of fall of the basidiospores. After inoculation of the agar in the ring with basidiospores, the dish *a* is removed and the dish *b* is covered and left until a mycelial mat has developed at the surface of the agar in the ring. B: another Petri dish in which has been set the glass ring of A, completed by adding the piece *f*, together with the agar and mycelial mat contained within the ring. The ring has been covered with a cover-glass. The arrows indicate the trajectories of basidiospores shot horizontally away from the mycelial mat. C: appearance of the agar, the mycelial mat, and the

Examination of the cover-glasses in the whole series of experiments showed that basidiospores were present upon them at all distances up to 1.0 mm. above the agar, but never at any greater height. From the 1.0 mm. must be deducted 0.1 mm. for mycelial projection, as already explained. Hence it may be concluded that the basidiospores of *Tilletia tritici* are shot upwards to a maximum distance of about 1 mm.

Only very few spores were shot to a height of 0.9 mm., but there were always many spores adherent to cover-glasses raised 0.5–0.6 mm. above the agar.

The maximum *horizontal* distance to which the basidiospores of *Tilletia tritici* are shot from their sterigmata in still air was determined as follows.

A glass ring 17 mm. in diameter and 6 mm. high, from which a piece of the side 11 mm. wide had been broken out, was placed on the bottom of a Petri dish so as to assume the position shown in Fig. 133, A, and the whole was then sterilised in hot air. Twenty cc. of agar medium were then poured into the plate, care being taken to keep the glass ring in the position shown in Fig. 133, A. The medium soon solidified, and then the agar within the ring was sown with basidiospores by the spore-fall method. To accomplish this, a Petri-dish culture was inverted over the base of the dish containing the ring, as shown in Fig. 133, A. The arrows in Fig. 133, A, indicate the path of fall of the basidiospores. The spores were allowed to fall for three hours. The upper Petri-dish base (*a*) was then removed, and the lower one (*b*) covered with its lid. The basidiospores in the closed Petri dish (*b*) soon germinated and, after 5–7 days, the mycelium had formed a thin white mat at the surface of the agar. At the end of this time, with the aid of a scalpel, the agar was cut downwards in planes coincident with the two flat ends of the glass ring and then the ring, together with the agar and mycelium contained within it, was lifted out of the dish

FIG. 133—*cont.*

discharged basidiospores when viewed with the microscope through the base of the Petri dish at the bottom of the ring shown in B after spore-discharge has proceeded for 2–3 hours. The scale enables one to measure the horizontal distance of discharge of the basidiospores. A and B, about natural size; C, magnification, about 56.

and completed by adding to it the sterilised fragment of glass shown at *f* in Fig. 133, A. The completed ring was then turned into an upright position, set in a sterile Petri dish, and covered with a sterile cover-glass (Fig. 133, B). The cover of the new dish was set in place, the dish covered with a bell-jar, and the whole left at room temperature for 2-3 hours. During this period, numerous basidiospores were discharged more or less horizontally away from the mycelium which was in a vertical plane and, after completing their trajectories in the still air of the ring-chamber, they fell on to the base of the Petri dish. At the end of the period allowed for spore-discharge, the Petri dish together with its adherent ring was inverted, and the spore-deposit was examined with the microscope. The appearance presented by one of the spore-deposits, when thus magnified, is shown in Fig. 133, C. On the left can be seen the mycelium growing at the surface of the agar, and toward the right the spores spread out on the glass surface of the Petri dish. Below is a scale in tenths of a millimetre, by the use of which one can read off the distance to which any individual spore has been discharged. Three separate experiments were made.

In the experiments just described, it was found that the majority of the spores had fallen at a distance of 0.4 to 0.6 mm. from the outer surface of the mycelium, and that comparatively few had been discharged to a distance exceeding 1.0 mm. The maximum horizontal distance of discharge was 1.4 mm.

The hyphal plexus of a mycelial mat of *Tilletia tritici*, with its scores of basidiospores projecting from their sterigmata (*cf.* the transverse section in Fig. 114, C, p. 232), is reminiscent of the hymenium of the Hymenomycetes.

The maximum horizontal distance of spore-discharge, as recorded by Buller,<sup>1</sup> is: for the non-tremelloid Hymenomycetes, 0.2 mm.; for the Tremellineae, 0.65 mm.; and for the Uredineae, 0.85 mm. In the three experiments with *Tilletia tritici* above described, several spores were discharged horizontally to a distance of 1.2 to 1.4 mm. We may therefore conclude that *Tilletia tritici* discharges its basidiospores to a greater distance than any other known member of the Basidiomycetes.

<sup>1</sup> A. H. R. Buller, *Researches on Fungi*, Vol. II, 1922, p. 169.

**The Spore-fall Method of Inoculating Wheat Seedlings.**—The discovery of violent basidiospore-discharge in *Tilletia tritici* makes it possible to place ripe discharged basidiospores upon the whole or any desired part of a developing wheat seedling. The *spore-fall method of inoculation*, as used in practice, will now be briefly described.

An agar slope bearing an active mycelial mat at its surface is inverted over about half a dozen germinating wheat grains, so that these are enclosed within the mouth of the test-tube. Numerous basidiospores are shot away from their sterigmata, fall down the tube, and settle on the primary leaf-sheath (coleophyllum) of each seedling. Here they readily germinate and produce mycelia which grow over the epidermal cells. The mycelia can be clearly seen when a leaf-sheath is removed from a seedling, is split in half longitudinally, and is flattened out in a drop of water under a cover-glass. Giemsa stain was found to be useful for differentiating the mycelia and the host-tissue.

**The Infection of Wheat Seedlings by Secondary Basidiospores of *Tilletia tritici* and *T. laevis*.**—By employing the spore-fall method just described, it was found possible to inoculate wheat seedlings solely with secondary basidiospores and thus to obtain mature plants bearing heads in which all the grains were diseased, *i.e.* in which every grain had developed into a typical bunt-ball.<sup>1</sup>

In February, 1928, two series of experiments were undertaken. The wheat varieties employed as host-plants were obtained from the State of Washington and were known to be moderately susceptible to bunt. The smut species were *Tilletia tritici* and *T. laevis*. The *T. tritici* material was obtained from the State of Washington and the *T. laevis* from Manitoba. The chlamydospores were sown on an agar medium in the usual manner; and the mycelial mats, when they had been growing for about a month, were used as a source of falling secondary basidiospores. Some of the experiments were made in the light, and others in the dark. The wheat grains were surface-sterilised in a mercuric chloride solution (1 gram mercuric chloride in 1000 cc. water) for ten minutes

<sup>1</sup> The earlier inoculation experiments were made at Winnipeg, but the later experiments, including those of which the results are embodied in the Table on p. 270, were made in the greenhouse of Macdonald College, McGill University.

and then thoroughly washed in sterile water. Immediately thereafter they were placed on the surface of sterilised greenhouse-soil held in a Petri dish, and then they were inoculated by means of the spore-fall method. The falling basidiospores were derived from (1) a vigorous test-tube culture, like that shown in Fig. 121 (p. 249), or (2) a vigorous plate culture, like that shown in Fig. 119 (p. 246). The whole apparatus was kept at a temperature of 10°–13° for 6–8 days, during which time secondary basidiospores were falling in large numbers on to the slowly developing seedlings. At the end of the inoculation period, the seedlings, together with attached soil, were carefully removed from the Petri dish and were placed on the surface of sterilised greenhouse-soil in a 6-inch pot, and then the soil was heaped upon their basal portions, care being taken not to injure the root system in any way. The pots were set in a cool greenhouse (about 15° C.) for the first three or four weeks and in a warmer situation subsequently. After about three months, when the plants were matured, the results of the experiments could be determined by observing whether or not the heads had become bunted. When a plant produced bunted heads, its chlamydospores were examined with the microscope and it was always found that the chlamydospores belonged to the same species as that which had been used for the inoculum.

*Infection of Germinating Wheat Seedlings by the Secondary Basidiospores of Tilletia tritici and T. laevis.*

	Experiment begun	No. of plants	Wheat variety	Fungus species	Light conditions during inoculation	Results			Date of harvesting
						Diseased	Healthy	Dead	
Series I Controls	Feb. 3, 1928	7	Kota	<i>T. laevis</i>	dark	1	5	1	June 21
	" "	7	Bluestem	<i>T. laevis</i>	"	3	4	0	"
	" "	3	Kota	none	"	0	3	0	"
	" "	4	Bluestem	none	"	0	4	0	"
Series II Controls	Feb. 14, 1928	4	Bluestem	<i>T. tritici</i>	daylight	2	2	0	June 21
	" "	3	Bluestem	<i>T. laevis</i>	dark	1	2	0	"
	" "	7	Kota	<i>T. laevis</i>	dark	2	5	0	"
	" "	3	Bluestem	none	daylight	0	3	0	"
	" "	3	Bluestem	none	dark	0	3	0	"
	" "	4	Kota	none	dark	0	4	0	"

The results of the two series of experiments are embodied in the Table on p. 270, from which it will be seen that out of a total of 28 inoculated plants 9 became diseased while 18 remained healthy and one died. The percentage of infection was therefore 32.14. Of the 17 control plants all remained healthy.

In a third and earlier series of experiments which was started on May 20, 1927, there were no control plants, but every grain was surface-sterilised in the manner already described. The plants were subjected to room temperature (about 20° C.). At this relatively high temperature the seedlings grew much faster than in the two series of experiments already recorded, and inoculation with falling basidiospores was stopped as soon as the coleophylla had attained a length of about two inches. During the inoculation period, the seedlings were exposed to diffused daylight. As before, some of the seedlings were inoculated with *Tilletia tritici*, and others with *T. laevis*.

Of a total of 38 plants inoculated in the third series of experiments 10 became diseased while the other 28 remained healthy. The percentage of infection was therefore 26.31. The plants inoculated with *T. tritici* basidiospores produced only *T. tritici* chlamydospores, while those inoculated with *T. laevis* basidiospores produced only *T. laevis* chlamydospores.

Sartoris,<sup>1</sup> under the experimental conditions used by him, failed to obtain infection of wheat plants either from the mycelium or from the basidiospores of *Tilletia tritici*. In the three series of experiments described above, we employed exclusively secondary basidiospores as a source of inoculum and we obtained results which clearly prove that the *secondary basidiospores of T. tritici and T. laevis can and actually do cause the infection of healthy wheat seedlings*. Thus the view held by the older workers on Bunt, namely, that the secondary basidiospores (their secondary conidia) are effective inocula for the Stinking Smut disease of Wheat has been confirmed by results obtained by a new and precise method.

When secondary basidiospores have fallen on to the moist surface of the coleophyllum, etc., of a wheat seedling, they germinate at once and send out long germ-tubes which creep over the

<sup>1</sup> G. B. Sartoris, "Studies in the Life History and Physiology of Certain Smuts," *American Journal of Botany*, Vol. XI, 1924, pp. 617-647.

surface of the epidermis where they can be very clearly seen when the colcophyllum has been removed and is examined under the microscope. Now, as the results of our three series of experiments clearly indicate, these germ-tubes must sometimes penetrate into the seedlings and cause infection there. Nevertheless, in spite of many observations on our part made both with and without the use of Giemsa stain, in no instance did we succeed in actually perceiving a germ-tube penetrating through the epidermis or growing within the epidermis or other tissues of the host-plant. So far as we are concerned, therefore, the exact place and mode of entry of the germ-tubes into wheat seedlings still remain to be elucidated.

**The Swelling of Bunted Wheat Grains in Water.**—Prevost<sup>1</sup> states that bunt-balls placed on the surface of water swell up considerably and that, within a few minutes, the spores in some of them are forced out *en masse* through some crack in the pericarp, with the result that they sink down through the water in the containing vessel like a cloud of descending smoke.

Prevost's experiments were repeated and confirmed (Fig. 134). It was found that the spores are forced out only from those bunt-balls which already have injured pericarps, and that bunt-balls with unbroken pericarps, although they absorb water freely so that they swell up and become turgid, do not liberate any of their chlamydospores.

Dry bunt-balls, when put into water, rise to the surface and float there for an indefinite time. In the course of a few hours the floating balls absorb much water. If, when absorption is complete, the balls are pressed beneath the surface of the water, they at once sink to the bottom.

Some intact floating balls were kept in a beaker in the laboratory and, after 6–8 weeks, masses of chlamydospores were observed to be exuding from them at the water-line. Their pericarps had been weakened and finally ruptured by species of *Alternaria*, *Cladosporium*, and *Mucor*, which had grown upon them saprophytically. According to Woolman and Humphrey,<sup>2</sup>

<sup>1</sup> B. Prevost, *Mémoire sur la Cause Immédiate de la Carie ou Charbon des Blés*, Paris, 1807, p. 3.

<sup>2</sup> H. M. Woolman and H. B. Humphrey, *Studies in the Physiology and Control of Bunt or Stinking Smut of Wheat*, U.S. Department of Agriculture, Bull. No. 1239, 1924, pp. 6–7.

bunt-balls over-wintering in the soil have their pericarps destroyed in the same manner.

Measurements of 75 dry and 75 wet chlamydospores of *Tilletia tritici* showed that the average diameter of dry and of wet spores was  $16.06 \mu$  and  $19.16 \mu$  respectively. Therefore, the ratio of the volume of an average dry spore to that of an average wet spore is about 1 : 1.65. When a bunt-ball swells up, therefore, the swelling is chiefly due to the pressure exerted by the swelling chlamydospores.

**The Reactions of the Promycelium to External Stimuli.**—As the chlamydospores of *Tilletia tritici* must often germinate in the interstices of the soil, it seemed worth while to determine whether or not the promycelia are heliotropic. The following experiment was therefore made. Chlamydospores were sown on 2 per cent. plain agar in a Petri dish and the dish was completely covered with black paper, except for an area 8 mm. square on one side through which alone light could pass. The Petri dish was then placed in a black wooden box, 6 inches high, 6 inches wide, 15 inches long, with one end open; and the box was then set on a table with its open end toward a window. In the course of 3–5 days, the chlamydospores germinated and produced numerous promycelia exposed to unilateral illumina-

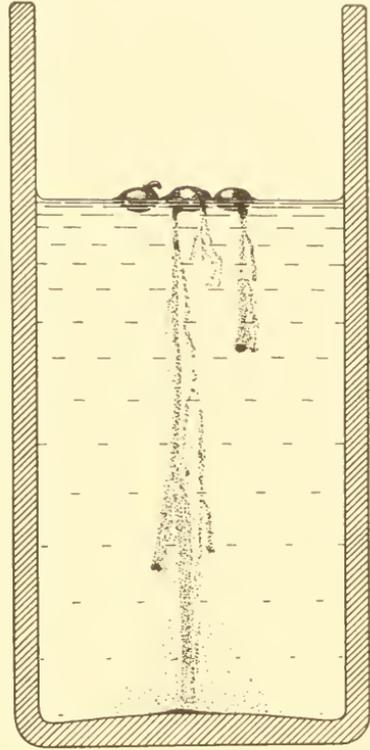


FIG. 134. — *Tilletia tritici*. The swelling of bunted wheat grains in water and the emission of their spores. Three bunted wheat grains have been placed in water contained in a glass vessel. The grains float, absorb water, and increase in volume. The swollen spores press against one another, escape *en masse* through a crack in the pericarp, and sink in the water. In the bunted grain on the left the escaping spores have been forced up into the air like paint from a tube of oil-paint. About one and one-third the natural size.

ation. The experiment was carried out twice, each time in duplicate.

When, after the promycelia were well grown, the Petri dishes were examined, it was observed that, in general, the promycelia were not directed toward the source of light, or away from it, or transversely to it, but that their axes were set at various angles to the incident rays. Hence it may be concluded that the promycelia of *Tilletia tritici* are not heliotropic.

To determine whether or not the promycelia are geotropic, some chlamydospores were sown on 2 per cent. plain agar in two Petri dishes, and the dishes were fixed in such a position that the surface of the agar was in a vertical plane. One of the dishes was exposed to diffuse daylight and the other kept in complete darkness. The chlamydospores germinated and, 5-6 days after they were sown, the direction of growth of the promycelia was determined with the microscope. The experiment was made on three separate occasions.

An examination of the plates showed that the promycelia had grown in all directions over the vertical surface of the agar and, therefore, that they had not been influenced in their direction of growth by gravity. Sartoris<sup>1</sup> states that promycelia developed on the surface of the primary leaf-sheath of a wheat seedling always grow upwards, and he came to the conclusion that the promycelia are negatively geotropic. However, the critical experiments just described do not confirm his deduction. It may be safely concluded that the promycelia of *Tilletia tritici* are ageotropic.

Chlamydospores were sown on 2 per cent. plain agar in Petri dishes, and the dishes were fixed in such a way that in some of them the surface of the agar was vertical, as in the preceding experiment, in others the surface of the agar looked directly upwards, and in yet others the surface of the agar looked directly downwards. In all the plates promycelia developed in the course of a few days. Invariably it was found that the ends of the promycelia and the tufts of sterigmata were directed away from the agar surface (cf. Fig. 114, A, p. 232). It appears from these observations that the promycelia of *Tilletia tritici* are negatively hydrotropic.

<sup>1</sup> G. B. Sartoris, "Studies in the Life History and Physiology of Certain Smuts," *Amer. Journ. Bot.*, Vol. XI, 1924, p. 621.

**The Phenomenon of Protoplasmic Migration.**—The advance of the protoplasm in a hypha toward the apical growing point and its basal delimitation by the formation of cross-walls is a phenomenon which is well known to occur in various fungi. Thus Buller<sup>1</sup> has described it in connexion with the development of the germ-tubes of *Polyporus squamosus*. However, up to the present, the conditions under which a new septum is formed do not seem to have been determined. In what follows an attempt will be made to throw some light on this problem.

Protoplasmic migration with the formation of septa occurs in *Tilletia tritici* in the promycelium, the sterigmata, the basidiospores, and in the mycelium arising either from an H-shaped pair of sterigmata or from a basidiospore (Fig. 114, A, B, and Fig. 130, A, B, C; pp. 232 and 260), so that it is a phenomenon of considerable importance in this fungus. It has been studied more especially in promycelia.

Some chlamydospores were germinated on the surface of sterile distilled water contained in a Syracuse watch-glass. Many of the promycelia grew along the surface of the water. As they elongated, their protoplasm migrated toward their apices, and in various promycelia all stages of protoplasmic migration and of septum-formation could be observed. A series of stages leading to the formation of a new and empty cell was followed in a single promycelium and is illustrated in Fig. 135.

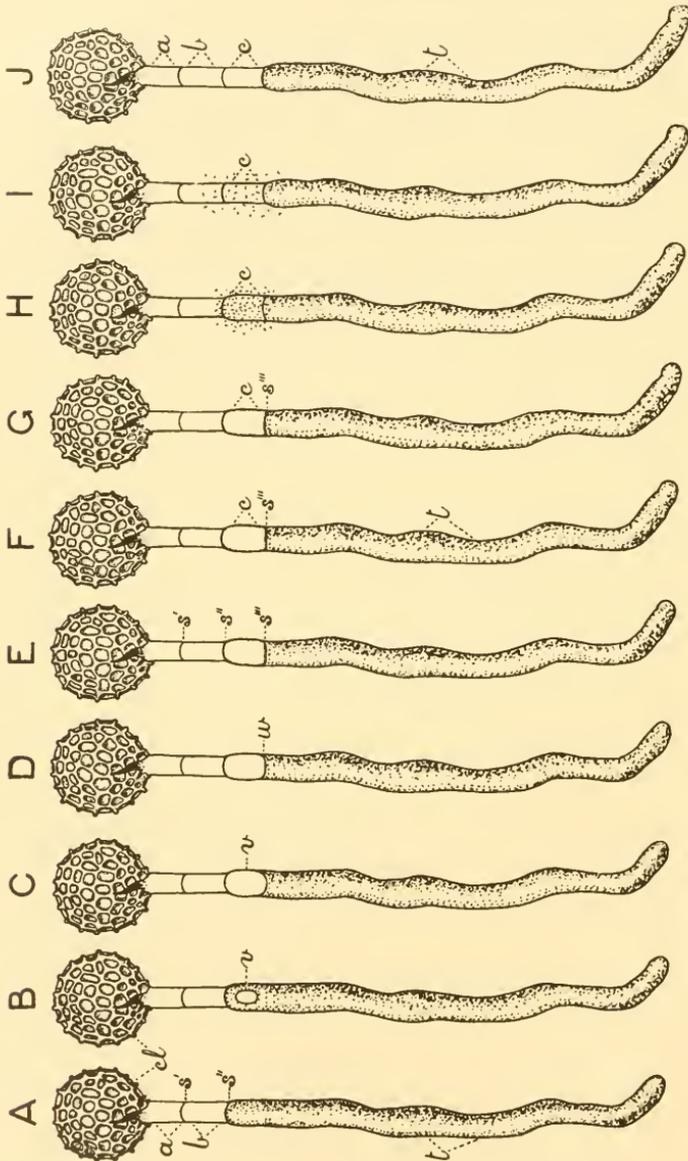
In Fig. 135, A, is shown a chlamydospore *cl* from which had grown out a promycelium which now consists of three cells—two clear basal cells, *a* and *b*, and a long terminal apical cell, *t*. The basal cells have lost their protoplasmic contents and therefore are dead and contracted laterally; while the apical cell is filled with protoplasm, is living and fully turgid, and is therefore uncontracted laterally. The two septa *s'* and *s''* are concavo-convex, the concavities being directed toward the apex of the promycelium. The septum *s''* separates the dead cell *b* from the living terminal cell *t*.

In Fig. 135, J, is shown the same promycelium as in A, but 3–4 hours later. J differs from A in having a longer promycelium and

<sup>1</sup> A. H. R. Buller, "The Biology of *Polyporus squamosus*, a Timber-destroying Fungus," *Journ. Econom. Biol.*, Vol. I, 1906, pp. 119–120.

in having three clear non-living collapsed basal cells, *a*, *b*, and *c*, instead of two. The various stages passed through from A to J will now be described.

In Fig. 135, B, a vacuole *v* has become visible. This vacuole



increases in size until it has the appearance shown in C. The apical wall of the vacuole, *w*, gradually flattens, as shown at D, and then a new septum, *s'''*, begins to develop, as shown at E. In F the septum has just been formed and, at this stage, it is quite flat. In F we now have four cells instead of three—two, dead, empty, laterally contracted, basal cells *a* and *b*, a highly vacuolated uncontracted cell *c*, and a terminal non-vacuolated uncontracted cell *t*. The layer of cytoplasm which covers the wall of the cell *c* is extremely thin, no thicker than the very thin cell-wall and scarcely visible with the microscope; but there can be no doubt that it is present, for otherwise the cell could not be turgid.

Undoubtedly, at the stage F in Fig. 135, the promycelium includes two living cells *c* and *t*. It therefore seems clear that the septum *s'''* was formed not at a free surface of the massive protoplasm of the cell *t*, but *in the protoplasm itself*. On one side of the septum, during its formation, there was the massive protoplasm of the cell *t*, and, on the other side, a very thin layer of protoplasm belonging to the highly vacuolated cell *c*. It is often stated in textbooks that the living part of the promycelium of *Tilletia tritici* is always unicellular; but, from the observations just recorded, it is evident that, temporarily at least, it is bicellular.

The stage F was observed to persist for an hour or more. The septum *s'''* then very gradually became slightly concavo-convex, as shown at G, thereby indicating that the turgidity of the cell *c* was diminishing. A little later the cell *c* died, as was shown by the

FIG. 135.—The phenomenon of protoplasmic migration and the formation of septa in the protoplasm of a promycelium of *Tilletia tritici*. A–J, successive stages in the development of a promycelium which grew in a film of water in a Syracuse watch-glass. Total time of observation, about 3·5 hours. A, a chlamydospore which has produced a promycelium in which the protoplasm has crept forward. There are two septa, *s'* and *s''*. The basal cells, *a* and *b*, are devoid of protoplasm and have collapsed. The terminal cell *t* is full of protoplasm. B, a vacuole *v* has now appeared. C, the vacuole is now much larger. D, the massive protoplasm is flattening where it abuts on the vacuole. E, a new septum *s'''* is being formed in the protoplasm. F, the new septum *s'''* has been formed and now separates a highly vacuolated living cell *c* from the living terminal *t* which is full of protoplasm. G–J, about an hour after F; the collapse of the cell *c*. G, the septum *s'''* is beginning to bulge into the cell *c*. H, the bulging has attained almost its maximum, the cell *c* is collapsing, and motile bacteria have been attracted chemotactically by the escaping cell-sap. I, the cell *c* has now completely lost its turgidity and the bacteria are leaving it. J, the bacteria have disappeared and the promycelium is now seen to consist of three short dead basal cells, *a*, *b*, and *c*, and of one long living terminal cell *t*. Magnification, 750.

further bulging of the septum  $s'''$  into it and by its lateral contraction (stages H to J).

As the cell  $c$  died, a remarkable event was witnessed. In the water, on the surface of which the promycelium was growing, there were a certain number of motile bacteria. These were evenly dispersed throughout the fluid, but were so few in number as scarcely to attract attention. However, at one particular moment, as shown at H, the bacteria swarmed toward the promycelium and congregated upon the surface of the cell  $c$ ; while, at that very moment, the cell  $c$  was observed to be collapsing (H-I). Evidently, the extremely thin layer of cytoplasm lining the cell-wall of the cell  $c$  had suddenly died, in consequence of which the cell-sap was escaping through the cell-wall, the cell itself was contracting laterally, and the escaping sap was stimulating the bacteria chemotactically and causing them to swarm toward the collapsing cell. The bacteria remained at and near the cell-wall of the cell  $c$  for only about one minute, and then they moved away again never to return. The dispersion of the bacteria doubtless coincided with a diminution of the chemotactic stimulus caused by the diffusion of the cell-sap into the surrounding medium. As soon as the bacteria had gone away from the cell  $c$ , the promycelium had the appearance shown at J in which there are three dead basal cells,  $a$ ,  $b$ , and  $c$ , and one living terminal cell  $t$ .

All the stages in the formation of a new basal cell cut off by a new septum have been described, so that we now have a clear picture of the manner in which protoplasmic migration and septation take place in a promycelium. Doubtless these phenomena, as they occur in the sterigmata, the basidiospores, and the mycelium arising from an H-shaped pair of sterigmata or from a basidiospore, run a similar course to that observed in a promycelium. It is probable that further observation will show that protoplasmic migration and septation take place in *Polyporus squamosus* and in many other fungi in the same manner as in *Tilletia tritici*.

**Conclusion.**—The investigations which have been recorded in this Chapter have led to a new conception of the basidium of the genus *Tilletia* and to a new mode of inoculating wheat seedlings. The observations made in the course of the work and the chief conclusions arrived at are summarised in the General Summary at the end of this volume.

## CHAPTER III

### THE SPHAEROBOLUS GUN AND ITS RANGE

Introduction—The Species of *Sphaerobolus*—Cultures—The Germination of the Gemmae and of the Spores and the Diploid Nature of the Germ-tubes—The Projectile—The Structure and Mechanism of the Gun—The Range of the Gun—The Range of *Sphaerobolus stellatus* of Kenora Origin—Miss Walker's Observations on the Range of Various *Sphaeroboli*—An Artificial Method for causing a *Sphaerobolus* Gun to discharge its Projectile—The Horizontal Range of *Sphaerobolus stellatus* of Winnipeg Origin—Summary of Observations on the Range of *Sphaerobolus* Guns—The Horizontal Range of Various Fungus Guns and of Expulsive Fruits—The Kinetics of the *Sphaerobolus* Gun—Relations of *Sphaerobolus* with Water—Relations of *Sphaerobolus* with Light—*Sphaerobolus* as a Coprophilous Fungus dispersed by Herbivorous Animals—*Pilobolus*, *Ascobolus immersus*, and *Sphaerobolus* as Three Fungi with Parallel Adaptations for a Coprophilous Mode of Life—*Sphaerobolus* as a Member of the More Specialised of Two Groups of Coprophilous Fungi—*Sphaerobolus* as a Lignicolous Fungus and the Problem of its Mode of Infecting Wood.

**Introduction.**—*Sphaerobolus* is a genus of the Gastromycetes, which includes an uncertain number of species and is of world-wide distribution. Its very delicate and wonderfully constructed fruit-bodies are commonly found in Europe and North America in groups upon rotting wood and sometimes on the dung of herbivorous animals. I myself have observed the fungus: in England, on old stumps, boards, sticks, sacking, and a fallen cone of *Cedrus Deodara*; and, in central Canada, on a board and wooden post lying on the ground, and on cow-dung and horse-dung plats scattered in meadows.

In the island of Trinidad, according to Rorer,<sup>1</sup> *Sphaerobolus stellatus* is found commonly on cow dung; and in New Zealand,

<sup>1</sup> J. B. Rorer, "A Preliminary List of Trinidad Fungi," *Board of Agriculture for Trinidad and Tobago, Circular No. IV*, Report of the Mycologist for the year ending March 31, 1911, Part II, issued Oct. 1911, p. 42.

according to Cunningham,<sup>1</sup> the same species grows on rotting wood and sticks lying on the forest floor and on rotting sacking. *S. stellatus* has also been found by Petch<sup>2</sup> on elephant dung in Ceylon and is recorded by Shirai and Hara<sup>3</sup> as occurring in Japan.

*Sphaerobolus* was first described and illustrated (Fig. 136) by Micheli<sup>4</sup> in 1729. He discovered that the glebal mass of the fruit-body is composed largely of spores and that the fruit-body, at the climax of its development, shoots away its glebal mass to a considerable distance. On account of the violent ejection of the glebal mass, Micheli called the fungus *Carpobolus*; but this name was changed by Tode<sup>5</sup> in 1790 to *Sphaerobolus*.<sup>6</sup>

The structure and discharge-mechanism of *Sphaerobolus* were described by Greville<sup>7</sup> in 1825, by Corda<sup>8</sup> in 1842, by Bonorden<sup>9</sup> in 1851, and in greater detail by Pitra<sup>10</sup> in 1870.

The older writers, like ourselves, were of course fascinated by the spectacle of a tiny fungus everting its inner peridium with lightning-like rapidity and casting away its ball of spores. Thus Greville,<sup>11</sup> in treating of *Sphaerobolus stellatus* in the third volume of his *Scottish Cryptogamic Flora*, says: "This is unquestionably the most wonderfully constructed plant which it has been my lot to describe in the present publication. That so great a degree of force should exist in a body not larger than the head of a pin,<sup>12</sup>

<sup>1</sup> G. H. Cunningham, "*Sphaerobolus stellatus* Tode, a Fungus with a Remarkable Method of Spore-dissemination," *New Zealand Journal of Science and Technology*, Vol. VI, 1923, p. 19.

<sup>2</sup> T. Petch, *in litt.*, 1921.

<sup>3</sup> M. Shirai and K. Hara, *A List of Japanese Fungi Hitherto Unknown*, Edition III, Japan, 1927, p. 368.

<sup>4</sup> P. A. Micheli, *Nova Plantarum Genera*, Florentiae, 1729, p. 221, Tab. CI.

<sup>5</sup> H. J. Tode, *Fungi Mecklenburgenses Selecti*, Luneburgi, 1790, p. 43.

<sup>6</sup> The name *Sphaerobolus* is derived from σφαῖρα, a ball, and βάλλω, I throw.

<sup>7</sup> R. K. Greville, *Scottish Cryptogamic Flora*, Edinburgh, Vol. III, 1825, No. 158, one coloured Plate with 14 Figs.

<sup>8</sup> A. C. J. Corda, *Icones Fungorum*, Bd. V, 1842, p. 66, Taf. VI.

<sup>9</sup> H. F. Bonorden, "Mykologische Beobachtungen. II. Über den Bau von *Sphaerobolus stellatus*," *Botanische Zeitung*, Bd. IX, 1851, p. 18; also *Handbuch der allgemeinen Mykologie*, Stuttgart, 1851, pp. 231-232.

<sup>10</sup> A. Pitra, "Zur Kenntnis des *Sphaerobolus stellatus*," *Botanische Zeitung*, Bd. XXVIII, 1870, pp. 681-689, 697-703, 713-719, Taf. XI.

<sup>11</sup> R. K. Greville, *loc. cit.*

<sup>12</sup> Greville is here minimising the size of the fungus or his pins had larger heads than those now most commonly used.

and that force, too, exerted in defiance of considerable resistance, seems to surpass the power of any theory to account for satisfactorily." A century has rolled by since Greville made those remarks and it is only in recent years, with the help of modern histological and biochemical methods, that it has been possible to explain the mechanism of the Sphaerobolus gun with any degree of completeness and finality.

In 1884, E. Fischer<sup>1</sup> gave a detailed account of the structure and development of the Sphaerobolus fruit-body and a general explanation of the mechanism by which the ball of spores is shot away. He concluded from his minute investigations that Sphaerobolus belongs not to the Nidulariineae, as was formerly believed, but to the Plectobasidiineae, and that the fungus is therefore more closely allied

to Scleroderma and Tulostoma than to Crucibulum and

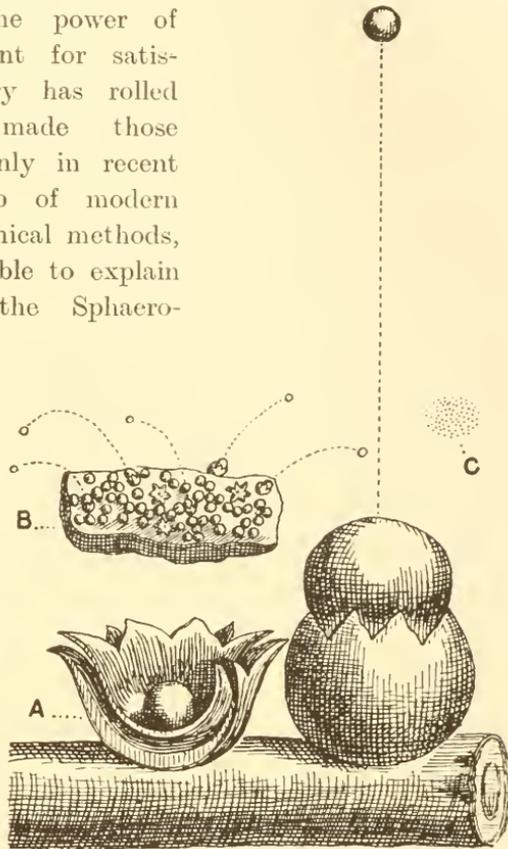


FIG. 136.—Micheli's illustration of *Sphaerobolus stellatus* (his *Carpobolus*). A, part of a drawing showing two fruit-bodies much enlarged on a stick; the fruit-body on the left, in section, exhibits the two layers of the stellate peridium bearing a glebal projectile shortly before discharge; in the fruit-body on the right the inner layer of the peridium has suddenly become everted and has shot away the projectile in the direction indicated by the broken line. B, fruit-bodies on a piece of wood, natural size: the broken lines indicate the direction of flight of several glebal projectiles. C, spores from the interior of the projectile. Copied by A. H. R. Buller from Plate 86 of Micheli's *Nova Plantarum Genera* (1729).

<sup>1</sup> E. Fischer, "Die Entwicklungsgeschichte der Gastromyceten," *Botanische Zeitung*, Bd. XLII, 1884, Nos. 28-31, pp. 433-443, 449-462, 465-470.

Cyathus.<sup>1</sup> Consequently he called the ball of spores not a *peridiolum*, as is still done by many systematists, but a *gleba*. His illustrations, showing the structure of the fruit-body in detail, are given in his account of the Plectobasidiineae in the *Pflanzenfamilien*<sup>2</sup> and have been reproduced by Zopf<sup>3</sup> and von Tavel.<sup>4</sup>

Other studies of the structure of *Sphaerobolus* have been made by Lydia Rabinowitsch (1894)<sup>5</sup> and Pillay<sup>6</sup> (1923)—both pupils of E. Fischer—and by Leva Walker (1927).<sup>7</sup> Rabinowitsch added details to our knowledge of the palisade (collenchyma) layer. Pillay

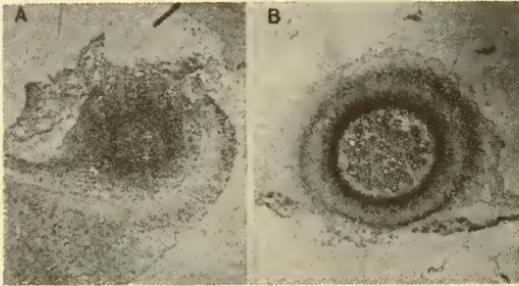


FIG. 137.—*Sphaerobolus iowensis*. Median sections of two young fruit-bodies. A, the primordia of the glebal mass and of the peridium are becoming differentiated from one another. B, a further stage in development: the glebal mass now contains many minute glebal chambers lined by young basidia, and the various layers of the peridium are being formed. Sections prepared and photographed by Leva B. Walker. Magnification, about 40.

showed that the germ-tubes of the spores of *S. stellatus* at once produce clamp-connexions, so that *S. stellatus* is homothallic.

Rabinowitsch and Pillay observed that in the strains of *Sphaerobolus stellatus* studied by them there was an entire absence of definite glebal chambers, but that the basidia tend to converge toward the middle of each glebal subdivision.

They therefore concluded that *Sphaerobolus* is more closely related

<sup>1</sup> E. Fischer, in *Die natürlichen Pflanzenfamilien* of Engler u. Prantl, 1900, Teil I, Abteil. I\*\*, p. 345.

<sup>2</sup> *Ibid.*

<sup>3</sup> W. Zopf, *Die Pilze*, Breslau, 1890, p. 85, Fig. 55. Zopf also shows the mycelium growing upon, and forming fruit-bodies upon, rabbit dung.

<sup>4</sup> F. von Tavel, *Vergleichende Morphologie der Pilze*, Jena, 1892, p. 119, Fig. 80.

<sup>5</sup> Lydia Rabinowitsch, "Beiträge zur Entwicklungsgeschichte der Fruchtkörper einiger Gastromyceten," *Flora*, Bd. LXXIX, 1894, pp. 414-418.

<sup>6</sup> T. P. Pillay, "Zur Entwicklungsgeschichte von *Sphaerobolus stellatus* Tode," *Jahrbuch der Philosophischen Fakultät II der Universität Bern*, Bd. III, 1923, pp. 197-219.

<sup>7</sup> Leva B. Walker, "Development and Mechanism of Discharge in *Sphaerobolus iowensis* n. sp. and *S. stellatus* Tode." *Journal of the Elisha Mitchell Scientific Society*, U.S.A., Vol. XLII, 1927, pp. 151-178, Plates XVI-XXV.

to the Plectobasidiales (Sclerodermales) than to the hymenium-bearing Gastromycetes such as the Lycoperdales, but that to a certain degree it occupies an intermediate position between the two groups. However, Miss Walker found that, whereas in *S. stellatus* true glebal chambers are never present and an orientation of the basidia is only to be seen in very young stages,

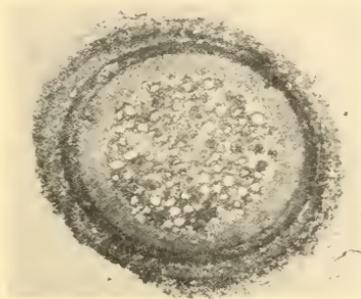


FIG. 138.—*Sphaerobolus iowensis*. A further stage in development of the fruit-body. The glebal chambers have increased in size, and the various layers of the peridium are distinguishable. Section prepared and photographed by Leva B. Walker. Magnification, about 30.

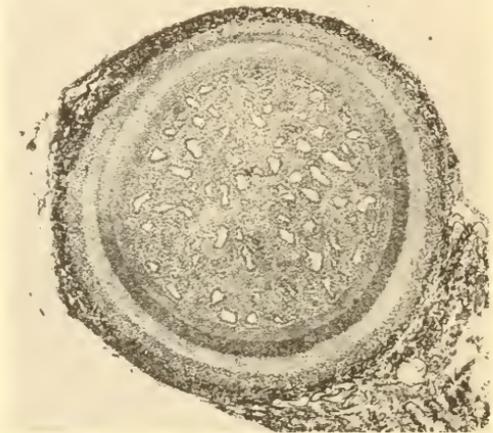


FIG. 139.—*Sphaerobolus iowensis*. A further stage in development of the fruit-body. The glebal chambers lined by basidia have now attained about their maximum size. The various layers of the peridium are still in course of development. Section prepared and photographed by Leva B. Walker. Magnification, about 40.

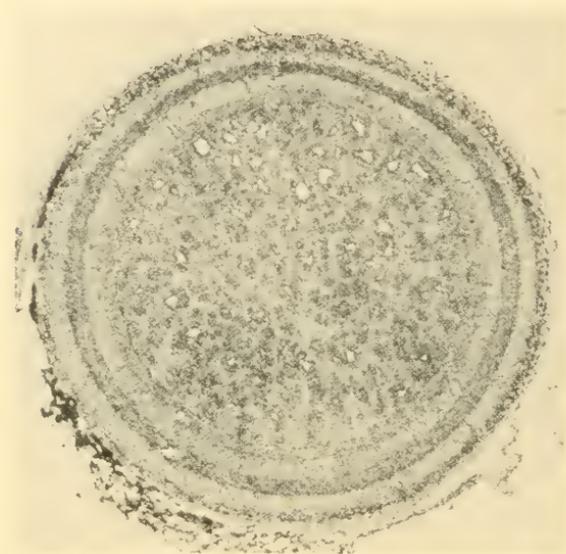
in *S. iowensis* true chambers are developed "which persist up to maturity and are very evident in dried unopened basidiocarps" and the basidia are arranged in definite hymenia, each hymenium lining the cavity of a glebal chamber (Figs. 137–141). Hence, while the absence of glebal chambers in *S. stellatus* would lead us to place *S. stellatus* in the Plectobasidiales (Sclerodermales), the presence of glebal chambers in *S. iowensis* would lead us to place *S. iowensis* near the Lycoperdales. But to separate *S. stellatus* and *S. iowensis*, which on account of their numerous points of resemblance are evidently but two species of a single genus, by placing them in

different orders would be taxonomically absurd. It is therefore best to keep the two species together and to accept Miss Walker's conclusion that the presence or absence of glebal chambers is not a good criterion for distinguishing between these orders of the *Gastromycetes*.<sup>1</sup>

**The Species of *Sphaerobolus*.**—Fischer,<sup>2</sup> in 1900, stated that there

were five species of *Sphaerobolus* but that only *S. stellatus* Tode was known in detail. Rea, in 1922, in his *British Basidiomycetae* enumerates three species: *S. stellatus* Tode, *S. dentatus* (With.) W. G. Sm., and *S. terrestris* (A. et S., non Tode) W. G. Sm., but it is uncertain whether or not *S. dentatus* and *S. terrestris* are really distinct from *S. stellatus*.

A critical comparative study of four North-American strains of *Sphaerobolus*



[FIG. 140.—*Sphaerobolus iowensis*. A further stage in development of the fruit-body. The glebal chambers are becoming filled with spores and thus obliterated. Toward the top, in the still open chambers, the spores are still adhering to their basidia. Section prepared and photographed by Leva B. Walker. Magnification, about 40.

has been made by Miss Walker.<sup>3</sup> She grew all the four strains in pure cultures and found that two of them were typical *S. stellatus*, that one of them was a new variety of *S. stellatus* which she named *S. stellatus* var. *giganteus*, and that another of them was an entirely new species which she named *S. iowensis*. The un-

<sup>1</sup> Leva B. Walker, *ibid.*, pp. 174-175.

<sup>2</sup> E. Fischer, in *Die nat. Pflanzenfamilien*, *loc. cit.*, p. 346.

<sup>3</sup> Leva B. Walker, *loc. cit.*, pp. 151-172, Plates XVI-XXV.

opened fruit-body of typical *S. stellatus* is 1·5–2·0 mm. in diameter, whereas that of its variety *giganteus* is 3–4 mm. in diameter. *S. iowensis* differs from *S. stellatus* in the absence from its peridium of an outer gelatinous layer and in having a distinctly chambered gleba. The typical form of *Sphaerobolus stellatus* may thus be described :

***Sphaerobolus  
stellatus* Tode<sup>1</sup>**

Basidiocarps more or less deeply embedded in the surrounding arachnoid mycelium and the substratum ; when young, spherical to oval, 1·5–2·0 mm. in diameter, whitish or pale yellow owing to the deep yellow of the interior showing faintly through the fine white filaments covering the exterior, the outer

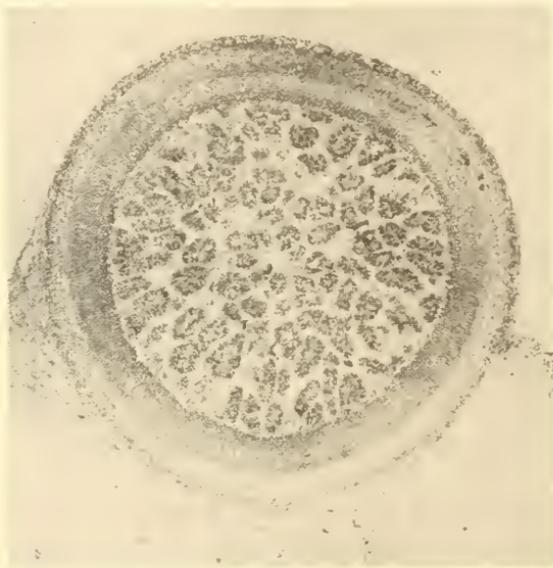


FIG. 141.—*Sphaerobolus iowensis*. Vertical section of a nearly mature fruit-body. The basidia have now broken down and disappeared and the spore-masses are held in place by tramal hyphae which are now beginning to disintegrate and form a soft gluey oily slime. The various layers of the peridium can now be readily distinguished and, centripetally, are : a layer of mycelium (lower half), a layer of pseudoparenchyma, a fibrous layer, a palisade layer passing into pseudoparenchyma above, and a thin pseudoparenchymatous layer completely surrounding the gleba, i.e. the wall of the glebal mass. Section prepared and photographed by Leva B. Walker. Magnification, about 40.

layer of the peridium decidedly gelatinous ; at maturity the peridium splitting open above so as to form an urceolus in which the glebal mass lies exposed to view, the rim of the urceolus divided into some 6–8 acute stellately arranged teeth, the interior surface of the urceolus down to the glebal mass orange-yellow.

<sup>1</sup> I have drawn up this description from my own and Miss Walker's observations.

The glebal mass solitary, spherical, devoid of glebal chambers, whilst in the urceolus becoming slimy on its exterior surface, at first paler, then orange-yellow to dark-brown, more highly coloured above than below, with the consistence of wax, adhesive when ejected, hardening on drying but not becoming brittle, turning black with age,<sup>1</sup> containing numerous spores and some gemmae. The ejection of the glebal mass accomplished by the sudden eversion of the inner layers of the peridium which, after the ejection, appear as a pearly vesicle crowning and attached to the outer membranes of the peridium. The basidia intermingled in the glebal subdivisions, short, swollen, more or less oval, without sterigmata, bearing at the apical end 4-9 sessile crowded spores, disappearing completely before the glebal mass is discharged. The spores colourless, smooth, oval but a few more or less globular,  $6-8 \times 4-5 \mu$ . Gemmae chiefly in the outer part of the gleba, more or less oval or elongated, larger than the spores, readily germinating in the gleba, the germ-tubes bearing clamp-connexions.

Scattered or crowded on twigs, sticks, decaying stumps, boards, posts, sawdust, old sacking, cordage, etc., and also on the dung of herbivorous animals, *e.g.* cows, horses, rabbits, hares, and elephants. Of world-wide distribution. Recorded in Europe, the United States, Canada, Trinidad, New Zealand, Japan, and Ceylon.

Miss Walker has thus described her new variety of *S. stellatus* and her new species *S. iowensis* :

#### ***Sphaerobolus stellatus* var. *giganteus***

“Basidiocarps having the colour, structure, and appearance of *S. stellatus* but being much larger—up to 4 mm, in diameter (usually about 3 mm.) in unopened basidiocarps and 5-6 mm. from tip to tip of peridium in opened basidiocarps. Spores globular with a slight apiculus,  $5-7 \times 6-8 \mu$ , mostly  $6-7 \mu$ .”

Found on dung (horse ?) at Starkville, Miss., U.S.A. Distinguished from typical *S. stellatus* by its large size and globular spores.

<sup>1</sup> At the end of five years glebal masses kept in a glass tube were found to have become quite black.

**Sphaerobolus iowensis** Walker

“Unopened basidiocarps 1–1.5 mm. in diameter. Peridium breaking stellately at the apex into 3–8 parts (usually 4–5), the tips becoming only slightly recurved so that the peridium of the opened fruit-body is somewhat cup-shaped. Interior of peridium cadmium-yellow when first opened, glebal mass raw umber<sup>1</sup> when first exposed, soon becoming almost black. Peridium separating into two regions, an outer and an inner. Outer peridium during its development and at maturity composed of two layers, an outer filamentous and an inner pseudoparenchymatic. Inner region of a layer of tangential hyphae and a palisade layer. Gleba containing during its development many definite chambers lined with basidia. Wall of glebal mass firm, composed of cells 6–15  $\mu$  in diameter. Contents of glebal mass soft and gluey, never drying hard. Spores 5–6  $\times$  6–10  $\mu$  (usually 5.6  $\times$  8  $\mu$ ).”

Found on old coniferous boards at Hunters, Iowa, U.S.A. Distinguished from *S. stellatus* by its chambered gleba and the absence of a gelatinous layer in the peridium.

The material for my own investigations on *Sphaerobolus* consisted of: (1) fruit-bodies of *S. stellatus* collected at Kenora in western Ontario and at the Manitoba Agricultural College near Winnipeg, and (2) cultures kindly sent to me by Dr. Leva Walker from the University of Nebraska, U.S.A.

**Cultures.**—*Sphaerobolus* can be readily cultivated on sterilised wood or on horse dung (Fig. 142). One obtains fruit-bodies growing on wood or dung, places them in a closed vessel in the laboratory, and allows them to discharge their glebal masses against a sterilised slide or glass plate. The projectiles stick to the surfaces which they strike, and most of them, when shot out of the *Sphaerobolus* guns, are free from contamination by bacteria. The projectiles caught on a glass slide or plate are removed with a sterilised needle and planted on sterilised agar plates. Each projectile sends out radially a pure white mycelium with numerous clamp-connexions on the

<sup>1</sup> Vide Ridgway, *Color Standards and Nomenclature*.

hyphae. Both Miss Walker and I have obtained *Sphaerobolus* in pure culture by the method just described.

Miss Walker transferred the mycelium from an agar plate to



FIG. 142.—*Sphaerobolus stellatus*. A culture made by Leva B. Walker, on horse dung, removed from a flask. A large number of sporocarps have come into existence in the white sheet of mycelium and some of them are now open and about to discharge their glebal balls. The projectiles were shot upwards to a maximum height of 14 feet. Photograph made in the early morning at the University of Nebraska. Magnification, 1·5.

flasks containing sterilised agar media, corn-meal mush, and partially rotted Willow wood, and she placed the cultures in the light. The Willow-wood cultures within a few weeks fruited abundantly, but the corn-meal and agar cultures never fruited. I, too, have not been able to obtain fruit-bodies on a malt-agar medium. After various trials, Miss Walker obtained the best results with the following media: Willow wood, half-rotted horse dung, sawdust (probably Willow or Ash), and mixtures of horse dung and sawdust.

I have cultivated the mycelium with success on sterilised horse dung, but my investigations have

mostly been made on wild fruit-bodies occurring on boards and on cow dung. When cow dung permeated with the mycelium was brought into the laboratory and kept moist, some scores of *Sphaerobolus* fruit-bodies appeared on its surface within a few days (Fig. 164, p. 332).

The Germination of the Gemmae and of the Spores and the Diploid Nature of the Germ-tubes.—When a glebal mass which has

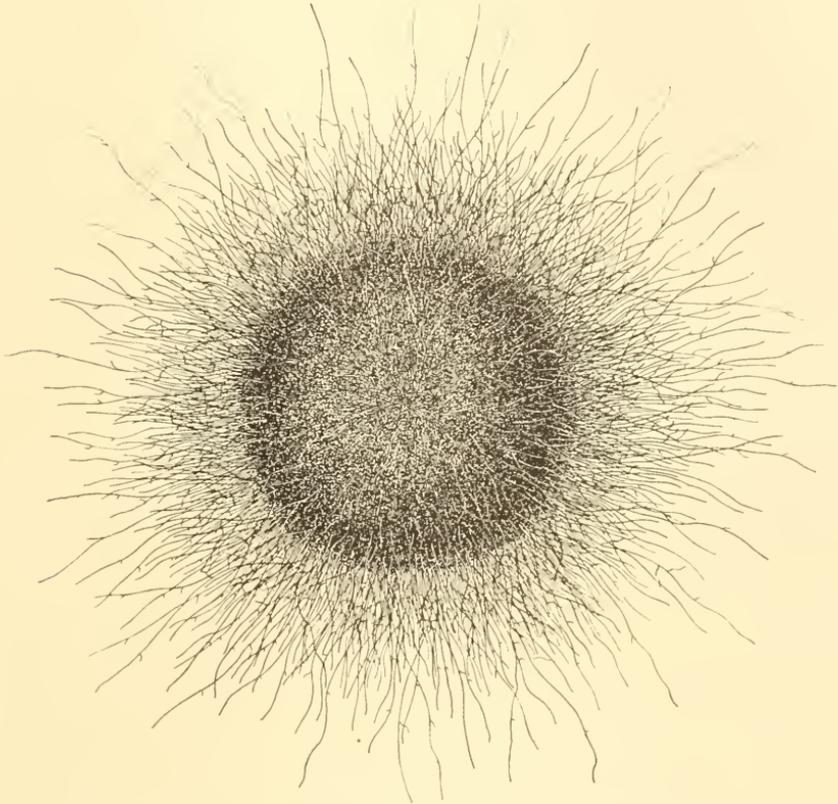


FIG. 143.—*Sphaerobolus stellatus*. A glebal mass which, after discharge from the fruit-body, was placed in sterilised tap-water where it germinated. Already, 60 hours after its immersion in water, it has sent out a copious radiating mycelium. This mycelium originated from the germination of numerous gemmae scattered among the spores in the gleba, and their germ-tubes penetrated the wall of the glebal mass. Glebal masses kept dry for upwards of ten years germinated in water in the manner here shown. Germinating glebae can be employed for making pure cultures. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 42.

been shot out of a fruit-body is placed in water, just as on malt-agar it germinates within 24 hours and sends out clamp-connexion-bearing hyphae on all sides (Figs. 143 and 144). These hyphae, as Pillay<sup>1</sup> observed, form an adhesive slime-disc (Schleimscheibe) where

<sup>1</sup> T. P. Pillay, *loc. cit.*, pp. 213-214.

they come into contact with the substratum. From the slime-disc hyphae grow radially outwards and in four days may surround it with a hyphal ring 6-8 mm. wide. Fischer tried to determine the origin of the radiating mycelium but failed on account of the presence of the thick slime in the gleba's interior. Pillay was more fortunate; for, with the help of microtome sections, he discovered that the hyphae spring not from the spores but *from the gemmae*.<sup>1</sup> He also observed that in a glebal mass three days old the spores are intact but ungerminated, and that in a glebal mass five to six days old the spores are breaking down, very few being still present. Fischer had also noticed this degeneration of the spores. Pillay thought he saw germinating spores in a glebal mass twice, but could not be sure; and he states that, in a glebal mass placed in water, while it is certain that almost all of the gemmae germinate it is equally certain that almost all of the spores degenerate.<sup>2</sup>

From the observations just described it is clear that the germination of a glebal mass as a whole is in reality due to the germination of some thousands of gemmae in the gleba's interior. When a moist glebal mass is opened and its contents are examined, one can often find among the ungerminated spores some gemmae which have already produced short germ-tubes, each gemma with its germ-tube consisting of 2-3 cells and each septum being provided with a clamp-connexion (Fig. 156, p. 311). Such germinated gemmae were illustrated by Fischer.<sup>3</sup> As one would expect, when a glebal mass as a whole germinates and the germ-tubes of the gemmae push their way out through the glebal envelope to the exterior, the germ-tubes in developing into the radiating mycelium continue to produce clamp-connexions. Pillay<sup>4</sup> found that the cells of the radiating hyphae contain typical pairs of nuclei. It is therefore certain that the mycelium produced by a germinating glebal mass is sexually diploid.

A glebal mass may retain its vitality for a very long time. Miss Walker<sup>5</sup> extracted from some conical pure-culture flasks some glebal masses which had been shot against the glass walls seven

<sup>1</sup> T. P. Pillay, *loc. cit.*, p. 214.

<sup>2</sup> *Ibid.*, pp. 214-215.

<sup>3</sup> E. Fischer, in *Die nat. Pflanzenfamilien*, *loc. cit.*, p. 345, Fig. 182, G.

<sup>4</sup> T. P. Pillay, *loc. cit.*, p. 214.

<sup>5</sup> L. B. Walker, *loc. cit.*, p. 173.

years previously, and she sowed them in a culture medium. They germinated more slowly than newly-discharged glebal masses, but the mycelium grew with the usual vigour. Recently, Miss Walker<sup>1</sup> sowed on agar plates some glebal masses of *Sphaerobolus stellatus* which had been kept dry in a culture flask for eleven years. Of fifteen of the glebal masses of one strain (her S I) thirteen produced a mycelium, and of twelve of the glebal masses of another (her S II) two produced a mycelium. She has thus proved that the glebal masses of *S. stellatus*, when dry, may retain their vitality for upwards of ten years.

My own observations on the retention of vitality by dried glebal masses of *S. stellatus* serve to confirm those of Miss Walker. In December, 1923, I placed a number of dry glebal masses in a test-tube which was closed with a cork. At the end of January, 1929, *i.e.* after five years and nearly two months, one of the glebal masses was placed in a hanging drop of

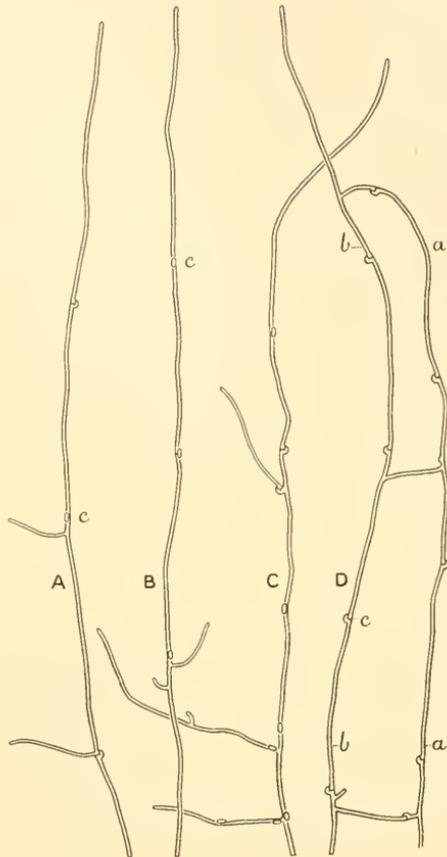


FIG. 144.—*Sphaerobolus stellatus*. Hyphae from the radiating mycelium produced by a germinating glebal mass (*cf.* Fig. 143). Terminal parts of hyphae; A, B, and C about 50 hours after the glebal mass was placed in water; and D 75 hours after. The hyphae all show clamp-connexions *c c* and were therefore in the diploid nuclear condition. In D the two hyphae *a a* and *b b* have anastomosed with one another in three places. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 380.

<sup>1</sup> L. B. Walker, *in litt.*, Feb., 1929.

sterilised tap-water and another in a hanging drop of dung-agar. In the course of a few days, both glebal masses germinated and, notwithstanding the competition of a *Mucor* and of bacteria, both of them sent out radially a septate mycelium which bore numerous clamp-connexions and exactly resembled that sent out by glebal masses in cultures made in a similar manner but in which the culture medium was free from other organisms. Thus it was proved that the glebal masses had retained their vitality in the dry condition for upwards of five years.

Another glebal mass which in my laboratory had fallen on to a sheet of tissue paper in November, 1920, and had been kept dry in a test-tube was placed in sterilised tap-water in February, 1929, *i.e.* after it had been kept dry for eight years and three months. For some days it showed not a sign of life, but eventually it sent out radially a typical mycelium made up of branched and septate hyphae bearing clamp-connexions.

The evidence which is available so far, therefore, indicates that the gemmae in dried glebal masses of *Sphaerobolus stellatus* may retain their vitality for at least eight to eleven years. This long retention of vitality by dried gemmae may well be due in part to the fact that the gemmae are embedded in a thick glutinous matrix inside the glebal mass and are therefore not exposed to the air.

The luxuriant development of the mycelium which grows out from a glebal mass placed in water is probably accomplished in part at the expense of the fatty matrix by which the spores and germinating gemmae are surrounded, and it may well be that the mycelium makes the fat available by excreting into it the enzyme lipase.

Because in a glebal mass placed in water the spores after a few days become disintegrated, it must not be concluded that the spores are valueless in propagating *Sphaerobolus* species. Under natural conditions in the open, it must often happen that the glebal masses of *Sphaerobolus* are swallowed by herbivorous animals, and then the fatty matrix and the spores which it envelopes are subjected to conditions differing greatly from those provided by a simple watery medium. As we shall see later, *Sphaerobolus* often occurs on the dung of cows, rabbits, etc., and it seems very probable that the fungus under such conditions has originated from spores which have

passed uninjured through the alimentary canal of the animals concerned.

Fischer<sup>1</sup> and subsequent workers have found that the spores of *Sphaerobolus* do not germinate readily. He tried feeding them to birds and subjecting them to high temperatures, but all in vain. At length he succeeded in inducing a few spores to germinate in a dung-decoction.

Pillay<sup>2</sup> and Miss Walker<sup>3</sup> both succeeded in germinating the spores of *Sphaerobolus* by placing them in water to which a trace of pepsin had been added. This is a very significant fact in connexion with the dispersion of the fungus by herbivorous animals, and we shall return to it again in a later Section.

Pillay<sup>4</sup> observed that the germ-tube of a spore of *Sphaerobolus stellatus* soon branches and from the first exhibits conjugate pairs of nuclei and develops clamp-connexions over the septa. Thus *S. stellatus* behaves like *Hypochnus terrestris* as investigated by Kniep.<sup>5</sup> Pillay's observations go to show that *S. stellatus* is not heterothallic like most of the Higher Fungi, but is homothallic—a conclusion supported by the subsequent work of Miss Walker.<sup>6</sup>

Miss Walker<sup>7</sup> observed that in the germ-tubes of the spores of *S. iowensis* the septa are far apart and that clamp-connexions are usually absent from them. However, in one instance, she clearly saw a clamp-connexion at a cross-wall and therefore concluded that *S. iowensis* "is probably homothallic."

Pillay<sup>8</sup> and Miss Walker<sup>9</sup> observed that there is a pair of nuclei in each young basidium and later only one nucleus—the fusion nucleus. They further found that the fusion nucleus divides and produces a number of nuclei which make their way into the spores. Each spore, when ripe, contains two nuclei which, during germination, as in *Hypochnus terrestris*, behave from the first as a conjugate pair.<sup>10</sup>

<sup>1</sup> E. Fischer, "Die Entwicklungsgeschichte der Gastromyceten," *Botanische Zeitung*, Bd. XLII, 1884, Taf. VII, Fig. 9.

<sup>2</sup> T. P. Pillay, *loc. cit.*, p. 215.

<sup>3</sup> L. B. Walker, *loc. cit.*, p. 172.

<sup>4</sup> T. P. Pillay, *loc. cit.*, p. 216.

<sup>5</sup> H. Kniep, "Beiträge zur Kenntnis der Hymenomyceten, I, II," *Zeitschrift für Botanik*, Bd. V, 1913, pp. 593-637.

<sup>6</sup> L. B. Walker, *loc. cit.*, p. 172.

<sup>7</sup> *Ibid.*, p. 173.

<sup>8</sup> T. P. Pillay, *loc. cit.*, pp. 205, 210-211, Fig. 4 c-o.

<sup>9</sup> L. B. Walker, *loc. cit.*, p. 168.

<sup>10</sup> T. P. Pillay, *loc. cit.*, p. 216.

**The Projectile.**—The glebal mass of *Sphaerobolus* is the largest known fungus projectile. In *S. stellatus*, at the time of its discharge (Figs. 145 and 160, *d, e*, p. 315), it is a viscid, smooth, shining, blackish-brown, solid, spherical body having a diameter of 1 to 1.25 mm. Its consistence is that of softened glue or beeswax, and it readily adheres to whatever it strikes. Notwithstanding its high content of fat, its specific gravity is greater than that of water, for it sinks when immersed therein.

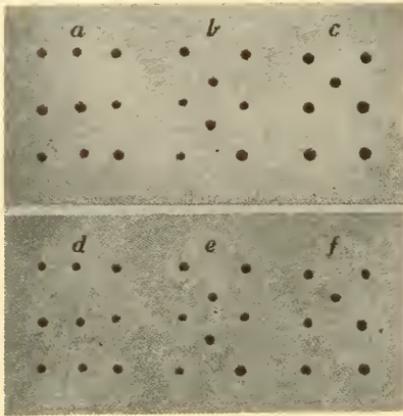


FIG. 145.—*Sphaerobolus stellatus*. Glebal masses after discharge from a horse-dung culture; *a*, kept moist since discharge the same morning; *b*, allowed to dry up since discharge; *c*, soaked in water for 24 hours since discharge; *d, e*, and *f*, the same as *a, b*, and *c* respectively, but photographed again next morning after they had all dried up. A comparison of *d* with *a* and of *f* with *c* shows that the glebal masses contract appreciably on drying. Natural size.

The glebal mass of *S. stellatus*, at the time of its discharge, consists of a thin, brown, *outer wall* (Fig. 146, *a*) and of a large, dirty-white, *internal core*. The wall, referred to by Pillay as the *sporangial wall*, is derived from the outer part of the innermost peridial layer (no. 6 in Figs. 148–150) and consists of slimy or adhesive matter composed of disintegrated cell-walls and brownish cell-contents of what were isodiametric cells. The core or *gleba* consists of a dense mass of fat in which are embedded: (1) *rounded or oval cells* which Pillay refers to as *cystidia*, (2) *spores*, and (3) *gemmae*. The

rounded or oval cells are larger than the spores and have a diameter of 6–16  $\mu$ . They lie just beneath the outer wall where they form a layer 2–3 cells deep, and they decrease in size centripetally<sup>1</sup> (Fig. 146, *b*, also some in view above the layer no. 6 in Fig. 150). The spores, which are colourless and very numerous, are well distributed in the gleba from its centre to the rounded cells (Fig. 146, *d*; the spores in Figs. 148–150 are darkly stained). The gemmae, which are oval or elongated colourless bodies, sometimes provided with one

<sup>1</sup> T. P. Pillay, *loc. cit.*, p. 209.

or more clamp-connexions, are far less numerous than the spores and they mostly occupy a peripheral position, near to and mixed with the rounded cells (Fig. 146, c).

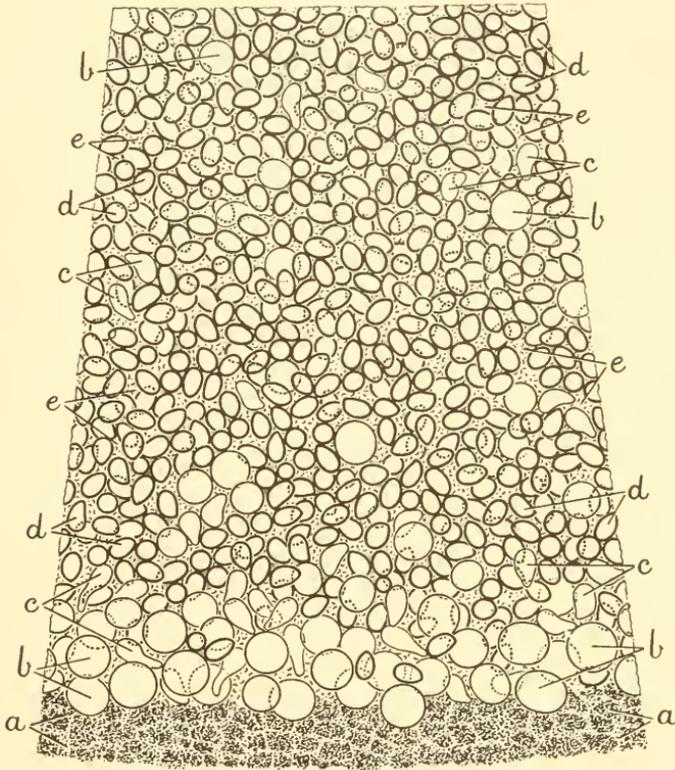


FIG. 146.—*Sphaerobolus stellatus*. A truncated sector of a glebal mass, 3-4 hours after discharge from the fruit-body: *a*, *a* the dark-brown glutinous outer wall consisting of the broken-down cells of the pseudoparenchymatous peridial layer no. 6. The gleba, enclosed by the glebal wall *a a*, now consists of the following elements: *cystidia b b*, forming a peripheral layer next to the glebal wall and also scattered sparsely among the spores; *gemmae c c*, some already showing germ-tubes, with thin walls; *spores d d*, oval and thick-walled; and the *matrix e e*, in which the cystidia, gemmae, and spores are embedded, consisting of a glutinous fatty substance which is very tough when dry. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 466.

The rounded or oval cells, the so-called cystidia, were included by Miss Walker<sup>1</sup> with the gemmae; but Pillay<sup>2</sup> regards them as

<sup>1</sup> L. B. Walker, *loc. cit.*, p. 155.

<sup>2</sup> T. P. Pillay, *loc. cit.*, pp. 209-210.

quite distinct from the gemmae : he states that they do not germinate either inside the glebal mass or when placed in culture media, and he insists that their function is entirely unknown.

The fatty mass making up the core of the projectile comes into existence as the tramal cells and basidia break down and disappear whilst the gleba is ripening. That the sticky substance does consist mostly of fat is indicated by the following observations : (1) oily-looking globules appear in the gleba as the tramal plates break down ; (2) the substance is insoluble in water but readily dissolves in ether, chloroform, xylol, or absolute alcohol ; and (3) the substance takes on a bright orange-red colour with the well-known fat-staining reagent Sudan III.

If with a freshly-discharged glebal mass one makes a smear on a glass slide and observes it with the microscope, the spores are seen to be immobilised by the solid fat in which they are embedded. However, as soon as one adds to the smear a drop of ether or chloroform, the matrix of the smear disappears and the tens of thousands of spores are all liberated so that they float about freely in the currents of the reagent.

After the projectile has been discharged and has become exposed to the atmosphere, it dries and shrinks somewhat, it becomes harder and tougher, and it sticks very tightly to any object which it may have struck ; but, even in the course of some years, it never becomes brittle but retains its elastic hard-glue-like consistence. The elasticity of old dry glebal masses can readily be demonstrated by pressing them down with a knife-blade and then setting them free again.

Evidence of the adhesiveness of the *Sphaerobolus* projectile, which is an important factor in the dispersal of *Sphaerobolus* species under natural conditions, is provided by the following facts. (1) When a projectile is shot against the side of a bell-jar or other container in the laboratory, it sticks where it strikes, mechanical force is required to dislodge it and, if left undisturbed, it remains in the position it has taken up indefinitely. (2) When a projectile is discharged in the open and happens to strike a leaf or a stem of a grass or other flowering plant, it sticks where it strikes and, despite wind and rain, as I have actually observed, remains *in situ* for some

days.<sup>1</sup> (3) When large water drops (0·17 cc.), falling from a tap at the rate of about one per second for a distance of one foot, are allowed to impinge on glebal masses which have been shot on to, and have stuck to, a pane of glass, the glebal masses are not dislodged in the course of five hours.

The projectile, owing to its fatty contents, forms, as we have seen, a glutinous mass which never becomes brittle, even when dried. It is clear, therefore, that the strongest winds cannot break it into pieces or disperse the spores and gemmae which it contains.

Not only is the projectile not dislodged from its place of attachment by water drops falling upon it (as recorded above) but, when submerged in water in a beaker in the laboratory, it does not disintegrate in the course of many hours. The projectile, therefore, is well-adapted to withstand the action of rain-storms under natural conditions.

Since the projectile has no means of its own for separating its spores and gemmae from the fatty matrix in which they are embedded, and since the projectile resists removal and disintegration by wind and rain, it is clear that, if the numerous spores and gemmae of a discharged projectile are ever to be disseminated under natural conditions, the projectile must find its way passively into some place where its fatty matrix can be dissolved. Such a position is actually provided by the alimentary canal of a herbivorous animal.

The sliminess of the exterior of a glebal mass just before and just after its discharge is due to a slimy liquid which collects in the open cup of the fruit-body and in which the glebal mass becomes partially submerged (Fig. 161, B, e, p. 316). This liquid, which Miss Walker<sup>2</sup> found can be drawn up with a capillary tube, doubtless functions as a *lubricating agent*: it prevents the projectile, before and at the time of discharge, from sticking to the palisade layer of the peridium, and it eases the separation of the projectile

<sup>1</sup> On November 14, 1923, I observed a projectile on a leaf of a Wolfberry bush (*Symphoricarpos occidentalis*) in a pasture of the Manitoba Agricultural College. After some rainy days had supervened and about a week after making my first observation, I returned to the pasture and found the projectile still in its old place on the leaf. For further field observations on *Sphaerobolus* projectiles *vide infra*.

<sup>2</sup> L. B. Walker, *loc. cit.*, footnote, p. 154. She adds that the liquid "contains a great deal of maltose as indicated by Flückiger's reaction and by osazone formation."

from the gun at the moment when the inner membranes of the cup attain their maximum degree of eversion.

The outer envelope of the glebal mass is dark reddish, changing to black with age. The pigment, which resides in the cell-walls,

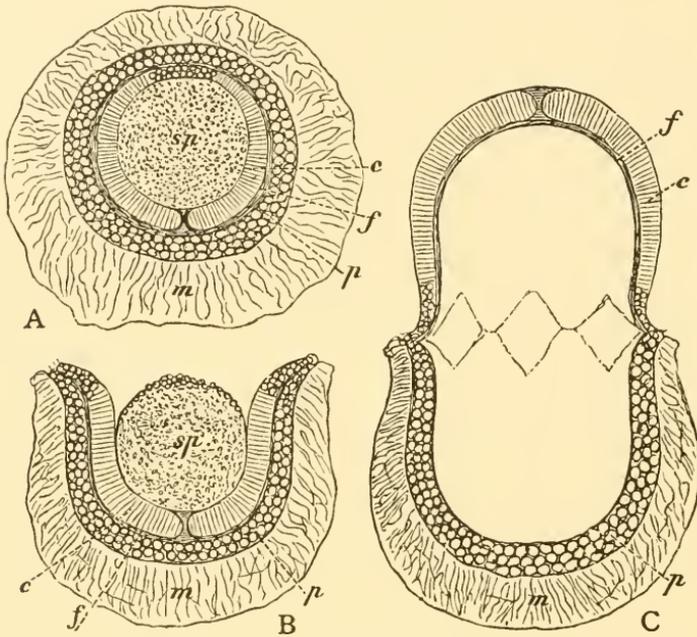


FIG. 147.—*Sphaerobolus stellatus*. Diagrammatic median vertical sections through the mature fruit-body. A, shortly before opening stellately; B, after opening stellately and just before the discharge of the gleba; C, just after the discharge and disappearance of the gleba. The four layers of the peridium, from without inwards, are: *m*, the gelatinous layer; *p*, the pseudo-parenchymatous layer; *f*, the fibrous layer; and *c*, the receptaculum or palisade layer. The gleba, *sp*, is shot away from the fruit-body by the sudden eversion of the peridial layers *f* and *c*. After E. Fischer. About 20 times the natural size.

may be merely a useless product of metabolic activity. On the other hand, there is the possibility that it serves as a light-screen and protects the colourless spores and gemmae lying in the gleba from injury by sunlight whilst the glebal mass is attached to herbage. Such a function may also be ascribed to the dark pigment in the black sporangial wall of *Pilobolus* and in the dark inner walls of the spores of *Ascobolus immersus*—two other fungi in which the pro-

jectiles (the sporangium in *Pilobolus*, a mass of eight cohering spores in the *Ascobolus*) are exposed to the sun whilst they are attached to grass in pastures.

**The Structure and Mechanism of the Gun.**—The glebal mass is cast out of the *Sphaerobolus* gun by a catapult mechanism of great

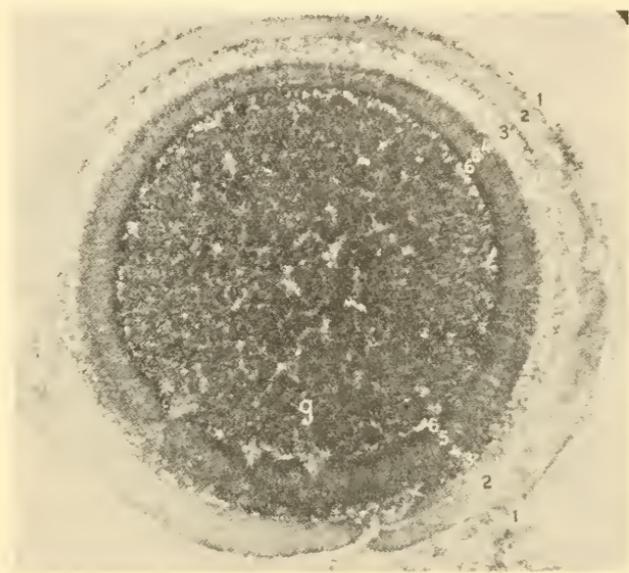


FIG. 148.—*Sphaerobolus stellatus*. Median vertical section of a nearly mature sporocarp. The peridium consists of six layers: no. 1, mycelial hyphae; no. 2, a gelatinous layer; no. 3, a pseudoparenchymatous layer; no. 4, a layer of interwoven, largely tangential hyphae; no. 5, a palisade layer becoming pseudoparenchymatous above; and no. 6, a thin layer of pseudoparenchyma surrounding the gleba, *g*. Section prepared and photographed by Leva B. Walker. Magnification, 46.

beauty and efficiency. At first the whole fruit-body is a spherical or somewhat oval ball firmly attached by its base to the substratum <sup>1</sup>

<sup>1</sup> According to Pillay (*loc. cit.*, pp. 198–204 and Figs. 1–3), so far as the origin of a fruit-body of *Sphaerobolus stellatus* is concerned, there are three structures to be considered: (1) a *mycelial sheet or cord*, which has an upper and a lower wall (Rindenschichten), (2) a *stroma* which is rounded, oval, or irregular-sided and has a wall of its own (Stromarinde), and (3) a rudimentary fruit-body (Anlage) which eventually becomes differentiated into a peridium and a gleba. He states that the stroma originates as a swelling in the middle region of a mycelial sheet or cord and

and then, as Fischer<sup>1</sup> taught us, the gleba is surrounded by four easily distinguished coats (Fig. 147, A), from without inwards as

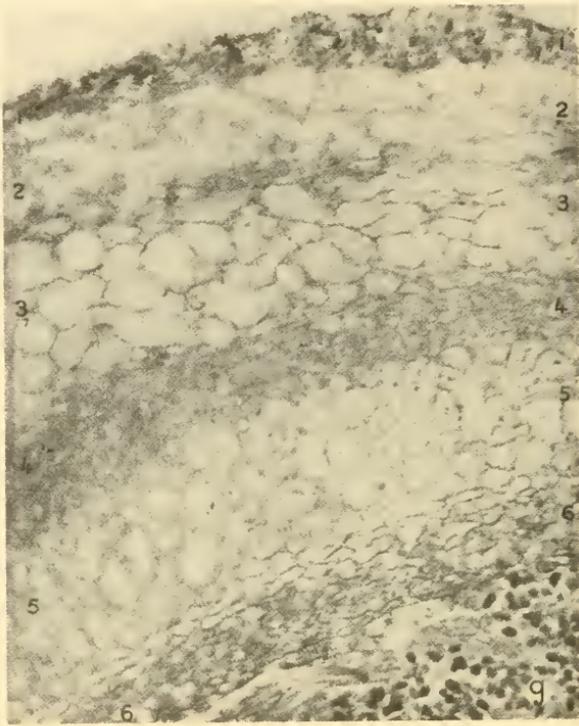


FIG. 149.—*Sphaerobolus stellatus*. The apical part of the peridium of a nearly mature sporocarp, highly magnified, consisting of six layers: no. 1, mycelial hyphae; no. 2, a gelatinous layer; no. 3, a pseudoparenchymatous layer; no. 4, a layer of interwoven largely tangential hyphae; no. 5, the palisade layer, here pseudoparenchymatous; and no. 6, a thin layer of pseudoparenchyma surrounding the gleba, *g*. Section prepared and photographed by Leva B. Walker. Magnification, about 350.

follows: (1) a gelatinous layer containing a network of hyphae with much swollen walls, (2) a pseudoparenchymatous layer, (3) a fibrous

that a rudimentary fruit-body (sometimes two) originates in its turn in the central part (Innengeflecht) of the stroma. As the rudimentary fruit-body grows, it bulges out from the substratum and ceases to be covered by the upper wall of the mycelial sheet or cord and by the upper and lateral walls of the stroma.

<sup>1</sup> E. Fischer, in *Die nat. Pflanzenfamilien*, *loc. cit.*, p. 346.

layer, and (4) a receptaculum (the palisade or collenchyma layer) made up of prismatic cells. Fischer regarded the first three layers

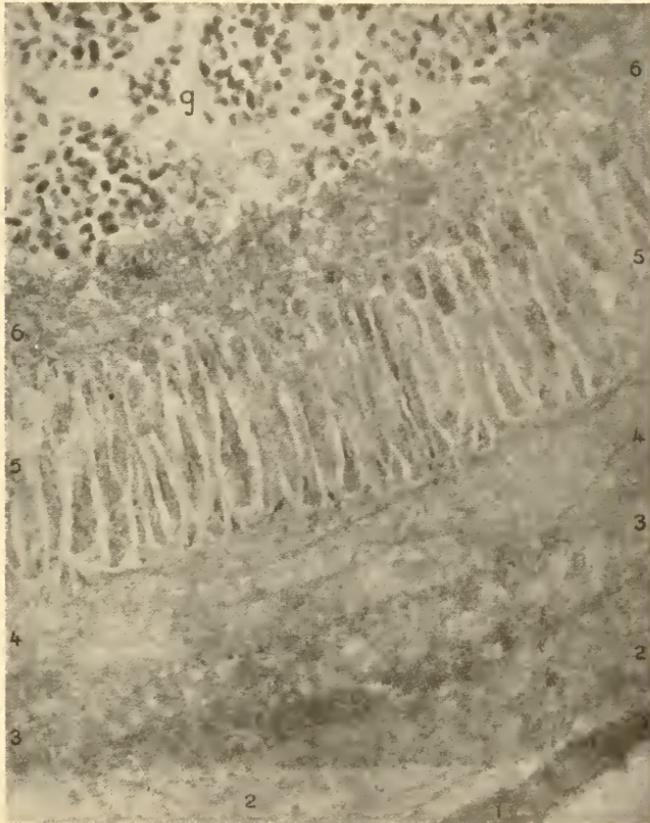


FIG. 150.—*Sphaerobolus stellatus*. The basal part of the peridium of a nearly mature sporocarp, highly magnified, consisting of six layers: no. 1, mycelial hyphae; no. 2, a gelatinous layer; no. 3, a pseudoparenchymatous layer; no. 4, a layer of interwoven largely tangential hyphae; no. 5, the palisade layer, here typically developed; and no. 6, a thin layer of pseudoparenchyma surrounding the gleba, *g*. Section prepared and photographed by Leva B. Walker. Magnification, about 350.

as parts of the peridium and the receptaculum as being derived from the gleba; but Miss Walker and I are inclined to regard all the four layers, including the receptaculum, as peridial.

Miss Walker,<sup>1</sup> with the help of the microtome and modern

<sup>1</sup> L. B. Walker, *loc. cit.*

microscopical technique, has re-investigated the layers which surround the glebal mass ; and her results confirm and extend those of Fischer already described. Her observations were as follows. In a median vertical section of a nearly mature sporocarp (Fig. 148) one can distinguish : (a) the peridium and (b) the gleba. The peridium (Figs. 148, 149, and 150), from without inwards, consists of the following six layers : no. 1, mycelial hyphae surrounding the exterior of the sporocarp ; no. 2, a gelatinous layer ; no. 3, a pseudoparenchymatous layer ; no. 4, a layer of interwoven, largely tangential hyphae ; no. 5, a palisade layer, becoming pseudoparenchymatous over the top of the sporocarp ; and no. 6, a thin layer of pseudoparenchyma surrounding the gleba. The gleba consists of hyphae, numerous spores, and some gemmae. The increase of the peridial layers to six from Fischer's four is due to the recognition and inclusion of the outermost layer, no. 1, and the innermost layer, no. 6. Just before the opening of the fruit-body, the broad outer gelatinous layer of the sporocarp nearly disappears. The sporocarp then breaks open in a stellate manner, the rupture taking place through all the layers of the peridium except the thin pseudoparenchymatous layer (no. 6) which immediately surrounds the gleba. The outer cells of this pseudoparenchymatous layer deliquesce, thus allowing all the outer peridial layers, nos. 1-5, to separate from the gleba surrounded by the innermost peridial layer, no. 6. The deliquescence begins in the pseudoparenchymatous layer (no. 6) at the top of the sporocarp and proceeds downwards, continuing after the sporocarp opens until shortly before the discharge of the gleba. The watery substance so produced can be seen within the open fruit-body and is often sufficient almost to submerge the gleba. It can be drawn off in a capillary glass tube. The outermost zone of the layer of the interwoven, largely tangential hyphae, no. 4, which corresponds to Fischer's fibrous layer, undergoes gelatinisation. This softening process begins basally and progresses upwards with the result that, in the opened fruit-body before the discharge of the projectile, a space is formed between the layers no. 4 and no. 3 (Fig. 161, A and B, c, p. 316). After this space has been formed, the upper everting membranes and lower non-everting membranes are only held together at the points of the

teeth. The palisade layer no. 5, being amply supplied with water resulting from the deliquescence or gelatinisation of layer no. 6 and part of layer no. 4, becomes highly turgid.

At or near its extreme base, as is shown in Figs. 147 and 148, the palisade layer is perforated by strands of filamentous hyphae. Doubtless these strands of hyphae serve to conduct food materials from the mycelium through the palisade layer to the gleba when the palisade layer is becoming specialised in structure in preparation for its mechanical activity and the gleba is developing and ripening its spores and gemmae.

As shown by Fischer in his illustrations reproduced in Fig. 147 and as is evident by comparing Miss Walker's photographs reproduced in Figs. 149 and 150, the uppermost cells of the palisade layer are not radially elongated but are pseudoparenchymatous. These pseudoparenchymatous cells constitute what is mechanically a weak spot in the palisade layer and therefore a spot where splitting can take place when the force which brings about the opening of the fruit-body becomes effective. Thus the *Sphaerobolus* fruit-body has a kind of *stomium* comparable with that of the capsule of a Fern sporangium.

My own investigations on the structure of the fruit-body of *Sphaerobolus stellatus* support and extend those of Miss Walker. The six layers of the peridium which she described have all been found and are shown on a smaller scale in Fig. 151 and much magnified in Fig. 154.

Fig. 151 shows a vertical section through a fruit-body about 24 hours before the discharge of the gleba. The fruit-body developed from a layer of mycelium (*a*) which covered a substratum of horse dung in a laboratory culture. The six layers of the peridium of the fruit-body, from without inwards, can be distinguished as follows: no. 1, a thin mycelial layer; no. 2, a thick gelatinous layer; no. 3, a fairly thick pseudoparenchymatous layer; no. 4, a thin fibrous layer made up of interwoven, largely tangential hyphae; no. 5, the palisade layer, composed of palisade cells below and becoming pseudoparenchymatous and orange-coloured above in the region where the peridium will break open; and no. 6, a thin layer of orange-coloured pseudoparenchyma surrounding the gleba and with

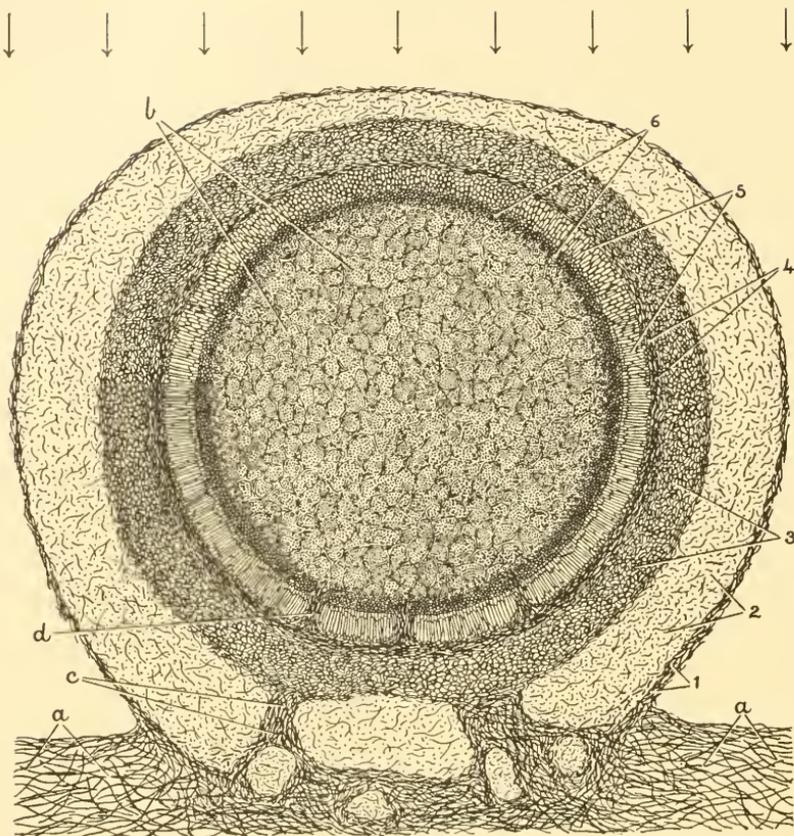


FIG. 151.—*Sphaerobolus stellatus*. Vertical section through a fruit-body, about 24 hours before the discharge of the glebal mass. The fruit-body was developed from a layer of mycelium *a* which covered the substratum (horse dung in a laboratory culture). The peridium of the fruit-body consists of the following six layers: no. 1, a mycelial layer; no. 2, a gelatinous layer; no. 3, a pseudoparenchymatous layer; no. 4, a fibrous layer made up of interwoven largely tangential hyphae; no. 5, the palisade layer, composed of palisade cells below and becoming pseudoparenchymatous and orange-coloured above in the region where the peridium will break open; and no. 6, a thin layer of orange-coloured pseudoparenchyma surrounding the gleba and with it forming the glebal mass. The gleba *b* still shows the partition-walls of its numerous chambers which contain great numbers of spores and a lesser number of gemmae and cystidia. At this stage the basidium-bodies have all disappeared but the fat cells have not yet liberated the fatty matrix in which the spores, gemmae, and cystidia are to become embedded. Hyphae which conduct food-materials to the developing gleba can be seen occupying passage-ways in the gelatinous layer at *c* and in the palisade layer at *d*. The fruit-body developed in such a way that its apex came to face the incident rays of light, the direction of which is indicated by the arrows. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 38.

it forming the glebal mass. The gleba (*b*) still shows the partition walls of its numerous chambers. The glebal chambers contain great numbers of spores and a less number of gemmae and cystidia. At this stage of development the basidium-bodies have all disappeared, but the *fat cells*, which will be described later (Fig. 159) have not yet liberated their contents destined to form the fatty matrix in which the spores, gemmae, and cystidia are shortly to become embedded. The fruit-body developed in such a way that its apex came to face the incident rays of light the direction of which is indicated by the arrows.

In Fig. 151, hyphae which doubtless conduct food-materials to the developing gleba can be seen occupying passage-ways in the gelatinous layer at *c* and in the palisade layer at *d*. The position of these passage-ways is brought out more clearly in Figs. 152 and 153.

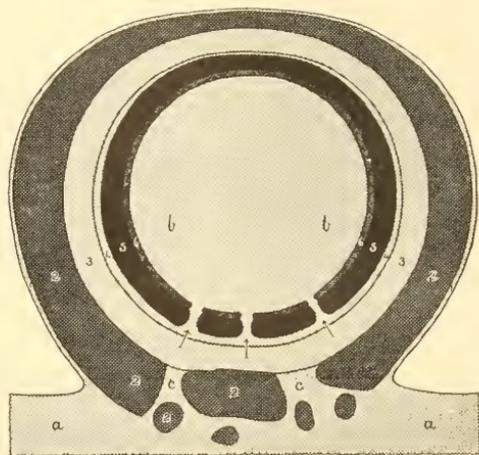


FIG. 152.—*Sphaerobolus stellatus*. Vertical section through a fruit-body and its underlying mycelium (the same as in Fig. 151), shaded diagrammatically to show more clearly the position of the passage-ways in the peridium. The passage-ways, *c c*, are in the gelatinous layer (no. 2), while the passage-ways in the palisade layer (no. 5) and the thin pseudo-parenchymatous layer (no. 6) are indicated by arrows which point to them. Hyphae pass through the passage-ways from the mycelium *a* to the developing gleba *b*. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 23.

Fig. 152 resembles Fig. 151 but is shaded diagrammatically with a view to emphasising the position of the passage-ways from the mycelium *a* to the developing gleba *b*. The gelatinous layer, no. 2, and the palisade layer, no. 5, are highly specialised in structure and function and are unsuited for conducting food-materials. The passage-ways through them, occupied by radially disposed ordinary hyphae, can be seen at *c c* and above the arrows respectively.

Glebal masses, after discharge, as already described, are en-

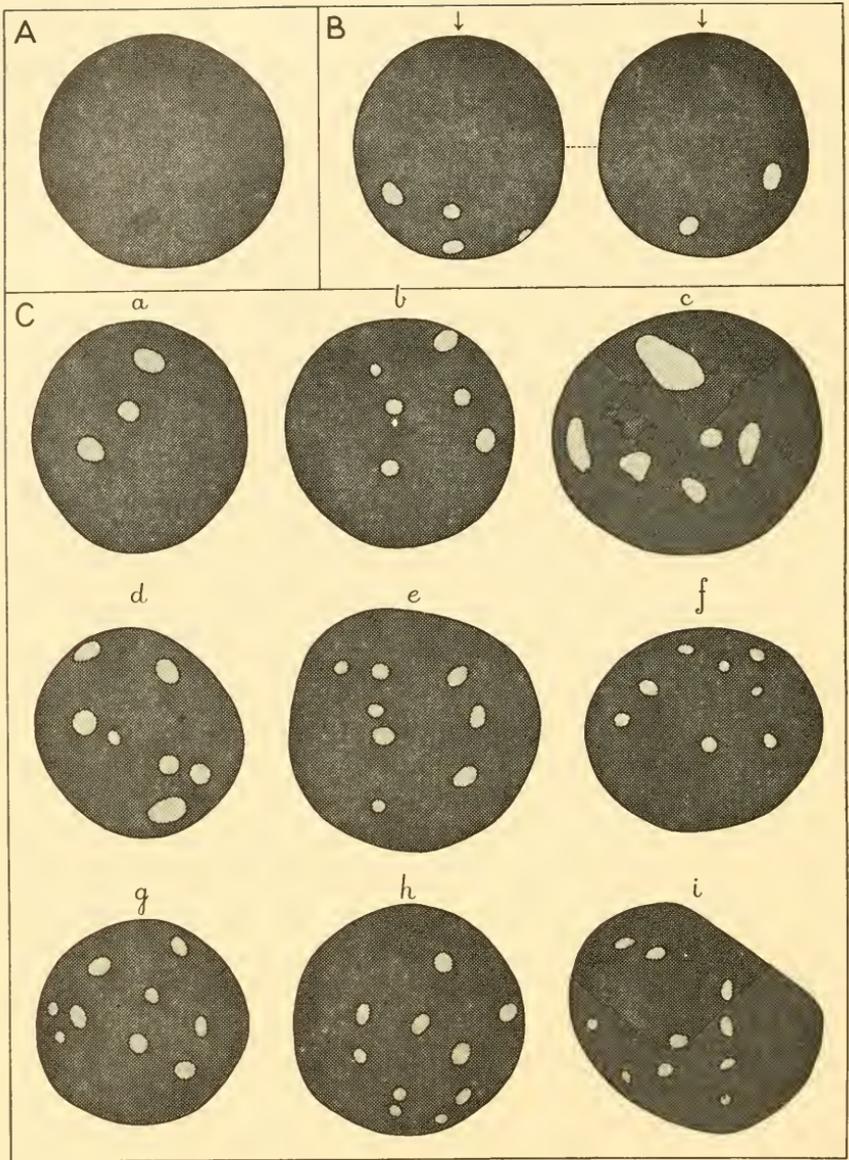
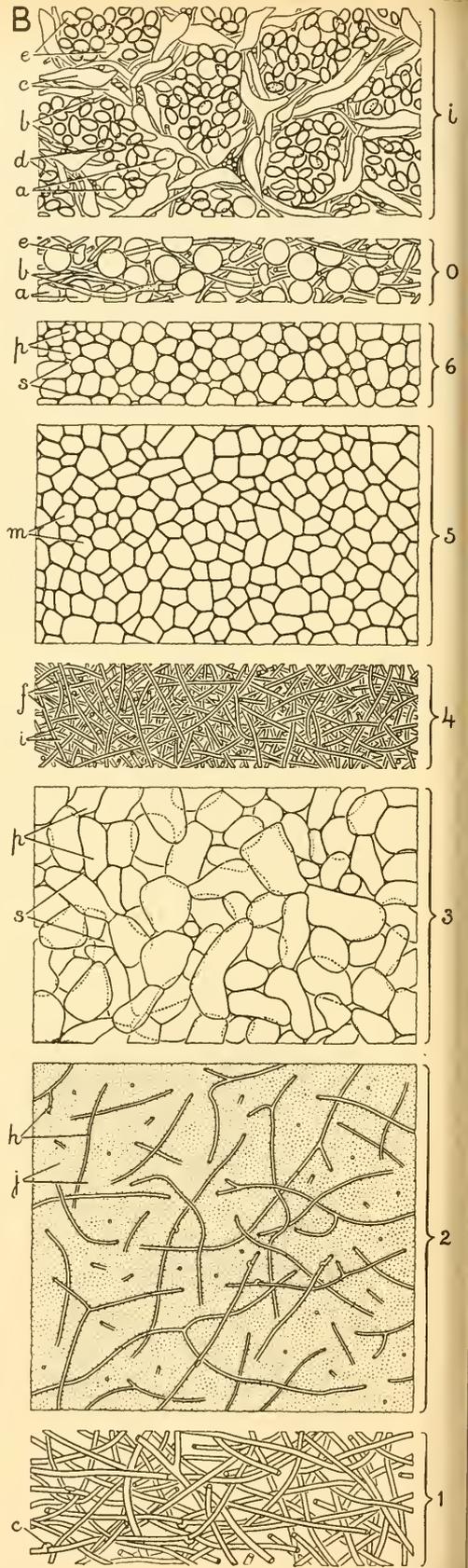
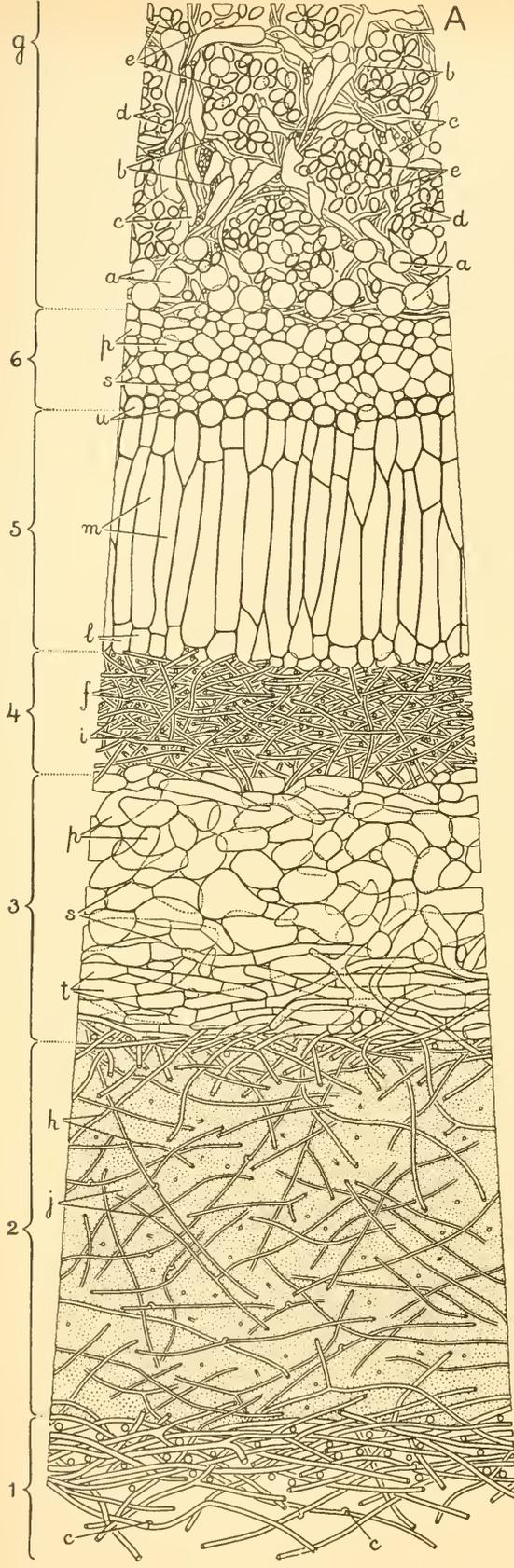


FIG. 153.—*Sphaerobolus stellatus*. Glebal masses, after discharge, drawn with the camera-lucida as if flat, to show the passage-ways through the thin pseudo-parenchymatous layer (peridial layer no. 6) which completely envelops the gleba. A, the upper half of a glebal mass: no passage-ways present. B, a glebal mass cut vertically downwards into two halves; the arrows point to the apex of each half; there are no passage-ways in the upper half of the gleba but several below. C, glebal masses seen from below, only their lower halves visible. The number of passage-ways is: in *a*, 3; in *b* and *c*, 6; in *d*, 7; in *e* and *f*, 8; in *g*, 9; and in *h* and *i*, 10. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 21.

veloped by a thin pseudoparenchymatous layer (peridial layer, no. 6, Fig. 146, *a*, p. 295, and Fig. 151). If such discharged glebal masses are examined with the low power of the microscope they are found to have, upon their exterior, lighter areas which mark the original position of the passage-ways. The passage-ways never occur in the upper half of a glebal mass (Fig. 153, A) but always in the lower half (Fig. 153, B). An examination of the lower halves of discharged glebae revealed the fact that the passage-ways vary much in size, number (three to ten), and arrangement (Fig. 153, C, *a-i*).

The histological structure of an unopened fruit-body which was approaching maturity and would have discharged its glebal mass next day is shown in Fig. 154, where A represents a median-vertical section of the fruit-body and B a series of tangential sections. The six layers of the peridium, nos. 1-6, and part of the gleba, *g*, will now be described in detail. In the *mycelial layer*, no. 1, the hyphae have thin walls and bear clamp-connexions. In the *gelatinous layer*, no. 2, the hyphae are very slender, bear clamp-connexions and are embedded in a gelatinous matrix formed by the swelling of their outer walls. In the thick *pseudoparenchymatous layer*, no. 3, the cells are swollen and rounded and separated by air-spaces. In the thin *fibrous layer*, no. 4, the hyphae are slender, thick-walled, interwoven, and disposed mostly in a tangential direction. In the *palisade layer*, no. 5, the cells are radially elongated, relatively thick-walled, and mutually adherent. In the thin *pseudoparenchymatous layer*, no. 6, the cells are small, rounded, and separated by air-spaces. The *gleba g* contains peripherally a layer of rounded cells *a a* known as *cystidia* and within is divided into small chambers several of which are in view. The partition walls of the chambers are made up of ordinary *thin-walled hyphae b b* which may bear clamp-connexions and of much-swollen *fat cells c c* whose glutinous contents have not yet been liberated but which eventually will be set free and come to form a matrix in which the spores, gemmae, and cystidia will be embedded. Loosely scattered in the chambers, from which the basidium-bodies have now disappeared, are: very numerous, oval, thick-walled *spores d d*; less numerous, thin-walled, more or less elongated *gemmae e e*; and a few spherical *cystidia* like those at *a a*.



A glebal mass, at the time of its discharge, is illustrated in Fig 146 (p. 295) and has already been described. Its gleba differs from the gleba shown in Fig. 154 in that the glebal chamber-walls have disappeared (*cf.* Figs. 146 and 154), the fat cells have broken down, and the spores, gemmae, and cystidia have all become embedded in a fatty matrix.

Fourteen of the large rounded cells known as cystidia which are present in a glebal mass at the time of its discharge (*cf.* Fig. 146, p. 295) are shown in Fig. 155. These cells form a layer at the periphery of the gleba (Figs. 146 and 154) and are scattered sparsely throughout the glebal mass among the spores.

Gemmae, peculiar oval or oval-elongated cells which are present in the gleba scattered among the spores before and after the glebal mass has been discharged, are illustrated in Fig. 156. On

FIG. 154.—*Sphaerobolus stellatus*. A, a vertical section, and B, tangential sections, of an unopened fruit-body, including all the layers of the peridium and part of the gleba, to show details of structure. The fruit-body was approaching maturity and would have discharged its glebal mass on the next day. A, a vertical section of the lower part of the fruit-body showing the peridium with its six layers, nos. 1-6, and the gleba *g*. The peridial layers are: no. 1, a *mycelial layer*, the hyphae with thin walls and having clamp-connexions *c c* at the septa; no. 2, a *gelatinous layer*, consisting of well-separated thin-walled hyphae *h* bearing clamp-connexions, the hyphae embedded in a gelatinous matrix *j* formed by the swelling of the outer hyphal walls; no. 3, a fairly thick *pseudoparenchymatous layer* with wide hyphal cells *t* in the transition zone below and pseudoparenchymatous cells *p* above, with air-spaces *s* between the elements; no. 4, a *fibrous layer* made up of interwoven, mostly tangential, thick-walled hyphae *f* with air-spaces *i* between them; no. 5, the *palisade layer*, made up for the most part of radially elongated, relatively thick-walled, mutually adherent cells *m*, with shorter cells *l* below and the uppermost cells *u* rounded and with walls specially thickened (the glebal mass eventually separates from the palisade layer just above the cells *u*); no. 6, a thin *pseudoparenchymatous layer* made up of small pseudoparenchymatous cells *p* with intercellular spaces *s* between them. The *gleba g* contains peripherally a layer of rounded cells *a a* known as cystidia and within is made up of small chambers, several of which are in view. The partition walls of the chambers are made up of ordinary *thin-walled hyphae b b*, which may bear clamp-connexions, and of much-swollen *fat cells c c* whose glutinous contents have not yet been liberated but will eventually be set free and come to form a matrix in which the spores, gemmae, and cystidia will be embedded. Loosely scattered in the chambers, from which the basidium-bodies have now disappeared, are: very numerous oval thick-walled *spores d d*; less numerous, thin-walled, more or less oval-elongated *gemmae e e*; and a few spherical *cystidia* like those already mentioned at *a a*.

B, a series of eight tangential sections through the peridium and gleba; letters with the same explanations as for A: nos. 1-6 represent tangential sections taken through the central parts of layers nos. 1-6 in A. The two drawings *o* and *i* represent tangential sections taken through the gleba, the former through the outer layer made up of cystidia, ordinary hyphae, and gemmae, and the latter through the inner region made up of glebal chambers. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 350.

germination gemmae produce clamp-connexions from the first (Fig. 156, B and C).

Some basidia of *Sphaerobolus stellatus* are shown in Fig. 157. One can find them in a fruit-body two days before the glebal mass should normally be discharged, but very shortly after this time (within 24 hours) their bodies and sterigmatic points disappear.

A basidium-body is short and swollen above, and at its base there is often a clamp-connexion (Fig. 157, A, B, G-I). The spores are oval to pear-shaped, pointed below, colourless, thick-walled, and crowded together on the top of the basidium. Sterigmata are absent. The number of spores on each basidium was found to vary from four to eight (Fig. 157, G-P). The shape of isolated spores and the thickness of the spore-wall are illustrated in Fig. 158.

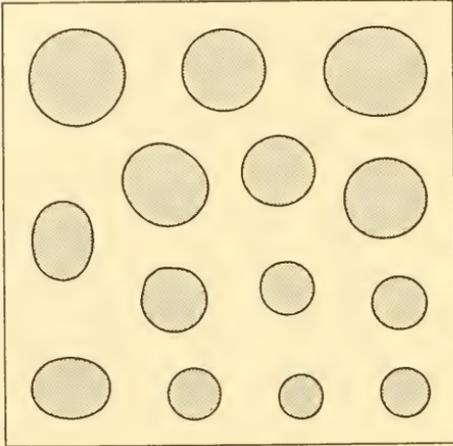


FIG. 155.—*Sphaerobolus stellatus*. Large rounded cells, known as *cystidia*, present in the glebal mass at the time of its discharge. These cells form a layer at the periphery of the gleba (cf. Fig. 146, p. 295) and are also scattered sparsely throughout the glebal mass among the spores. The fourteen *cystidia* represented serve to indicate variations in size. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 1060.

Hyphae from the walls of the glebal chambers are shown in Fig. 159. Ordinary hyphae bearing clamp-connexions are represented at *a*, *b*, and *c* and *fat cells* at *d-t*.

The fat cells, which hitherto appear to have escaped notice, are much swollen and of irregular shape. Eventually their walls break down and their contents are liberated. Thus the fatty matrix in which in a discharged glebal mass the spores, gemmae, and *cystidia* are embedded comes into existence.

At a certain stage in its development, the fruit-body, yielding to an expansive pressure exercised upon it by the palisade layer, opens at its top in a stellate manner (Fig. 142, p. 288); and then one can see the ball-like gleba lying, as it were, at the bottom of a tiny

cup (Fig. 160, *d, e*, p. 315). The rim of the cup is orange-yellow in colour and is produced outwards into from six to nine delicate teeth. The cup constitutes the Sphaerobolus gun and has a diameter of only about 2 mm.

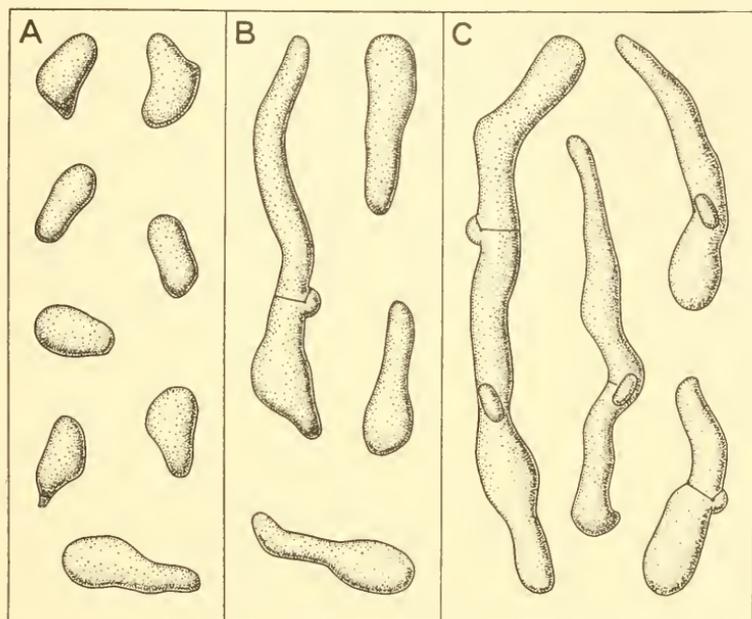


FIG. 156.—*Sphaerobolus stellatus*. Gemmae, peculiar oval or oval-elongated cells, which are present in the gleba scattered among the spores before and after the glebal mass has been discharged: A, gemmae from the gleba of an unopened fruit-body, 24 hours before the glebal mass would have been discharged. The gemmae have not yet germinated. B, gemmae of a glebal mass dissected 1–2 hours before the glebal mass would have been discharged, left in water for 2–3 hours. The gemmae have begun to germinate and one shows a clamp-connection. C, gemmae of a glebal mass which had been discharged about 3 hours and then had been kept moist. The gemmae have germinated: three of them are two-celled and one three-celled; and every septum is provided with a clamp-connection. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 1060.

After the fruit-body has opened stellately, the rim of the cup, owing to continual expansion of the highly turgid palisade layer, gradually increases in diameter and bends outwards (Figs. 147, B, p. 298, and 161, A and B). When a certain stage in this process has been reached, the two inner membranes of the cup, *i.e.* the palisade

layer and the fibrous layer, yield to the strain which has been set up and suddenly turn inside out, thus slinging the glebal mass violently away. After discharge of the glebal mass, the everted

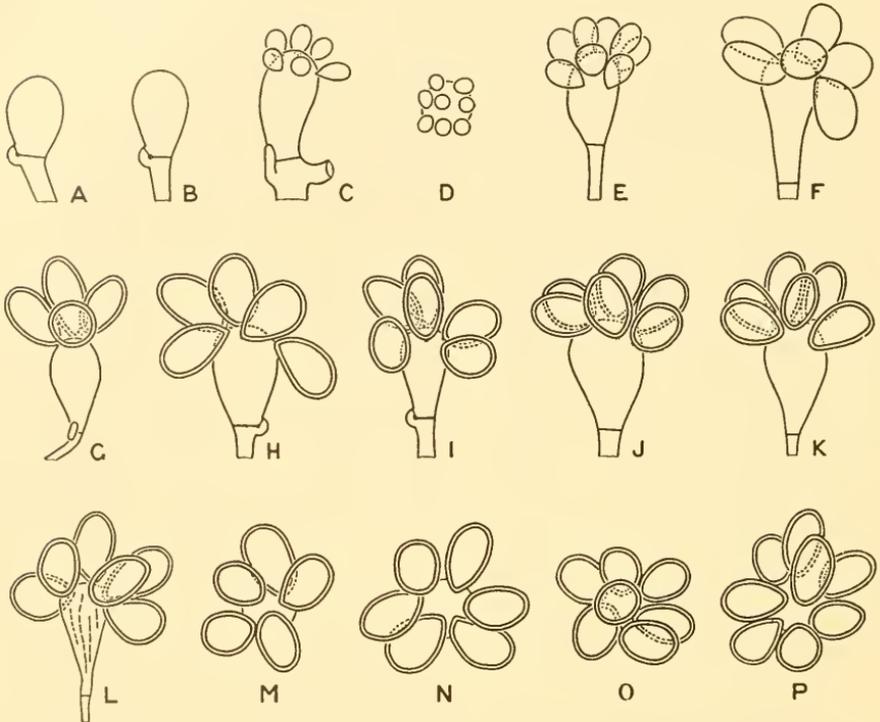


FIG. 157.—*Sphaerobolus stellatus*. Basidia as seen in sections of the gleba made two days before the glebal mass would have been discharged. A and B, two very young basidia, each with a clamp-connexion at the basal septum. C, a basidium with six young spores, seen from the side. D, a basidium with eight young spores, seen from above. E, an older basidium with eight older spores. F, a still older basidium bearing six spores, the spore-walls of which are still very thin. G-L, six basidia seen from the side, and M-P four basidia seen from above, all bearing nearly mature spores with thickened walls. The basidium G bears four spores, H and M five spores, I, J, K, L, and N six spores, O seven spores, and P eight spores. The basidium-body of L is becoming autodigested. Clamp-connexions can be seen at the base of the basidia G, H, and I. In J, K, and L, they may have been present at the back of each septum. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 1132.

membranes have a balloon-like appearance; and they are usually left standing over the cup, attached to its teeth and covering its orifice (Figs. 147, C, and 160, *f, g, h*).

The force which brings about the discharge of the *Sphaerobolus*

gun is undoubtedly located in the inner everting membranes, *i.e.* in the fibrous layer and the palisade layer which are firmly attached to one another. Of this I have obtained convincing evidence by means of a simple experiment which was carried out as follows. After a fruit-body had opened stellately and shortly before it normally would have discharged its projectile, the inner membranes of the fruit-body together with the glebal mass at their base were dissected away from the outer membranes, as shown in Fig. 161, C, and set on a moist surface in a damp-chamber. After some time, the little cup, consisting of inner membranes only, suddenly became everted in the usual manner (Fig. 161, D) and discharged the projectile to a distance. This experiment was repeated several times. It was also found that the isolated inner membranes discharged the projectile when these membranes and the projectile, after being dissected out of an opened fruit-body, were completely submerged in water.

Miss Walker<sup>1</sup> investigated the cause of glebal discharge and she, too, concluded that the inner membranes of the fruit-body are alone responsible for the ejection of the glebal mass. In the course of her work she removed the glebal mass from opened fruit-bodies and replaced it with paraffin balls mixed with

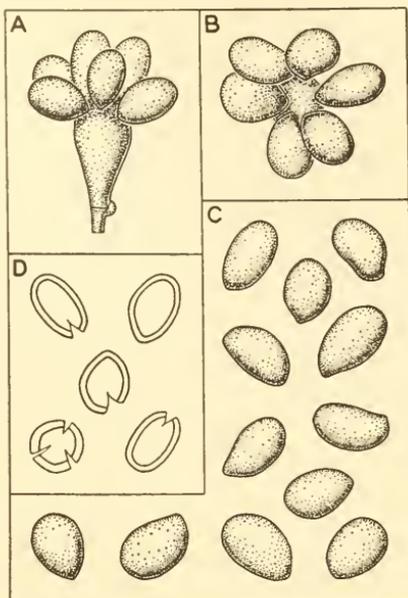


FIG. 158.—*Sphaerobolus stellatus*. Basidiospores produced in the glebal chambers. A, six spores borne on a single basidium which has a clamp-connexion at its base. The spores are crowded and attached to minute projections on the basidium-body, typical sterigmata not having been developed. B, a six-spored basidium, seen from above. C, twelve isolated mature spores, to show their variations in shape and size. D, some crushed spores, to show the thickness of the walls. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 1060.

<sup>1</sup> L. B. Walker, *loc. cit.*, pp. 156-157.

sand, little pebbles, or drops of mercury, and she had the satisfaction of seeing these artificial projectiles thrown out of the

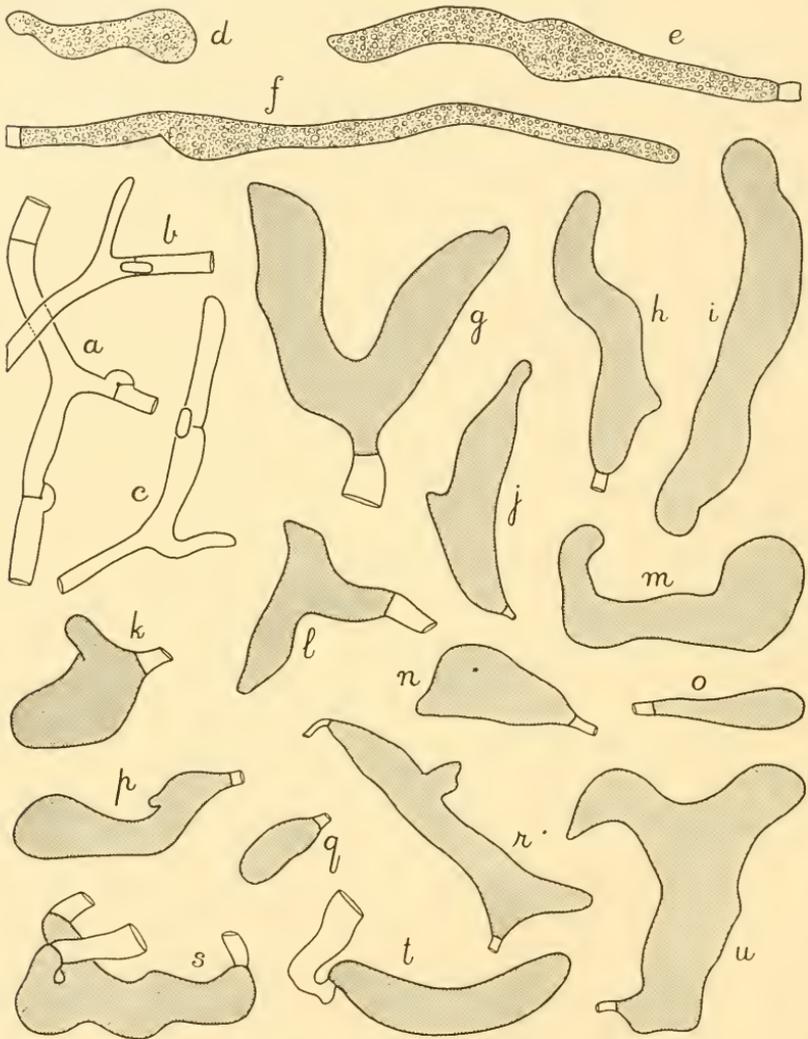


FIG. 159.—*Sphaerobolus stellatus*. Fat cells from the walls of the glebal chambers (cf. Fig. 154, g); for comparison with them some ordinary hyphae from the walls of the glebal chambers are shown at a, b, and c. The fat cells d, e, and f contained numerous tiny fat-droplets, while the fat cells g-u contained highly refractive, homogeneous contents. The variations in the size and shape of the fat cells may be realised by inspecting the drawings d-u. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 1060.

cups in the usual manner.<sup>1</sup> She gives the following account of her observations :

“When a fruit-body first breaks open, the glebal mass is firmly attached to the peridium at the base but can be removed with a needle. After the basidiocarp has been open an hour or more, the glebal mass lies as a ball entirely free from the peridial walls and may be rolled over or removed with great ease. These glebal

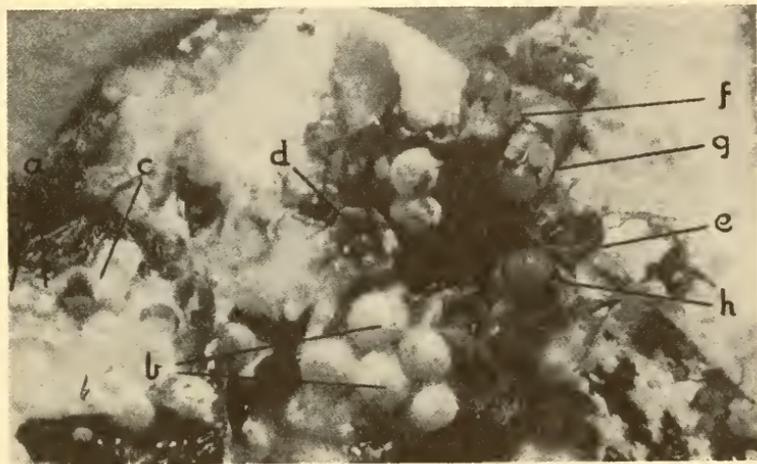


FIG. 160.—*Sphaerobolus stellatus*. A pure culture on Willow wood. *a*, very young sporocarps; *b*, full-grown sporocarps covered with the mycelial layer of the peridium; *c*, a sporocarp opening; *d* and *e*, two sporocarps which have opened stellately and now show the glebal projectile in the centre of each cup; *f*, *g*, and *h*, three sporocarps which have discharged their projectiles, the pearly dome over each of their mouths being the everted, elastic, combined palisade and fibrous layers of the peridium. Enlarged to three times the natural size from a photograph made by Leva B. Walker.

masses, which are slightly heavier than water, were removed at various periods after opening and tiny balls of paraffin mixed with sand to weight them to about the same specific gravity as the original mass were substituted. These were as readily discharged as the glebal masses. Pebbles of similar size were used as substitutes and also drops of mercury the size of or larger than the glebal masses. These were as readily discharged as the original projectile. During the time intervening between the substitution of the artificial ball for the glebal mass and the discharge, water accumulated in the

<sup>1</sup> Using pebbles and small shot, I have successfully repeated these experiments.

peridial cup and all changes took place normally. Thus it was clearly demonstrated that the glebal mass plays no part in producing the discharge.

“That the outer peridial region takes no part in causing the

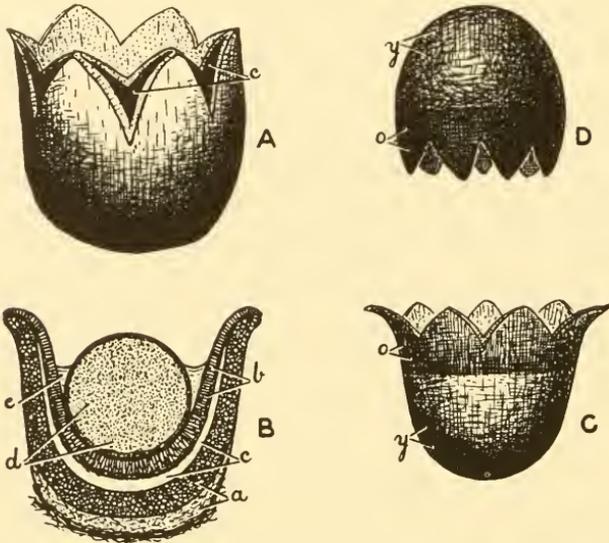


FIG. 161.—*Sphaerobolus stellatus*. A, a diagram of a fruit-body which has opened stellately and is about to discharge its projectile; *c*, a space between the outer fixed and the inner eversible sets of membranes into which one can insert the pointed end of a pin and so cause the projectile to be discharged. B, a median-vertical section through A, showing: *a*, the fixed outer set of membranes; *b*, the eversible inner set of membranes; *c*, the space between the two sets of membranes; *d*, the gleba; and *e*, liquid surrounding the gleba in the cup. C, the inner eversible set of membranes after removal from a fruit-body; *o*, the orange-coloured upper part; *y*, the yellowish-white lower part. D, the same as C, but directly after eversion. About ten times the natural size.

discharge is quite evident from the following facts: first, that before the discharge takes place the inner and the outer peridial parts separate from each other in the sinuses of the notches until a needle point may easily be inserted. This removes the possibility that gas formation between the peridial layers is in any way responsible for the discharge. Second, there are no morphological structures suggestive of such a function.

“Thus, by elimination, the force necessary for the discharge must be confined to the inner peridial region. We can easily see that in the structure of the palisade layer with its very unusually long, narrow, radially placed cells, free on their inner ends and closely bound together on their outer ends by the layer of small tangential hyphae (*cf.* Fig. 162, A), we have an ideally constructed mechanism for producing the discharge. The palisade cells, if turgid, would tend to expand on the inner end while held closely together on the outer end by the tangential filaments. That these cells are extremely, almost explosively, turgid is evident when free-hand sections of fresh materials are cut at the time of the discharge or just after the discharge. Faint pops may even be heard as the cells are cut.”

As we have seen from the remarks just quoted, Miss Walker considers that both of the inner membranes play a part in bringing about eversion and the discharge of the glebal mass: the inner fibrous layer is a non-expanding basal plate to which the inner ends of the elongated palisade cells are attached, while the palisade layer is an expansive layer which is free to increase its area only on its upper surface.

It seems to me that the fibrous layer, on account of its thinness and the looseness of the individual hyphae of which it is composed, can play but a relatively minor rôle in eversion; and it may well be that, if the fibrous layer were to be removed from the palisade layer, the isolated palisade layer might evert on its own account. The palisade layer is relatively very thick, and its cells are firmly attached to one another so that there are no intercellular spaces between them (Fig. 162). My study of the palisade layer inclines me to believe that the layer is so organised that its upper cells or the upper parts of its much-elongated cells which are more or less pear-shaped tend to expand more than its lower cells or the lower parts of its elongated cells and that, in consequence, the palisade layer tends to expand far more on its upper surface than on its lower (*cf.* A and B in Fig. 162). Thus it seems to me that, just before the discharge of the Sphaerobolus gun, in the U-shaped pocket, the upper part of the palisade layer is in a state of great compression while the lower part of the palisade layer is in a state of great tension. In the opened fruit-body, as expansion takes place by the rim

bending outwards, the two upper everting membranes, except at the teeth, separate from the lower non-everting membranes, so that there is a space formed between them (Fig. 161, A and B). It may be that, while this space is being formed and afterwards, the fibrous layer serves to prevent the expanding palisade layer from pushing downwards as a whole and thus itself becomes stretched and brought into a state of tension. In any case, in the dual everting

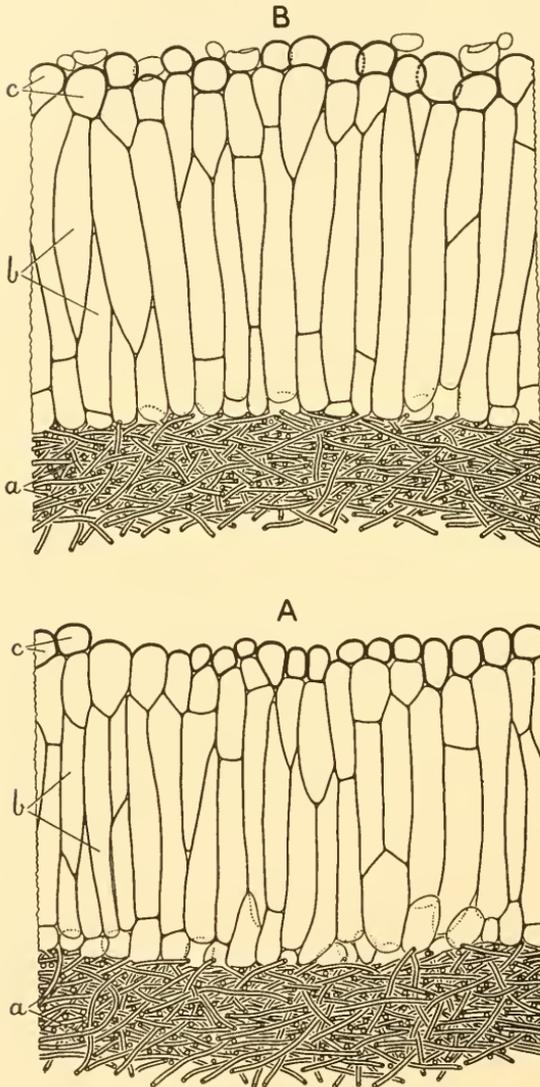


FIG. 162. — *Sphaerobolus stellatus*. A portion of the double peridial membrane which, by turning inside out, projects the glebal mass to a distance: A, before eversion; B, after eversion. A: the membrane is concave; already it has separated below from the thick pseudoparenchymatous layer of the peridium (layer no. 3) and above from the glebal mass (not here represented) which it supports (cf. Fig. 151, p. 304). It consists of two peridial layers firmly attached to one another: *a*, the fibrous layer, made up of thick-walled, interwoven, predominantly tangential hyphae, and *b*, the palisade layer, made up for the most part of radially elongated, thick-walled, palisade cells covered above with rounded cells with still thicker walls. B, the same as A, but after the eversion of the membrane: the membrane is now convex, and the upper parts of the palisade cells have increased in width. The thin-walled broken cells shown above (not seen in A) are the remains of the outer part of the thin pseudoparenchymatous layer (peridial layer no. 6) which underwent autodigestion when the glebal mass was separating from the palisade layer. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 380.

membrane, it is the tendency of the upper part to increase in surface area opposed by the tendency of the lower part to decrease in surface area that eventually leads to a condition of stress and strain being set up in the whole, with a climax that results in eversion. This climax arrives when the rim of the cup has been bent outwards sufficiently to allow the main part of the everting membranes to become broadly U-shaped in form.

The general mode of action of the dual everting membrane of *Sphaerobolus* can be illustrated very simply by means of a working model consisting of a strip of writing paper, 6 × 2 inches, and a small coin for a projectile. One places the coin in the middle of the piece of paper. Then, holding the two ends of the paper with a finger and thumb of each hand, one pushes the two ends toward one another until the centre part of the paper is broadly U-shaped. The coin now lies at the bottom of the U. Then one bends the ends of the paper more and more outwards until, suddenly, eversion of the central U-shaped part of the paper takes place and the coin is shot some 6–8 inches upwards into the air. The same experiment can be performed with more effect with a thin strip of cardboard or mica. Using a sheet of cardboard, 8 × 10 inches, taken from the back of a writing-pad, I was able to shoot a five-cent or ten-cent piece to a height of over 6 feet and to make it hit the ceiling of my office.

The expansion of the palisade layer is caused by the lateral swelling of the individual palisade cells, and this swelling appears to be due to increasing turgescence. Both Miss Walker and I, by means of microchemical tests with iodine, have observed that the palisade cells of unopened fruit-bodies are densely filled with glycogen and that this glycogen disappears during the few hours intervening between the opening of the fruit-body and the discharge of the projectile. That the glycogen is converted into sugar was to be expected, and actual evidence that this supposition is correct has been obtained by Miss Walker assisted by Miss Andersen.<sup>1</sup> These investigators tested the palisade layer for sugars with Flückiger's reaction and the phenylhydrazine reaction and found

<sup>1</sup> Leva B. Walker and Emma N. Andersen, "Relation of Glycogen to Spore-ejection," *Mycologia*, Vol. XVII, 1925, pp. 154–159.

that reducing sugars were present. One of these sugars was maltose.

Glycogen exerts almost no osmotic pressure, whilst maltose and allied sugars exert a high osmotic pressure. There is therefore every reason to suppose that, in the palisade cells, whilst glycogen is being transformed into sugar, the osmotic pressure of the cell-sap is gradually increased by several atmospheres; and we may conclude that it is the increase in the osmotic pressure of the cell-sap of the palisade cells which is chiefly responsible for the discharge of the *Sphaerobolus* gun.

Since the fruit-bodies of *Sphaerobolus* normally open and discharge their projectiles in the morning and since darkness delays opening and enfeebles discharge, it seems probable that the light and heat of the fore-noon hours have an important influence on the transformation of the glycogen into sugar. In all probability, also, the transformation of glycogen into sugar is aided by an enzyme—glycogenase—but this has not yet been proved.

“Le glycogène,” said Errera,<sup>1</sup> “est l’amidon des Champignons,” and he regarded it as the reserve carbohydrate of fungi. Since, as Miss Walker and Miss Andersen<sup>2</sup> point out, it occurs in the palisade layer of *Sphaerobolus* before expansion takes place, in the stipes of *Coprini* before their elongation, in the asci of the *Discomycetes* before they become stretched and (I may add) in the unexpanded stipe of *Phallus impudicus*, and since it disappears and is replaced by sugar in all these organs as they expand, we may conclude with Miss Walker and Miss Andersen that one use of glycogen in fungi is to provide a substance which can readily be transformed into one or more soluble substances of high osmotic value.

When a fruit-body opens stellately, the inner surface of the palisade layer, which becomes exposed to view above the projectile, is bright orange-yellow. Apparently, the orange-yellow pigment, which resides within the cells of the palisade layer, has not been investigated; but it probably resembles that of *Pilobolus*, the *Uredineae*, *Dacryomyces deliquescens*, *Peziza aurantia*, etc., in

<sup>1</sup> L. Errera, “Sur le glycogène chez les Basidiomycètes,” *Bull. de l’Acad. roy. de Belgique*, 3 sér., T. VIII, 1884, and *Mém.*, T. XXXVII, 1885.

<sup>2</sup> L. B. Walker and E. N. Andersen, *loc. cit.*, pp. 157–159.

being a lipochrome and one of the carotins.<sup>1</sup> The exposure of the pigment to sunlight just as the fruit-body is most actively preparing to discharge its projectile suggests that the pigment is not a mere waste product of metabolic activity but plays some part in the development of the discharge mechanism. Light, as observed by Miss Walker,<sup>2</sup> not only hastens the opening and discharge of the fruit-bodies but increases the vigour with which discharge takes place. As already suggested, light may hasten the transformation of the glycogen in the palisade cells into sugar. If this is so, it may well be that the transformation process is in some way facilitated by the absorption of light by the orange-yellow pigment.

The supposition that the osmotic pressure in the cells of the palisade layer is, in the main, the force which causes the ejection of the glebal mass is supported by what we know concerning the relations of the fruit-body with water. The dependence of the discharge mechanism of *Sphaerobolus* on water supply will now be discussed.

In contrast with the explosive fruits of *Geranium palustre*, *Viola canina*, and many Mimosaceae, Caesalpiniaceae, Sterculiaceae, Acanthaceae, etc. (*vide* the Table on p. 337), where the expulsion of the seeds takes place as a result of desiccation and the contraction of a special layer of the fruit-wall, the *Sphaerobolus* gun can discharge its projectile only when it is sufficiently supplied with water. There can be no doubt that, at the moment of discharge, the palisade layer of the peridium is in a highly turgid condition. If a mature fruit-body of *Sphaerobolus*, just before or just after opening stellately, be placed in an atmosphere saturated with water vapour, sooner or later it will eject its glebal mass; but if, on the other hand, it be allowed to dry, no discharge takes place, the discharge mechanism being rendered inoperative as soon as the osmotic pressure in the individual cells of the palisade layer becomes reduced below a certain minimum.

<sup>1</sup> Cf. J. Zellner, *Chemie der Höheren Pilze*, Leipzig, 1907, pp. 12, 139-142.

<sup>2</sup> L. B. Walker, "Development and Mechanism of Discharge in *Sphaerobolus iowensis* n.sp. and *S. stellatus* Tode," *Journal of the Elisha Mitchell Scientific Society*, U.S.A., Vol. XLII, 1927, p. 156. Cf. L. B. Walker and E. N. Andersen, *loc. cit.*, pp. 156-157.

One of the difficulties with which I had to contend in determining the range of *Sphaerobolus* was in keeping the little guns sufficiently moist after they had been taken from a damp-chamber and had become exposed to the dry air of a large room. In the depth of winter, at Winnipeg, the air in the laboratories and dwelling-rooms becomes extraordinarily dry. To counteract transpiration, therefore, it was found necessary to moisten the board or dung around each opened fruit-body or to place pieces of wet filter paper, etc., on the substratum so that they just touched the lower part of the outer peridium.

The experiments now to be described demonstrate not only that the discharge of the *Sphaerobolus* gun has nothing to do with desiccation and is favoured by a good supply of water, but also that the elasticity of the cell-walls is a factor in the eversion of the dual inner membrane of the peridium.

One day at noon, the dual membranes (palisade layer and fibrous layer combined, *vide* Fig. 161, C, p. 316) of eight fruit-bodies which had opened stellately were removed and, together with the enclosed glebal masses, were submerged beneath the surface of some water contained within a crystallising dish. Thereafter, in the course of a few minutes, in succession, all of the eight membranes suddenly everted and discharged their projectiles; and some of them everted with such force that the impact of the projectiles as they struck the side of the crystallising dish beneath the water could be heard at a distance of several feet.

Some dual everting membranes, after removal from stellately opened fruit-bodies, were placed in one or another of the three following reagents: (1) 10 per cent. potassium nitrate, (2) water to which a little 95 per cent. alcohol was added, (3) water to which a little iodine was added. Spontaneous eversion did not take place in any of these liquids.

In the solution of potassium nitrate the membranes soon contracted so that the teeth, instead of remaining spread radially outwards, curved tangentially inwards, while the main part of the membranes below the teeth became softened. This was doubtless due to exosmosis and consequent reduction of the turgidity of the living cells. The semiglobose main part of the membranes, however,

did not entirely lose its elasticity ; for, when it was pushed upwards from below by pressure exerted by a pin, it everted, although very feebly.

The alcohol and iodine solutions must have killed every cell in the membranes subjected to them, yet the membranes behaved in these solutions like those which had been immersed in 10 per cent. potassium nitrate, *i.e.* they contracted but did not entirely lose the elasticity of the semiglobose lower part ; for, on pressing the membranes below in the manner already described, sudden eversion took place, albeit feebly.

The results of the experiments just described seem to show that, while the osmotic pressure in the palisade cells is of primary importance in providing the force for the ejection of the Sphaerobolus projectile, the elasticity of the rather thick white walls of the palisade cells, and possibly also that of the walls of the hyphae of the fibrous layer, is an additional factor in the process.

If the cell-walls of the palisade layer and the fibrous layer were lignified and stiff, like those of wood cells, instead of being non-lignified and highly elastic like those of collenchymatous cells, the discharge mechanism of the Sphaerobolus gun—despite the high osmotic pressure in the cells of the palisade layer—would not work. We thus see that the successful operation of the discharge mechanism depends not entirely upon osmotic pressure but also upon the mechanical properties of cell-walls.

We have seen that the inner everting set of membranes of the stellately opened Sphaerobolus gun is attached to the outer non-everting membranes *solely by the tips of the 6–9 teeth*, the two sets of membranes being separated by an air-space everywhere else including the tooth-sinuses (Fig. 161, A and B, p. 316). The attachment of the two sets of membranes to one another at the tips of the teeth is a very firm one, so firm indeed that, normally, it is not broken when the projectile is suddenly discharged (*cf.* Figs. 147, p. 298, and 172, p. 361). Some idea of the mechanical strain tending to separate the inner and outer sets of membranes from one another at the moment when the projectile leaves the gun may perhaps be gained when one considers that the inner set of membranes (as will be shown in a later Section) turns inside out

in one-fifteen-hundredth to one-thousandth part of one second and, in so doing, attains a final axial velocity of 20–30 feet per second.

The tooth-tip adhesion of the two sets of peridial membranes is doubtless of considerable importance for the efficient working of the Sphaerobolus gun ; for, if the two sets of membranes were to break apart at the moment when the inner set had attained its maximum state of eversion, the inner set of membranes would follow after the projectile and interfere with its flight.

Occasionally, at the moment of discharge of the projectile, the inner set of membranes does actually break away from the outer set and travel for a few inches through the air ; but the rarity of this phenomenon indicates that it is due to some unusual imperfection in the development of the fruit-body.

The tooth-tip mode of attachment of the inner and outer sets of membranes is such that the inner set, when everting, is given the maximum possible *working distance* for pressing against the projectile. It is this long working distance—about 3 mm.—which in a large degree is responsible for the high efficiency of the Sphaerobolus gun.

As the eversion of the inner set of membranes takes place during the discharge of the gun, the openings between the sinuses of the inner and outer teeth become diamond-shaped (Fig. 147, C, p. 298). At the same time, air rushes through these openings into the enlarging central cavity of the fruit-body and thus the formation of an internal vacuum which would interfere with the efficient working of the gun is prevented. The air-passage function of the diamond-shaped openings will be further treated of in the Section on the kinetics of the gun.

The outer non-everting set of membranes is of importance for the working of the Sphaerobolus gun in that it fixes the gun to a substratum, holds the inner set of membranes by its periphery as it everts, and prevents the inner set of membranes from following after the projectile and interfering with its flight.

The slimy liquid which collects in the stellately opened Sphaerobolus fruit-body just before discharge and which wets and partially submerges the glebal mass (Fig. 161, B, p. 316) has already been

referred to as a *lubricating agent* which prevents the projectile from sticking to the gun and eases the separation of the projectile from the gun at the moment when the inner set of membranes has attained its maximum state of eversion. By placing a sheet of glass in front of discharging Sphaerobolus fruit-bodies I have been able to ascertain that, when discharge takes place, some seven or eight drops of the slimy liquid are shot away from the gun. These drops are much smaller than the projectile and doubtless, under natural conditions, are not shot nearly so far.

The projectile lying in a stellately opened fruit-body just before discharge is spherical in form. If a glass plate is placed about 2 inches in front of such a fruit-body, the projectile, on being discharged, impinges upon it with great violence and flattens out like a leaden bullet, thus becoming shallowly plano-convex. As the projectile flattens out, its diameter increases from about 1-1.25 mm. to about 1.5-2.25 mm.

The Sphaerobolus gun opens stellately, slowly and silently ; but, when it discharges its projectile, it emits an audible sound. Also, when a projectile impinges upon and flattens out against a solid body such as a sheet of glass or the ceiling of a room, the sound of the impact can be readily heard. Comparative observations<sup>1</sup> have taught me that the Sphaerobolus gun is not only the largest and the most powerful but also the loudest of all fungus guns.

**The Range of the Gun.**—In 1920, at the time my investigations on Sphaerobolus were begun, the horizontal distance and the vertical height to which the Sphaerobolus gun is able to throw its projectile had not been exactly measured. The horizontal distance of discharge was said : by C. G. Lloyd<sup>2</sup> to be from one to five inches, depending on the vigour of the plant ; by G. Masee<sup>3</sup> to be a foot or more ; and by de Bary<sup>4</sup> and Zopf<sup>5</sup> to be a metre and upwards.

<sup>1</sup> *Vide* these *Researches*, in the forthcoming Volume VI.

<sup>2</sup> C. G. Lloyd, *Mycological Notes*, Cincinnati, U.S.A., No. 33, 1909, pp. 432-433.

<sup>3</sup> G. Masee, *British Fungi, with a Chapter on Lichens*, London, not dated, 1911 (?), p. 469.

<sup>4</sup> A. de Bary, *Vergleichende Morphologie und Biologie der Pilze*, Leipzig, 1884, p. 353. In the English edition, de Bary's "metre" has unfortunately been translated as a "millimetre"! *Vide* A. de Bary, *Comparative Morphology and Biology of the Fungi*, Oxford, 1887, p. 328.

<sup>5</sup> W. Zopf, *loc. cit.*, p. 375.

The vertical distance of discharge was found by E. Fischer<sup>1</sup> to be up to and over one metre. As we shall see, all these estimates were far too low.

In what follows we shall consider *seriatim* three sets of range experiments made respectively : (1) by myself on *S. stellatus* growing on boards obtained at Kenora on the Lake of the Woods (Ontario) ; (2) by Miss Leva Walker on *S. stellatus* and its variety *giganteus* and on *S. iowensis*—all of U.S.A. origin ; and (3) by myself on *S. stellatus* growing on cow dung at Winnipeg. In connexion with the last set of experiments, an artificial method for discharging a Sphaerobolus gun will be described.

**The Range of Sphaerobolus stellatus of Kenora Origin.**—Dean E. M. Freeman of the University of Minnesota informed me that he once had a piece of wood bearing Sphaerobolus fruit-bodies upon a table in a room and that, whenever one of the little guns went off, he could distinctly hear the projectile strike the ceiling. Stimulated by this astonishing statement I determined to measure the range of the Sphaerobolus gun for myself. For several seasons I was unable to do this owing to lack of the necessary fungus material. However, in September, 1920, I found a few Sphaerobolus fruit-bodies upon a board in a wood at Kenora on the Lake of the Woods ; and I put them, along with the woody substratum to which they were attached, in a cardboard box and took them to my hotel. The next morning, at 11 A.M., I opened the box and found that four of the fruit-bodies had already discharged their projectiles and that a fifth had opened stellately (*cf. d, e*, in Fig. 160, p. 315). Bearing Freeman's observations in mind, I placed this fifth fruit-body on my dressing-table so that the mouth of the little cup looked directly upwards. After waiting and watching for about an hour, I suddenly saw the inner membranes turn inside out and appear above the cup like a beautiful pearl (*cf. f, g, h*, in Fig. 160) ; and I distinctly heard a little bang as the gun was discharged. The gleba had disappeared. I therefore stood upon the dressing-table and looked for it. I quickly found it adhering to the ceiling above my head and at a height of 5 feet 11·5 inches above the fruit-body which had discharged it. Thus was confirmed Freeman's observation that in a

<sup>1</sup> E. Fischer, "Die Entwicklungsgeschichte der Gastromyceten," *loc. cit.*

dwelling room the Sphaerobolus gun can shoot its projectile from a table to the ceiling.

A few weeks after making the preliminary observation just recorded, I procured a piece of a board bearing fruit-bodies at Kenora, brought it to Winnipeg, washed it with water, and put it under a bell-jar exposed to the light. Then, each day for about a week, a few fruit-bodies came to maturity and discharged their glebal masses between 10 A.M. and 2 P.M. With this material the range of the Sphaerobolus gun was determined with some exactitude.

A fruit-body, which was just about to discharge its glebal mass, was placed on a table in the laboratory. The glebal mass was shot upwards to the ceiling and stuck there at a height of 6 feet 10·5 inches above the fruit-body. A sheet of tissue-paper was then attached to the ceiling and the fungus guns were aimed at it from below. Some of the fruit-bodies hit the tissue paper with their projectiles when the vertical distance to be traversed was 7 feet 3·5 inches. The maximum height to which a glebal mass was shot in any of these experiments was 7 feet 8·5 inches. The glebal masses of five fruit-bodies failed to strike the tissue-paper when the fruit-bodies were 7 feet 9·5 inches below it. These observations therefore indicate that the vertical range of the *Sphaerobolus stellatus* guns used in my experiments was about 7 feet 8·5 inches.

In order to measure the horizontal range of the Sphaerobolus gun, I took a fruit-body which had opened stellately in a damp-chamber, and set it on a table so that its axis was inclined upwards at an angle of  $45^\circ$  (*cf.* Fig. 163, *b*), and I placed tissue-paper from 6 to 12 feet away, on tables of the same height as the first, so that the glebal mass, after its discharge, might land upon it. I watched and waited, waited and watched. At length, after about an hour had passed, the glebal mass was suddenly shot from the gun. As the discharge took place, I heard the snap and saw the inner membranes of the gun become everted; but the projectile disappeared and was not to be found upon the tissue-paper. However, on searching about, I discovered that the glebal mass had been shot beyond the extreme edge of the sheets of tissue-paper and had lodged upon a flat-topped stand at a distance of 14 feet 1 inch from the fungus gun which had discharged it. The glebal mass was identified by its

size, colour, and viscosity, by its having become flattened out like a bullet as a result of striking the stand, and by its spore-contents as seen beneath the microscope. The stand was actually 18 inches higher than the fungus gun. Had it stood at the same level, there can be no doubt that the horizontal distance to which the glebal mass would have been propelled would have measured at least 15 feet.

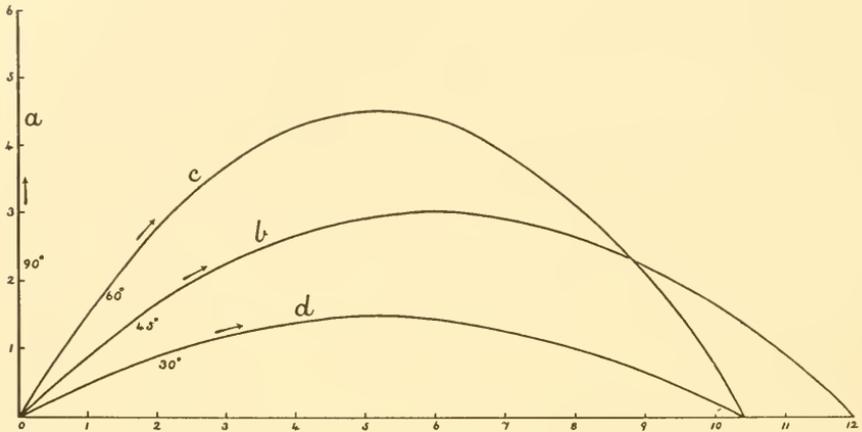


FIG. 163.—Graph illustrating the motion of projectiles discharged from a point with the same initial velocity but at different angles of elevation. The resistance of the air is not taken into consideration. In *a* the projectile is discharged vertically upwards (angle of  $90^\circ$  with the horizontal) and attains a maximum height of six units. In *b* the projectile is discharged at an angle of  $45^\circ$ : it rises to a height of three units and has a horizontal range of twelve units. This is the maximum range that can be attained and is equal to twice the height of *a*. In *c* the angle of discharge is  $60^\circ$  ( $15^\circ$  more than in *b*) and the horizontal range is 10.4 units. In *d* the angle of discharge is  $30^\circ$  ( $15^\circ$  less than in *b*) and the horizontal range is again 10.4 units.

Another *Sphaerobolus* fruit-body was inclined so that its axis made an angle not of  $45^\circ$  but of only  $40^\circ$  with a horizontal plane. The glebal mass, upon being shot away, struck the horizontal sheet of tissue-paper which had been prepared to receive it. The projectile was found to have struck the tissue-paper at a distance of 10 feet 8 inches from the fungus gun which discharged it. Had the gun been inclined at a slightly greater angle, it is probable that the horizontal distance of discharge of its projectile would have been several feet greater.

A third *Sphaerobolus* fruit-body which had developed in the

laboratory and which had a projectile distinctly under the average size, was inclined at an angle of about  $45^\circ$  to a horizontal plane. The projectile, upon being discharged, struck the horizontal sheet of tissue-paper at a distance of 11 feet 10·5 inches from the fungus gun which had discharged it.

It thus appears, from direct observation on fruit-bodies of *S. stellatus* growing on a board of Kenora origin, that the *Sphaerobolus* gun can fire its projectile about 7 feet 9 inches vertically upwards into the air and, when inclined at an angle of  $45^\circ$ , about 15 feet horizontally. Thus, as one would expect from the theory of ballistics (cf. Fig. 163), the horizontal range of the gun is about twice the vertical range. The range of the *Sphaerobolus* gun is astonishingly great when one remembers that the diameter of the gun scarcely exceeds 2 mm. and the diameter of the projectile scarcely 1 mm.

**Miss Walker's Observations on the Range of Various Sphaeroboli.**—Miss Walker,<sup>1</sup> working at the University of Nebraska at the same time as myself, but independently, investigated the range of the gun of three forms of *Sphaerobolus*: *S. stellatus*, *S. stellatus* var. *giganteus*, and *S. iowensis*. She noticed that in her cultures, when discharge took place, the projectiles were hurled to the top of the flasks and that they struck there with such force that they made a click loud enough to attract the attention of persons in an adjoining room. She therefore decided to determine the height to which the projectiles could be shot vertically upwards. She grew the fungi in pure cultures on dung so that the fruit-bodies developed on the horizontal upper surface of the medium and looked more or less upwards (Fig. 142, p. 288).

In her first attempt at finding the vertical range of the *Sphaerobolus stellatus* gun, Miss Walker set her culture at the base of a large glass museum jar which was 7 feet high; but the tube was not high enough, as many of the glebal masses were shot to its top.

With sheet-celluloid Miss Walker then constructed a long cylinder about 5 metres high and 23 cm. in diameter and closed at

<sup>1</sup> L. B. Walker, "The Forceful Ejection of the Glebal Mass by *Sphaerobolus*," *Publications of the Nebraska Academy of Science*, Vol. X, 1922, pp. 23-25; also "The Development and Mechanism of the Discharge in *Sphaerobolus iowensis* and *S. stellatus*," 1927, *loc. cit.*, pp. 157-159.

the top. She set this cylinder in a stairway, upright, over a culture bearing mature fruit-bodies about to discharge their projectiles; and then, since light exercises a favourable influence on discharge, she illuminated the culture by placing on one side of it a 100-Watt electric globe and reflector. Since the fruit-bodies of *Sphaerobolus* are heliotropic only when very young and not at all when nearly mature, the unilateral illumination of the cultures used in the experiments did not affect the direction in which the fruit-bodies shot away their projectiles. The cultures were kept in an east window exposed to daylight until just before they were used for experiment. The projectiles stuck where they struck and hence it was possible to determine the height to which they had been shot in the cylinder. Miss Walker's observations are embodied in the accompanying Table.

*Vertical Range of Sphaerobolus Guns.*

Height of Discharge in Metres	Number of Projectiles		
	<i>S. stellatus</i>	<i>S. stellatus</i> var. <i>giganteus</i>	<i>S. iowensis</i>
4·0-4·5	2	0	0
3·5-4·0	9	0	3
3·0-3·5	11	2	4
2·5-3·0	32	10	5
2·0-2·5	55	16	8
1·5-2·0	82	29	10
1·0-1·5	122	32	10
0·0-1·0	No count.	No count.	No count.

As will be seen by reference to the Table, a great many projectiles struck the lower part of the cylinder. This was doubtless chiefly due to the fact that the fruit-bodies concerned pointed somewhat laterally instead of vertically upwards.

The greatest height of discharge observed by Miss Walker was 4 metres 40 cm. (nearly 14·5 feet) for *Sphaerobolus stellatus*, which for the present is a world record in fungus gunnery. This height is almost twice that observed by me (7·7 feet) with *S. stellatus* of Kenora origin. Possibly Miss Walker's fruit-bodies grown on dung

were somewhat larger and more vigorous than mine grown on an old board. Also it must be noted that, whereas I had at my disposal relatively few fruit-bodies, she was able to experiment with many hundreds.

On account of the large size of the fruit-bodies of *Sphaerobolus stellatus* var. *giganteus*, one might have expected that this fungus would shoot its projectiles higher than *S. stellatus* or *S. iowensis*; but, as the Table shows, it did not do so. The failure of *S. stellatus* var. *giganteus* to beat its competitors may well have been due to the fact that, as Miss Walker states, *S. stellatus* var. *giganteus* did not form basidiocarps in sufficient number to make a satisfactory test.

In the experiments with *Sphaerobolus iowensis*, the counted number of projectiles was only 40, but three of them were shot to a height of 3·5–4·0 metres. This suggests that, if as many projectiles had been shot away by *S. iowensis* as by *S. stellatus*, a still greater height of projection for *S. iowensis* might have been recorded.

If every one of the fruit-bodies in Miss Walker's experiments with *Sphaerobolus stellatus* had discharged its projectile vertically upwards, it is probable that some of them would have been shot to an even greater height than 4 metres 40 cm.; but this record is sufficient to indicate the marvellous efficiency of the Sphaerobolus gun.

The horizontal range of *Sphaerobolus stellatus* and of *S. iowensis* was also investigated by Miss Walker.<sup>1</sup> As a result of a relatively small number of experiments, she found that both these species can shoot their projectiles somewhat farther than 5 metres. The maximum horizontal range<sup>2</sup> observed by her for *S. stellatus* was 5 metres 5 cm., or approximately 17 feet 3 inches. As we shall see shortly, working with the same species, I have observed an even greater horizontal range than this, namely, 18 feet 7 inches.

**An Artificial Method for Causing a Sphaerobolus Gun to Discharge its Projectile.**—In 1870, Pitra<sup>3</sup> discovered that, some time after a Sphaerobolus fruit-body has opened stellately and just

<sup>1</sup> L. B. Walker, "The Development and Mechanism of the Discharge in *Sphaerobolus iowensis* and *S. stellatus*," 1927, *loc. cit.*, p. 158.

<sup>2</sup> L. B. Walker, *in litt.*

<sup>3</sup> A. Pitra, "Zur Kenntnis des *Sphaerobolus stellatus*," *Bot. Zeit.*, Bd. XXVIII, 1870, pp. 702–703.

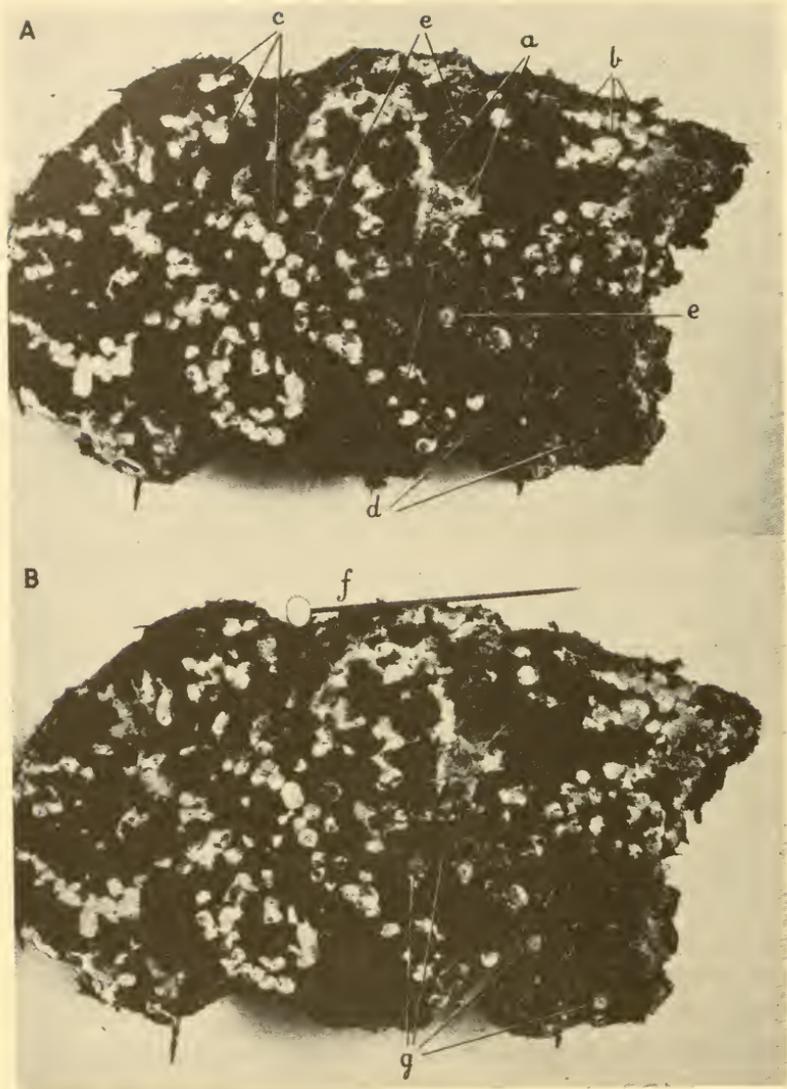


FIG. 164.—*Sphaerobolus stellatus*. Discharge of glebae with the help of a pin. A, a piece of cow-dung plat kept moist in the laboratory, ten days after it had been collected in a meadow at the Manitoba Agricultural College, Winnipeg; a, mycelium; b, young fruit-bodies; c, older fruit-bodies which opened next day; d, four fruit-bodies which have opened stellately and have thus exposed their glebae in preparation for their discharge; e e, fruit-bodies which have already discharged their glebae and now show their everted inner wall-membrane. B, the same as A, after an interval of about two minutes. In this interval the pin *f* was pushed in succession between the outer and inner wall-membranes of all the four fruit-bodies shown at *d* in A, with the result that the guns discharged their projectiles: the everted inner wall-membranes are shown at *g* in B. Photographed in the morning between 11 A.M. and noon. Natural size.

before it is about to discharge its projectile, it is possible to cause the fruit-body to discharge its projectile at any moment with the help of mechanical pressure exerted by means of a pin. In a fruit-body about to discharge its projectile there is always a space between the upper everting membranes and the lower non-everting membranes except at the points of the teeth (Fig. 161, A and B, p. 316). I found that, if one pushes the end of a pin down the sinus between two teeth into the space just described so that the end of the pin presses very slightly against the everting membranes, the everting membranes at once turn inside out and shoot away the projectile in a normal manner.

Using a pin (shown at *f* in Fig. 164, B) in the manner just described, I caused the four stellately-opened fruit-bodies shown at *d* in Fig. 164, A, to discharge their projectiles. The same four fruit-bodies after discharging their projectiles are shown at *g* in Fig. 164, B: the everted membranes can now be seen as a pearly dome covering each of the fruit-body mouths.

**The Horizontal Range of *Sphaerobolus stellatus* of Winnipeg Origin.**—In November, 1923, I found *Sphaerobolus stellatus* growing on cow-dung plats in pastures at the Manitoba Agricultural College, near Winnipeg. Some of the dung was taken to the laboratory and kept moist in large crystallising dishes. Numerous fruit-bodies soon developed on the surface of the dung (Fig. 164) and, about a week after the dung had been collected, they began to discharge their glebal masses.

To determine approximately the horizontal range of the *Sphaerobolus* guns which had developed on the cow dung my procedure was as follows. Between 12 noon and 1 P.M. on December 23, 1923, when a number of fruit-bodies were about to discharge their glebal masses, I removed a piece of dung (like that shown in Fig. 164) from the culture dish, held it in my hand 2 feet 6 inches above the floor of the laboratory and then, by means of the pin-trigger method already described, caused a number of fungus guns to discharge their projectiles in succession. Each gun was inclined upwards at an angle of 45° or less from the horizontal and was so directed that its projectile, on falling, would land on one or other of some large sheets of white paper spread over a series of tables some

feet away from the gun's mouth. Miss Dorothy Newton and W. F. Hanna kindly assisted me in observing the place of fall of each projectile and in measuring the horizontal distance to which each projectile had been discharged.

About a dozen projectiles were discharged. Several of them fell between 10 and 12 feet from the mouths of the guns. This relatively short distance may have been due to the guns having been less vigorous than others, to their having been vigorous but not sufficiently matured at the moment of their discharge, or to the angle of discharge having been either too great or too small.

The four greatest horizontal distances of discharge were as follows :

No. 1	.	.	.	.	.	18 feet 7 inches
No. 2	.	.	.	.	.	16 feet 4 inches
No. 3	.	.	.	.	.	16 feet 6 inches
No. 4	.	.	.	.	.	16 feet 5 inches

Up to the present 18 feet 7 inches is a world record for horizontal distance in fungus gunnery.

The inner everting membranes of one of the fruit-bodies which was about to discharge its projectile were dissected away from the non-everting membranes and held lightly between the first finger and thumb of the left hand at an angle of about 40° with the horizontal. A very slight pressure given with the right hand with the head of a pin and applied to the base of the little cup set the everting process in motion, with the result that the projectile was discharged to a horizontal distance of 13 feet 6 inches. This experiment affords further evidence that the force of the discharge of the *Sphaerobolus* gun resides in the inner everting peridial membranes only.

When a culture dish which stood on a table in the laboratory was uncovered so that the fruit-bodies looked freely upwards, one of them discharged its projectile with such force that the projectile struck the ceiling about 7 feet above the dung. The impact of the projectile with one of the metal plates of the ceiling was so violent that it was readily heard by both myself and others and the projectile bounced back to the table again where it fell on to a glass

plate. There can be no doubt that the projectile would have been shot several feet higher if its flight had not been stopped by the ceiling.

No attempt was made to determine the maximum vertical range of the fruit-bodies.

**Summary of Observations on the Range of Sphaerobolus Guns.**—

On the theory of ballistics, neglecting the resistance of the air, the maximum horizontal range of a gun is twice the vertical range. Taking the resistance of the air into consideration, the horizontal range should be less than twice the vertical range; and the smaller the projectile, the more closely should the two ranges approximate. Miss Walker, as we have seen, obtained a maximum vertical range of 14 feet 5 inches and yet a maximum horizontal range of only 17 feet 3 inches. It was to be expected that the horizontal range would be greater. In all probability, had her fungus guns all been directed upwards at an angle of rather less than  $45^\circ$ , and had they been as numerous and as vigorous as those used for determining the vertical range, the maximum horizontal range would have been found to exceed at least 20 feet. There is still room for a new and even more wonderful record in fungus artillery than any so far obtained.

The maximum ranges hitherto obtained by Miss Walker and by myself may be tabulated as follows.

*Range of the Sphaerobolus stellatus Gun.*

Observer	Where the fungus strain was obtained	Substratum of culture	Maximum range observed	
			vertical	horizontal
Walker	Nebraska, U.S.A.	Sterilised horse dung	14 feet, 5 inches	17 feet, 3 inches
Buller	Kenora, Central Canada	A naturally infected board	7 feet, 9 inches	15 feet, 0 inches
Buller	Winnipeg, Central Canada	Naturally infected cow dung	7 feet and upwards, limit not determined	18 feet, 7 inches

**The Horizontal Range of Various Fungus Guns and of Explosive Fruits.**—The following Table gives the approximate horizontal range of certain typical fungus guns as measured by myself.

*The Horizontal Range of Fungus Guns.*

Species	Gun	Projectile	Range
<i>Psalliota campestris</i>	basidium	basidiospore	0·1 mm.
<i>Coprinus sterquilinus</i>	basidium	basidiospore	0·2 mm.
<i>Puccinia Malvacearum</i>	basidium	basidiospore	0·5–1·0 mm.
<i>Tilletia tritici</i>	hyphal cell having a sterigma	secondary basidio- spore	0·5–1·0 mm.
<i>Puccinia graminis</i>	aecidium	aecidiospore	4–8 mm.
<i>Aleuria vesiculosa</i>	ascus	ascospore	1–2·5 cm.
<i>Empusa muscae</i>	conidiophore	conidium	2–3 cm.
<i>Ascobolus immersus</i>	ascus	ascospores	1 foot, 2 inches
<i>Pleurage curvicolla</i>	ascus	ascospores	2 feet, 7·5 inches
<i>Pilobolus longipes</i>	sporangiophore	sporangium	8 feet, 7·5 inches
<i>Sphaerobolus stellatus</i>	basidiocarp	glebal mass	18 feet, 7 inches

The *Sphaerobolus* gun, as is evident from a comparison of the data given in the Table, easily outranges that of *Ascobolus* and of *Pleurage* and even that of *Pilobolus*. It is undoubtedly not merely the largest but also the most powerful of all fungus guns.

The fruits of many Phanerogams have various mechanisms for shooting out their seeds. The Table on p. 337, which is taken from Kerner and Oliver's *Natural History of Plants*,<sup>1</sup> gives the horizontal range for a few of these fruits.

As we have seen, *Sphaerobolus stellatus* shoots out its glebal mass to a distance of about 18·5 feet, or about 5·75 metres. By reference to the data in the Table on p. 337 it will therefore be evident that *Sphaerobolus* outranges species of *Cardamine*, *Viola*, *Dorycnium*, and *Geranium*, but is outranged by species of *Lupinus*, *Acanthus*, *Hura*, and *Bauhinia*. The outranging fruits, however, are much

<sup>1</sup> A. Kerner von Marilaun, *The Natural History of Plants*, translated and edited by F. W. Oliver, London, Vol. II, 1895, p. 839.

larger than the fungus fruit-body and their seeds are heavier than the glebal mass; and it is probably safe to say that the Sphaerobolus fruit-body easily outranges any phanerogamic fruit of its own size.

*The Horizontal Range of Expulsive Fruits.*

Name of Plant	Shape of seed	Longest diameter of seed in mm.	Shortest diameter of seed in mm.	Weight of seed in grams	Range of projection in metres
Cardamine impatiens .	ellipsoidal	1.5	0.7	0.005	0.9
Viola canina . . . .	oval	1.6	1.0	0.008	1.0
Dorycnium decumbens .	spherical	1.5	1.5	0.003	1.0
Geranium columbinum .	spherical	2.0	2.0	0.004	1.5
Geranium palustre . .	cylindrical	3.0	1.5	0.005	2.5
Lupinus digitatus . .	cubical	7.0	7.0	0.08	7.0
Acanthus mollis . . .	bean-shaped	14.0	10.0	0.4	9.5
Hura crepitans . . .	lenticular	20.0	17.0	0.7	14.0
Bauhinia purpurea . .	lenticular	30.0	18.0	2.5	15.0

**The Kinetics of the Sphaerobolus Gun.**—In *Sphaerobolus stellatus*, the distance from the bottom of the inside of the stellately opened cup, upon which the glebal mass lies, to the top of the everted inner peridial membrane when this forms a balloon-like covering to the top of the cup after the glebal mass has been discharged (*cf.* Figs. 136, 160, and 172, pp. 281, 315, and 361) is about 3 mm. Pressure is therefore exerted upon the glebal mass before it leaves the gun over a distance of about 3 mm. As the Sphaerobolus gun before discharge is only about 2 mm. high, this distance is remarkably great, and no doubt it is a main factor in making the Sphaerobolus gun so efficient.

It has been possible to measure or estimate: (1) the height to which a Sphaerobolus projectile may be shot, (2) the diameter of the projectile, (3) the specific gravity of the projectile, and (4) the distance over which the propelling peridium acts on the projectile before the projectile leaves the gun.

With the help of the data just enumerated we shall first calculate mathematically: (1) the initial velocity of the Sphaerobolus

projectile, (2) the time taken in the flight of the projectile, and (3) the time taken in the discharge of the gun, *i.e.* in everting the inner peridial membrane; and this will lead to (4) a discussion of the function of the air-spaces between the sinuses of the outer and inner peridial teeth. We shall then calculate mathematically: (5) the momentum of the projectile at the moment of leaving the gun, (6) the kinetic energy of the projectile at the moment of leaving the gun, (7) the average force of the propelling peridium while this acts upon the projectile, and (8) the rate of developing energy in the gun. In concluding this Section, (9) the results of the calculations will be summarised.

(1) *Initial velocity of the projectile.* From the equation:

$$v^2 = 2gs$$

where  $v$  = the initial velocity,  $g$  the acceleration due to gravity, and  $s$  the vertical height to which a projectile is discharged when shot vertically upwards, *neglecting the resistance of the air* it can be calculated that a Sphaerobolus projectile, if shot vertically upwards to the maximum observed height of 14·5 feet, has an initial velocity of about 30·4 feet per second.

A similar calculation to that just made shows that, if a Sphaerobolus projectile is shot vertically upwards to a height of only 7 feet instead of 14·5 feet, the initial velocity of the projectile would be about 21 feet per second instead of about 30 feet per second.

A Sphaerobolus projectile is only about 1·25 mm. in diameter, and therefore its surface area relatively to its mass is large. The resistance offered to its flight through the air must be considerable. It seems clear, therefore, that a projectile which has been shot vertically upwards to a height of 14·5 feet must have had an initial velocity which exceeded 30 feet per second, and that a projectile which has been shot vertically upwards to a height of 7 feet must have had an initial velocity which exceeded 21 feet per second.

(2) *Time taken for the flight of the projectile.* Employing the equation:

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

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 of the air* it can be calculated that, when a sphere is shot vertically upwards from rest to a height of 14·5 feet or falls to the ground from that height, the time required for each movement is approximately 0·95 second. Taking into account the resistance of the air, we may conclude that a projectile of Sphaerobolus which is shot 14·5 feet vertically upwards doubtless takes upwards of one second to reach its highest point and upwards of one second to fall from the highest point back to earth again, or upwards of two seconds for the double journey.

Similarly, it can be calculated that, *neglecting the resistance of the air*, when a sphere is shot vertically upwards to a height of 7 feet instead of 14·5 feet or falls 7 feet instead of 14·5 feet, the time required to carry out the movement is approximately 0·65 second. Taking into account the resistance of the air, however, we are justified in concluding that the actual time for the upward or downward journey would exceed 0·65 second. If we assume the actual time to be 0·75 second, then the projectile after discharge and before striking the earth would be in the air for about 1·5 seconds.

(3) *Time taken in the discharge of the gun.* Just before the discharge of a Sphaerobolus gun, the dual everting membrane can be seen to have a cup-like form and the glebal mass can be seen resting within it; and *immediately after the discharge* the everting membrane can be seen everted and forming a pearly balloon-like covering to the top of the fruit-body; but *during discharge* the eye cannot perceive any movement either of the everting membrane or of the projectile, *i.e.* one does not perceive the actual discharge of the gun. The reason why the eye cannot perceive the discharge of the Sphaerobolus gun is that the movements of the everting membrane and of the projectile are *too rapid for the eye to follow*.

The time taken for the eversion of the inner dual membrane of the Sphaerobolus gun can be readily calculated. Let us assume that the projectile has been shot to the maximum observed height of 14·5 feet. From a calculation already made we know that the initial velocity of the projectile, as this leaves the everting membrane, is about 30 feet per second. Since this velocity is due to the

pressure of the membrane on the projectile, we may assume that the membrane at the moment of complete eversion has a velocity of about 30 feet per second. The membrane starts from rest and its base is raised through a distance of about 3 mm. Now if a body starts from rest and travels for  $t$  seconds, finishing with a velocity of  $v$  feet per second, its average velocity is  $\frac{1}{2} v$  during this time and

$$s = \frac{1}{2} vt$$

where  $s$  is the distance travelled over. Applying this equation to the problem in hand, we have  $s = 3 \text{ mm.} = \frac{30}{3048}$  feet,  $v = 30$  feet per second, and  $t$  is the time taken for the eversion of the membrane. Therefore

$$t = \frac{30}{3048} \times \frac{2}{30} = \frac{1}{1524} \text{ second.}$$

Thus we see that the time taken for the discharge of our Sphaerobolus gun, *i.e.* for the eversion of the inner membrane, is only about one-fifteen-hundredth part of one second. No wonder, therefore, that the eye cannot observe the eversion movement.

If the projectile were shot upwards to a maximum height of 7 feet instead of 14.5 feet so that its initial velocity were only about 21 feet per second instead of about 30 feet per second, the time taken for the eversion of the inner membrane would be increased from about one-fifteen-hundredth of a second to about one-thousandth of a second. Even so, the eversion movement is a very sudden one and it is therefore not surprising that, on actually watching Sphaerobolus guns which shot their projectiles to a measured height of 6-7 feet, I was not able to perceive the inner membrane become everted or the projectile leave the gun.

(4) *Function of the air-spaces between the outer and inner peridial teeth.* As already mentioned in a previous Section, after a fruit-body has opened stellately the inner eversible peridial membrane separates slightly from the outer non-eversible peridial membrane, except at the tips of the teeth, so that a shallow concavo-convex cavity filled with air is formed between the two membranes, as shown in Fig. 161, B (p. 316). Since the separation of the inner and outer membranes extends to the teeth, excepting their tips,

the cavity formed between the peridial membranes opens to the outer air at the rim of the cup between the points of the teeth, *i.e.* at each tooth-sinus (Fig. 161, A). This being so, it is obvious that, before the discharge of the gun, an arrangement is provided by which it is possible for air to pass from the outer atmosphere into the peridial cavity. As discharge of the gun takes place and the inner peridial membrane becomes everted, the tooth-sinus apertures increase in size and finally become diamond-shaped, as shown in Fig. 147, C (p. 298).

We have seen that when a *Sphaerobolus* projectile is shot vertically upwards to a maximum height of 7 feet the eversion of the inner peridium takes place in about *one-thousandth of one second*, and that when it is shot vertically upwards to a maximum height of 14.5 feet the eversion of the inner peridium takes place in about *one-fifteen-hundredth of one second*. This exceedingly rapid eversion of the inner peridial membrane necessitates a correspondingly rapid enlargement of the cavity between the inner and outer peridial membranes; and this exceedingly rapid enlargement of the peridial cavity must tend to cause the formation of a partial vacuum within the cavity's interior. Now, if the inner and outer peridial membranes were completely attached to one another all around the rim of the stellately opened cup instead of only at the points of the teeth, as discharge of the gun took place the inner peridial membrane during its eversion would have to do its work against the pressure of the atmosphere; but, owing to the existence of the tooth-sinus apertures, which are so constructed that they enlarge as discharge takes place, this mechanical difficulty does not arise. It is obvious that, when the discharge of a *Sphaerobolus* gun takes place, a blast of air must rush into the enlarging peridial cavity through every tooth-sinus aperture, so that any slight negative air-pressure which may be formed in the cavity is quickly reduced to zero. From this discussion we may conclude that the tooth-sinus openings between the inner and outer peridial membranes have the function of permitting of the rapid entrance of air into the central peridial cavity during the discharge of the gun.

The enlargement of the peridial cavity during the discharge of the *Sphaerobolus* gun may be roughly estimated at 4-6 cubic mm.

The air which suddenly enters the cavity through the tooth-sinus apertures as the inner peridial membrane becomes everted must be equal in volume to the enlargement of the cavity, *i.e.* roughly 4-6 cubic mm.

(5) *Momentum of the projectile when leaving the gun.* If momentum be expressed by  $M$ , mass by  $m$ , and velocity by  $v$ , all in corresponding units, we can write :

$$M = mv.$$

Let us calculate the momentum of a Sphaerobolus projectile which has been shot to a maximum height of 14.5 feet. We know that the projectile's initial velocity is 30 feet per second. Let us assume that the projectile's radius was 0.625 mm. and its density was 1.25 (the projectiles sink in water). Since

$$\text{mass} = \text{volume} \times \text{density}$$

and, for a sphere,

$$\text{volume} = \frac{4}{3} \pi r^3$$

where  $r$  is the radius of the sphere, we have

$$\text{mass} = \frac{4}{3} \pi r^3 \times \text{density}$$

and, where  $M$  = momentum, we have

$$M = \frac{4}{3} \pi r^3 \times \text{density} \times \text{velocity}.$$

The number of cubic mm. in one cubic foot =  $(305)^3 = 28,000,000$ .  
Hence for our projectile :

$$\text{volume} = \frac{4}{3} \times \frac{22}{7} \times \frac{0.625^3}{28,000,000} = \frac{1}{28,000,000} \text{ cubic feet approximately.}$$

The weight of one cubic foot of water is 62 lbs. Hence for our projectile :

$$\text{density} = \frac{1.25}{1} \times \frac{62}{1} = 77.5 \text{ pounds per cubic foot.}$$

The initial velocity of our projectile is known to be 30 feet per second. Hence, for our projectile, with the mass expressed in pounds and the velocity in feet per second, we have :

$$M = \frac{1}{28,000,000} \times \frac{77 \cdot 5}{1} \times \frac{30}{1} = 0 \cdot 00008 \text{ units}$$

*i.e.* a Sphaerobolus projectile shot to a maximum height of 14·5 feet has, on leaving the gun, an initial momentum of 0·00008 units.

(6) *Kinetic energy of the projectile when leaving the gun.* The kinetic energy of the projectile can be found from the equation :

$$\text{K.E.} = \frac{1}{2} mv^2$$

where K.E. = the kinetic energy,  $m$  the mass, and  $v$  the velocity. Therefore for our projectile which was shot up 14·5 feet high and which therefore had an initial velocity of 30 feet per second

$$\begin{aligned} \text{K.E.} &= \frac{1}{2} \times \frac{78}{28,000,000} \times \frac{30 \times 30}{1} \\ &= 0 \cdot 0012 \text{ foot poundals} \\ &= \frac{0 \cdot 0012}{g} = \frac{0 \cdot 0012}{32} \\ &= 0 \cdot 000037 \text{ foot pounds} \end{aligned}$$

or

*i.e.* the kinetic energy of a projectile of Sphaerobolus when just leaving a gun which can discharge it to a maximum height of 14·5 feet is 0·0012 foot poundals or 0·000037 foot pounds.

(7) *Average force of the propelling peridium when acting upon the projectile.* It is known that

$$Fs = \frac{1}{2} mv^2$$

where  $F$  is the average force exerted on a projectile before it leaves the gun,  $s$  is the distance that the projectile goes in the gun whilst still subjected to its pressure,  $m$  is the mass of the projectile, and  $v$  is the initial velocity of the projectile on leaving the gun. Applying this equation to the Sphaerobolus gun in the case of the discharge of a projectile to the maximum observed height of 14·5 feet, we have :

$$s = 3 \text{ mm.} = \frac{30}{3048} \text{ feet, } m = \frac{78}{28,000,000} \text{ lbs., and } v = 30 \text{ feet per second.}$$

$$\begin{aligned} \text{Therefore } F &= \frac{1}{2} \times \frac{78}{28,000,000} \times \frac{30 \times 30}{1} \times \frac{3048}{30} \\ &= 0 \cdot 13 \text{ poundals} \end{aligned}$$

$$\text{or } F = \frac{0 \cdot 13}{g} = 0 \cdot 004 \text{ lbs.}$$

*i.e.* the average force exerted by the inner peridium upon the projectile as it everts through a distance of 3 mm. is 0·13 poundals or 0·004 lbs.

(8) *Rate of developing energy in the Sphaerobolus gun.* In calculating the horse power (rate of developing energy) of the Sphaerobolus gun, let us consider again the case of the projectile shot to the maximum height of 14·5 feet. As we saw under (6), 0·000037 foot pounds of energy are imparted to the projectile before it leaves the gun ; and, as we saw under (2), this amount of energy must be imparted in  $\frac{1}{1524}$  second, *i.e.* the time taken for the

inner peridial membrane to become everted. The imparting of kinetic energy to the projectile therefore takes place at the rate of

$$0\cdot000037 \times 1524 = 0\cdot055 \text{ foot pounds per second.}$$

Now one horse power = 550 foot pounds per second. If H.P. = horse power, we have

$$\text{H.P.} = \frac{\text{foot pounds per second}}{550}$$

So that for our Sphaerobolus gun we have :

$$\text{H.P.} = \frac{0\cdot055}{550} = \frac{1}{10,000}$$

*i.e.* the Sphaerobolus gun does work at the rate of one ten-thousandth part of one horse power.

(9) *Summary of the calculations.*

Initial velocity of the projectile :

if shot 7 feet high . . . . upwards of 21 feet per second ;

if shot 14·5 feet high . . . upwards of 30 feet per second.

Time taken for the projectile to reach its highest point :

if shot 7 feet high . . . . upwards of 0·65 second ;

if shot 14·5 feet high . . . upwards of 0·95 second.

Time taken in the discharge of the gun :

if it shoots the projectile 7 feet high . . . . about  $\frac{1}{1066}$  second ;

if it shoots the projectile 14·5 feet high . . . about  $\frac{1}{1524}$  second.

- Momentum of a projectile shot 14·5 feet high,  
 when leaving the gun . . . . 0·00008 units.
- Kinetic energy of a projectile shot 14·5 feet high,  
 when leaving the gun . . . . 0·000037 foot pounds.
- Average force of the propelling peridium,  
 when acting on the projectile . . . . 0·13 pounds,  
 or . . . . 0·004 pounds.
- Rate of developing energy in a Sphaerobolus gun which shoots a  
 projectile 14·5 feet high . . . .  $\frac{1}{10,000}$  horse power.

**Relations of Sphaerobolus with Water.**—Without a sufficient supply of water the Sphaerobolus gun can neither open stellately nor, if opened stellately, discharge its projectile. As explained in an earlier Section, this is due to the fact that the force employed in the opening and discharge of the gun is, in the main, the osmotic pressure in the cells of the palisade layer of the peridium.

The unopened fruit-bodies of *Sphaerobolus stellatus* can withstand desiccation for several months without any loss of vitality. Thus a piece of wood bearing young fruit-bodies was collected at Minaki (western Ontario) in the summer of 1928, was allowed to become air-dry in the laboratory at Winnipeg and, after having been kept dry for about four months, was re-moistened; whereupon the fruit-bodies at once revived, continued their development, and eventually discharged their glebal masses. This revival after desiccation is a phenomenon exhibited by the fruit-bodies of Schizophyllum, Panus, Corticium, Stereum, Dacryomyces, Calocera, and many other Basidiomycetes which grow upon sticks and other masses of wood, *i.e.* upon a substratum which rapidly dries up in dry weather and readily re-absorbs water when precipitation occurs. It is certain that, under natural conditions, the fruit-bodies of *Sphaerobolus stellatus* often persist through periods of summer or autumn drought in a desiccated condition and, subsequently, upon the advent of rain, absorb water rapidly, revive, and continue their development.

When pieces of wood or dung containing the mycelium of *Sphaerobolus stellatus* are collected in the open or when artificial

cultures of the mycelium made in the laboratory are allowed to dry up, on the addition of water after the lapse of several weeks or months the mycelium revives and continues its development. Thus, in withstanding desiccation and in reviving when moistened, the mycelium of *S. stellatus* resembles that of many lignicolous fungi, e.g. *Polystictus versicolor*, and of many coprophilous fungi, e.g. *Coprinus sterquilinus*.

A piece of cow dung permeated with the mycelium of *Sphaerobolus stellatus* was allowed to dry up in the autumn of 1923 and was kept in the dried condition for a little over five years. It was then moistened, but the mycelium failed to revive and soon became covered with moulds. From this experiment, it is evident that the mycelium of *S. stellatus*, if kept dry, loses its vitality in the course of a few years.

As already recorded, the observations of Miss Walker combined with my own afford evidence that the gemmae in dried glebal masses of *Sphaerobolus stellatus* retain their vitality for at least 8–11 years.<sup>1</sup> If one places a glebal mass which has been kept dry for 5–8 years in a hanging drop of water, the gemmae within it send out clamp-connexion-bearing hyphae which radiate from the glebal mass and soon form a widely-extending mycelium. From mycelium produced in this way one can start fresh artificial cultures.

When, under natural conditions, a glebal mass has been shot on to the leaves of herbage, etc., it sticks so tightly and is so insoluble in water that even heavy rain can neither wash it from its place of attachment nor break up its contents. Thus at Winnipeg I observed that a whole day's rain failed to disturb a glebal mass which I had previously found resting on an upper leaf of a Wolfberry bush (*Symphoricarpos occidentalis*) several feet above the ground.<sup>2</sup> The significance of the resistance of the glebal masses of *Sphaerobolus* to the action of rain-water will be discussed in connexion with the mode of dispersion of the spores.

**Relations of *Sphaerobolus* with Light.**—Brefeld<sup>3</sup> showed that in

<sup>1</sup> *Vide supra*, pp. 290–292.

<sup>2</sup> *Cf.* foot-note on p. 297.

<sup>3</sup> O. Brefeld, *Untersuchungen aus dem Gesamtgebiete der Mykologie*, Heft VIII, Leipzig, 1889, pp. 289–290.

the absence of light the mycelium of *Sphaerobolus stellatus* is unable to form fruit-bodies, but that when once fruit-bodies have come into existence and have advanced to a certain stage they can ripen in the dark, although more slowly than in the light. If a culture bearing fruit-bodies is placed in a dark chamber, the more mature fruit-bodies continue to discharge their glebae for many hours. This continuance of glebal discharge in the entire absence of illumination has been verified by various observers, including the writer.

In unilateral light, the fruit-bodies of *Sphaerobolus stellatus* and, doubtless, of all species of *Sphaerobolus* develop in such a way that: when they open stellately, their mouths face the incident rays of light; and, when they discharge, their projectiles are shot toward the source of the light. I called attention to these facts in 1920.<sup>1</sup> At the time, Miss Walker was not able to accept them; but, in 1927, she<sup>2</sup> supported them with observations of her own. One of two experiments, made in my laboratory, which afford convincing evidence that light influences the direction in which a fruit-body develops will now be recorded.



FIG. 165.—Heliotropism of *Sphaerobolus stellatus*. Fruit-bodies developing on the top of a board which rested on the floor of a large glass case. The black arrows indicate the direction of the incident light. Each fruit-body developed in such a way that its long axis became parallel to the rays of light and so that its apex came to face the light. The fruit-body behind the white arrow has just discharged its projectile toward the source of maximum light, and its everted double inner membrane is now visible at its mouth. Board found at Kenora, Ontario, Canada. Natural size.

<sup>1</sup> At the Chicago meeting of the Botanical Society of America, held Dec. 20, 1920. As it happened, Miss Walker and I read papers on *Sphaerobolus* in succession.

<sup>2</sup> L. B. Walker, 1927, *loc. cit.*, p. 159.

I placed a board of coniferous wood, upon which some *S. stellatus* fruit-bodies were developing, flat upon the floor of a large cubical glass case which was situated on a table next to a wall and about 21 feet distant from each of two windows. About twelve fruit-bodies developed on the horizontal upper side of the board and, during their growth to maturity, which was completed in the course of two or three weeks, they were unilaterally illuminated each day by daylight coming from the two windows (Fig. 165). Under these conditions the fruit-bodies did not project straight upwards from the board but, at an early stage in their growth, inclined themselves toward the direction of the greatest illumination so that the longitudinal axis of each of them came to make an angle of somewhat less than  $45^\circ$  with the upper surface of the board. The result of the fruit-bodies having their apices turned toward the light in this way was that, when the fruit-bodies came to discharge their glebae, the glebae hit and stuck to the side of the glass case which was nearest the windows, *i.e.* nearest the source of the brightest light.

Miss Walker,<sup>1</sup> in treating of the relations of *Sphaerobolus* with light, says: "Buller in unpublished work previously mentioned showed a positive heliotropic reaction, and experiments conducted by the writer entirely confirm his results. That fruit-bodies in flask cultures point in various directions is due to reflections and refractions of light in the culture flask. When cultures were placed several feet from a window and arranged so that no reflections of light were possible, all basidiocarps formed pointed directly toward the source of the light."

Both Miss Walker<sup>2</sup> and I have observed that the directive action of light on *Sphaerobolus* is limited to very young fruit-bodies and does not affect fruit-bodies which are maturing. At present, we do not know how light causes a *Sphaerobolus* fruit-body to develop so that its apex faces the incident rays, and it is possible that Miss Walker's assumption that the phenomenon is one of heliotropism may be incorrect. There are three theoretical possibilities for the mode of action of the light. Light may influence

<sup>1</sup> L. B. Walker, 1927, *loc. cit.*, p. 159.

<sup>2</sup> *Ibid.*

the fruit-body : (1) morphogenically, *i.e.* by causing it, from the first, to develop, as does the fertilised egg in the Fucaceae,<sup>1</sup> with its apex directed toward the source of light ; (2) heliotropically, *i.e.* by causing it to turn its apex after this has been formed, toward the light by means of unequal lateral growth ; and (3) first of all morphogenically and then, if the direction of the light should be altered, heliotropically. Further experiment alone can teach us which of these three possibilities corresponds to the facts of nature.

Light is a decisive factor in the time of opening of the fruit-bodies ; for, in artificial cultures exposed to daylight in the laboratory, a certain number of fruit-bodies come to maturity each day and discharge their glebal masses in the morning or noon hours, and no discharges take place at night. This diurnal periodicity is precisely similar to that of the sporangiophores of *Pilobolus* and of the asci of a fruit-body of *Ascobolus immersus*.

Bright sunlight, according to Miss Walker,<sup>2</sup> promotes the vigour with which *Sphaerobolus* guns discharge their projectiles ; and, as already suggested, this may possibly be due to light favourably affecting the transformation of glycogen into sugar.

We thus see that light acts on *Sphaerobolus* in several different ways : (1) it is essential for fruit-body formation, (2) it causes the fruit-body to develop so that its apex faces the strongest incident rays, (3) it causes the fruit-body to open in the day instead of at night, and (4) it increases the vigour with which a fruit-body discharges its projectile.

**Sphaerobolus as a Coprophilous Fungus dispersed by Herbivorous Animals.**—*Sphaerobolus* is most frequently found on stumps, sticks, boards and other pieces of worked wood, sacking, etc. ; and systematists, as a rule, have described it as lignicolous. Thus Dr. T. Petch, writing to me concerning his observations in England, says : “ I know the fungus well and used to find it until I was tired of seeing

<sup>1</sup> Hans Winkler, “ Ueber den Einfluss äusserer Factoren auf die Theilung der Eier von *Cystosira barbata*,” *Ber. d. D. Bot. Gesell.*, Bd. XVIII, 1900, pp. 298–305. Winkler showed that the first cell-wall of the fertilised egg is formed perpendicularly to the incident rays of light, and that the daughter-cell facing the light produces the free branching system while the daughter-cell away from the light produces the rhizoids.

<sup>2</sup> *Vide supra*, p. 321.

it; but it usually occurred, in my experience, on pieces of wood in hedge bottoms. I used to find it in quantity when I was hunting for Mycetozoa during the winter months, December–January. It was usually on worked wood—pieces of fencing posts, or railings, or the surface of cut branches—all of which tend to drift into the bottom of a hedge in England.”

Notwithstanding that *Sphaerobolus* has been most frequently found on wood, there is now a considerable amount of evidence which goes to show that *Sphaerobolus* is not only lignicolous but also coprophilous.

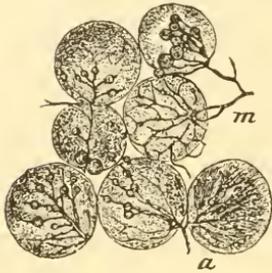


FIG. 166.—Rabbit dung invaded by *Sphaerobolus stellatus*. At *m* mycelial cords; at *a* the mycelium is spreading in a more fan-like manner over a dung ball; at the surface of the dung balls numerous fruit-bodies are being developed. From Zopf's *Die Pilze* (1890, p. 85). Natural size.

The fruit-bodies of *Ascobolus immersus*<sup>1</sup> and of *Pilobolus*<sup>2</sup> are beautifully adapted for securing the dispersal of their spores by herbivorous animals. Now, as will be brought out in detail shortly, the general characteristics of the *Sphaerobolus* gun and projectile are precisely similar to those of the *Ascobolus immersus* and the *Pilobolus* gun and projectile. A perception of this fact led me to enquire whether or not *Sphaerobolus* is primarily coprophilous and whether or not it finds its way to wood, at least sometimes, through animal agency. In what follows an

attempt will be made to answer these questions.

Some records from mycological literature and some observations of my own which afford evidence that *Sphaerobolus* is coprophilous and that it can, and often does, vegetate and fruit upon the dung of such various herbivorous animals as the elephant, the horse, the cow, the hare, and the rabbit, will now be given.

According to Zopf,<sup>3</sup> *Sphaerobolus stellatus* occurs on the dung of hares and rabbits in Germany; and he has given us an illustration of its mycelium growing over and connecting several of the tiny dung-

<sup>1</sup> These *Researches*, Vol. I, 1909, pp. 251–257.

<sup>2</sup> These *Researches*, in the forthcoming Volume VI.

<sup>3</sup> W. Zopf, *Die Pilze*, Breslau, 1890, p. 85, Fig. 55.

balls (Fig. 166). Miss W. M. Page, in the autumn of 1925, at Oxshott Woods in England, found *Sphaerobolus stellatus* on rabbit dung.<sup>1</sup>

Dr. Petch, in Ceylon, found *S. stellatus* on elephant dung; and he further reports that *S. rubidus* B. and Br. has been collected twice in Ceylon, once by Thwaites and once by himself, and on each occasion on elephant dung.<sup>2</sup>

Miss Walker's *Sphaerobolus stellatus* var. *giganteus* was originally found by Dr. H. B. Brown at Starkville, Mississippi, U.S.A., "evidently growing upon half-rotted horse dung."<sup>3</sup> Miss Walker also obtained a strain of *S. stellatus* from a Carnation bench in a greenhouse at Lincoln, Nebraska; and she informed me that it appeared to be "growing upon little sticks or straws which possibly had been brought in with the manure for the bench."<sup>4</sup> The late C. G. Lloyd<sup>5</sup> once informed me that Father Langlois, in Louisiana, U.S.A., had collected *S. stellatus* "on manure," presumably therefore on horse manure. Mr. E. H. Ellis,<sup>6</sup> a member of the British Mycological Society, has found *S. stellatus* in England twice on horse dung: in 1925, in the pine woods at Oxshott (Surrey); and, in 1927, on a path through some woods at Mountfield, near Battle (Sussex).

Rorer,<sup>7</sup> in his list of the fungi of Trinidad, describes *Sphaerobolus stellatus* as "a fungus which is found commonly on cow dung." The late Dr. C. H. Kauffman<sup>8</sup> informed me that he found *Sphaerobolus* on cow dung in Grant's Pass, Oregon, U.S.A., in December, 1925.

For many years I had known and thought of *Sphaerobolus* only as a lignicolous fungus; but, in 1919, having recognised that the characteristics of its gun and projectile resembled those of *Ascobolus immersus* and of *Pilobolus*, which are coprophilous fungi *par excellence*, I resolved to find *Sphaerobolus* on dung for myself, if that were possible. I therefore began to look for *Sphaerobolus* on horse dung

<sup>1</sup> Winifred M. Page, *in litt.*, 1929.

<sup>2</sup> T. Petch, *in litt.*, 1921.

<sup>3</sup> L. B. Walker, *in litt.*, 1921.

<sup>4</sup> *Ibid.*

<sup>5</sup> C. G. Lloyd, *in litt.*, 1920.

<sup>6</sup> E. H. Ellis, *in litt.*, 1929. Mr. Ellis kindly supplied me with a photograph showing *S. stellatus* on an Oxshott horse-dung ball.

<sup>7</sup> J. B. Rorer, "A Preliminary List of Trinidad Fungi," *Board of Agric. for Trinidad and Tobago, Circ. No. 4*, Report of Mycologist for year ending March 31, 1911 (Part II), issued Oct. 1911, p. 42.

<sup>8</sup> C. H. Kauffman, personal communication, 1926.

and cow dung in the woods and meadows of central Canada. At first, this effort was all in vain ; but, in 1923, as will now be related, it was crowned with success, a success that was far beyond my expectation.

On October 16, 1923, in a pasture at the Manitoba Agricultural College (a few miles from Winnipeg) I collected some old horse dung



FIG. 167.—An old cow-dung plat, obtained in a meadow at the Manitoba Agricultural College, Winnipeg, November 14, 1923, which contained the mycelium of *Sphaerobolus stellatus* within and concealed rudimentary fruit-bodies of the same fungus in the exterior crevices between its different layers. Seen from above. About one-half natural size.

bearing fruit-bodies of *Stropharia semiglobata*, and I took the dung to the laboratory at the University of Manitoba and kept it moist in a covered crystallising dish. After three weeks, to my surprise, there came up on the dung a few fruit-bodies of *Sphaerobolus stellatus*. Having thus obtained a clue to the occurrence of *Sphaerobolus* on dung under natural conditions, I asked my colleague Dr. G. R. Bisby, who had made a special study of the fungi in the woods and prairie grounds of the Agricultural College without ever finding *Sphaerobolus*, to accompany me on an excursion into the College pastures, and

I ventured to promise him that I would show him *Sphaerobolus* growing there. Accordingly, in the afternoon of November 14, 1923, Dr. Bisby and I visited two pastures (about a quarter of a mile apart) at the Agricultural College and sought for *Sphaerobolus* on a considerable number of old dung plats. The day was a gloomy



FIG. 168.—An old cow-dung plat, obtained in a meadow at the Manitoba Agricultural College, Winnipeg, November 14, 1923, broken open to show the characteristic mycelium of *Sphaerobolus stellatus* in its interior. About one-half natural size.

one and very mild for the time of the year, the winter's frost not having yet set in. A drizzle of rain added to the gloom and failing light brought an early termination to our foray; but, in the course of an hour and a half, we succeeded in finding fourteen cow-dung plats bearing fruit-bodies of *Sphaerobolus stellatus* and four cow-dung plats containing the white strands characteristic of the *Sphaerobolus* mycelium (Figs. 167–169); and we also found one horse-dung ball bearing young fruit-bodies.

The cow-dung plats in which *Sphaerobolus* was found were not fresh and green but old and, to a very large extent, rotted and exhausted. They no longer bore fruit-bodies of *Piloboli*, *Ascoboli*, or *Coprini* and, when broken across, were found to be traversed by open channels, doubtless borings which had been made by the

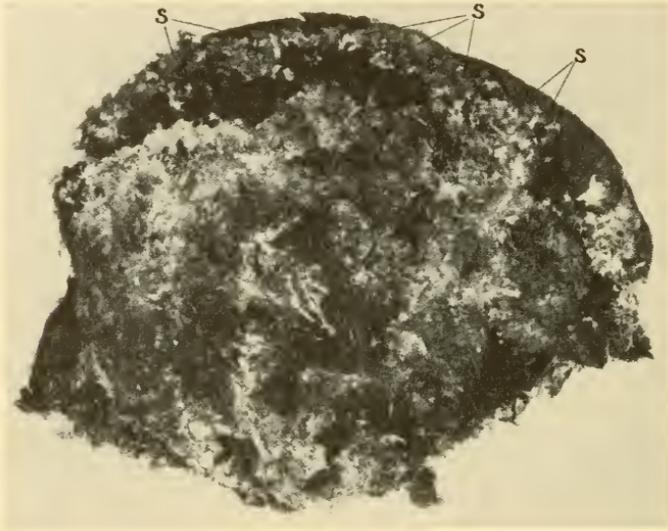


FIG. 169.—A layer of an old cow-dung plat, obtained in a meadow at the Manitoba Agricultural College, Winnipeg, November 14, 1923, showing an interior surface. Throughout the dung is the characteristic mycelium of *Sphaerobolus stellatus* which, along the edge at *s, s, s* has produced a line of young fruit-bodies. In the laboratory these fruit-bodies completed their development and discharged their projectiles. Natural size.

larvae of a large dung beetle that had disappeared.<sup>1</sup> The plats containing the fungus could be identified by the characteristic white mycelial strands which were particularly numerous between the successive droppings (Figs. 168 and 169) and which in fourteen plats had grown toward the periphery of the dung and had there formed fruit-bodies. As could be seen by breaking plats in the middle into

<sup>1</sup> One of the cow-dung plats which did not bear *Sphaerobolus* contained at its base, just above the grass, a number of large, somewhat irregularly shaped sclerotia of *Coprinus Rostrupianus*. This was my first finding of this species. I have often found it since in a similar situation.

pieces, the mycelium in a plat was often limited, except for extensions to the periphery here and there, to a larger or smaller inner core, the mass of dung occupied by the mycelium being readily distinguishable from the surrounding unoccupied mass of dung by its whiteness. The fruit-bodies on the exterior of a dung plat usually occurred in rows, each row situated in a crevice between two successive droppings, *i.e.* in a place where the mycelium had reached the light and had received the stimulus necessary for fruit-body formation. Fig. 167 shows one of the cow-dung plats seen from above. It was found to contain the mycelium of *Sphaerobolus stellatus* and also a number of rudimentary fruit-bodies concealed in the exterior crevices between the different layers. Fig. 168 shows a similar dung plat broken open, with the characteristic mycelium of *S. stellatus* in its interior. Fig. 169 shows an interior surface of another dung plat. Here, the mycelium has extended to what was a crevice between two layers of the dung and has formed there a row of about twenty fruit-bodies (*s*). When this piece of dung was set in a damp-chamber, the fruit-bodies completed their development and discharged their glebal masses in the course of about a week.

Whenever pieces of the cow-dung plats infected with the mycelium of *Sphaerobolus* were kept moist in a glass vessel exposed to daylight in a warm laboratory, within a week they produced an abundant crop of fruit-bodies and soon a certain number of the fruit-bodies opened each morning and discharged their glebal masses. One such piece of cow dung bearing fruit-bodies is shown in Fig. 164 (p. 332). Fruit-bodies thus obtained provided me with material for determining approximately the horizontal range of the *Sphaerobolus* gun, and it was a fruit-body developed on cow dung which shot its projectile the record horizontal distance of 18 feet 7 inches.<sup>1</sup>

The glebal mass of *Sphaerobolus* resembles the sporangium of *Pilobolus* in that it is very adhesive and, after being discharged, sticks to anything which it may happen to strike. Bearing this in mind, I examined the phanerogamic vegetation surrounding two of the *Sphaerobolus* dung plats and had the satisfaction of finding many glebal masses which had been shot on to it. Within a few

<sup>1</sup> *Vide supra*, p. 334.

feet of the first plat examined, I found numerous glebal masses attached to the stems and leaves of grass-plants (Fig. 170); and around the second plat, which happened to be close to a small patch



FIG. 170.—Glebal projectiles of *Sphaerobolus stellatus* found adhering to grass haulms and leaves around a cow-dung plat in a pasture at the Manitoba Agricultural College, Winnipeg, November 14, 1923. Natural size.

of outlying bush, I found : (1) one glebal mass, on the upper side of a horizontal leaf of a Wolfberry shrub (*Symphoricarpos occidentalis*), 5 feet horizontally from the plat and 18 inches above the ground ; (2) one glebal mass, on a terminal twig of a Rose-bush, one foot horizontally from the plat and 2 feet 1 inch above the ground ; (3) one glebal mass, on the stem of a Wolfberry bush, almost

directly above the plat and 1 foot 10 inches above the ground ; (4) two glebal masses on the leaves and one on the inflorescence of a Golden-rod plant (*Solidago serotina*) which was 1 foot 6 inches distant horizontally from the plat ; and, finally, (5) a number of glebal masses on the stems and leaves of grasses which were growing



FIG. 171.—*Sphaerobolus stellatus* and its dispersal. Cattle feeding in open woods and on prairie land in Manitoba swallow discharged glebal masses attached to grass and thus serve as distributors of the fungus. Photograph taken in East Kildonan, Manitoba, by the Manitoba Department of Agriculture. The trees are mostly Elms (*Ulmus americana*).

close to the margin of the plat. These observations teach us that the glebal masses of *Sphaerobolus* can be, and sometimes actually are, shot away in large numbers from dung-plats in pastures on to the surrounding herbage in the same general manner as are the sporangia of *Pilobolus*.<sup>1</sup>

<sup>1</sup> In October, 1924, Dr. Bisby and I again found *Sphaerobolus stellatus* on old cow-dung plats in the pastures of the Manitoba Agricultural College, but in less abundance than in the previous year. In November, 1928, in the same pastures we failed to find the fungus even in a single plat, but the weather during the preceding summer had been unusually wet and therefore possibly unfavourable to the growth

How did *Sphaerobolus stellatus* come to invade the cow-dung plats in the first instance? It is most unlikely that glebal masses should have been shot on to the plats from fruit-bodies growing on stumps of trees, etc.; for (1) the plats were lying out in the open at a considerable distance from the nearest woods, and (2) in those woods, neither before nor since November 14, 1923, have any fruit-bodies of *Sphaerobolus* ever been found. It is also most unlikely that the wind should have blown to the plats either glebal masses as wholes or individual spores or gemmae; for (1) a glebal mass is so adhesive that it cannot be dislodged from its substratum even during a gale, and (2) the spores and gemmae cannot escape from a glebal mass attached to grass, etc., because they are embedded in a glutinous matrix which resists disintegration by wind and rain.<sup>1</sup> The simplest and most satisfactory answer to our question seems to be as follows: the cows (Fig. 171) some months previously to November 14 had eaten herbage like that which I found around the two cow-dung plats as already described, *i.e.* herbage to which glebal masses were attached; the spores or gemmae, or both, had passed unharmed down the alimentary canals of the cows concerned and had been extruded in the faeces, where they germinated; subsequently, after the dung-plats had become much altered and largely exhausted by other fungi and by insects, the mycelium derived from the *Sphaerobolus* spores or gemmae, or both, grew freely in the core of the dung plats and eventually produced fruit-bodies at the dung-plats' surface.

In all probability, whenever *Sphaerobolus* is found on the solid excreta of herbivorous animals, the infection of the substratum, in the first place, has been due to the animals concerned having swallowed one or more glebal masses along with herbage to which the masses were attached.

From a consideration of: (1) the general structure and the

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of the mycelium. In the first half of November up to the 16th, 1931, we again found *S. stellatus* in a pasture at the Agricultural College. It was present on many cow-dung plats, on one mass of horse dung, and on a soft-wood board and a piece of packing-case cardboard which had been thrown down near an infected cow-dung plat. Again, a number of glebae were found which had been discharged from fruit-bodies developed on dung and which had stuck to surrounding grass-blades, etc.

<sup>1</sup> *Vide supra*, p. 297.

physiological properties of the Sphaerobolus gun and projectile, (2) the fact that the fungus under natural conditions is not infrequently found on the dung of various herbivorous animals, and (3) the fact that the fungus grows and fruits well on horse dung in laboratory cultures, I have been led to the conclusion that *Sphaerobolus primarily is a coprophilous fungus* and that its *occurrence on wood*, while due occasionally perhaps to the germination of glebae which have been shot on to the surface of the wood from neighbouring fruit-bodies, *must in general be accomplished through the agency of animals*. A discussion of the manner in which Sphaerobolus comes to infect wood will be deferred to a subsequent Section. In the meanwhile an attempt will be made to show how Sphaerobolus resembles two other unrelated fungi which exhibit special adaptations for a coprophilous mode of existence.

**Pilobolus, Ascobolus immersus, and Sphaerobolus as Three Fungi with Parallel Adaptations for a Coprophilous Mode of Life.**—It is a remarkable fact and an illustration of parallelism in the course of organic evolution that in Pilobolus, *Ascobolus immersus*, and Sphaerobolus, three fungi which belong respectively to the Phycomyces, the Ascomycetes, and the Basidiomycetes—the three great divisions of the Fungi proper—the sporocarps are either converted directly into guns (Pilobolus, Sphaerobolus) or else produce guns (*Ascobolus immersus*) which have many characteristics in common (Fig. 172). Let us now consider what these characteristics are.

(1) *Massiveness and Parabolic Trajectory of the Projectile.* The Pilobolus,<sup>1</sup> *Ascobolus immersus*,<sup>2</sup> and Sphaerobolus guns all shoot away projectiles which are relatively massive, so massive indeed that, immediately after leaving the guns, they are not carried off by the wind as are the ascospores of the Pezizae and most species of *Ascobolus*<sup>3</sup> or as are the basidiospores of the Uredineae, *Tilletia*,

<sup>1</sup> For a description of the Pilobolus gun and projectile, *vide* these *Researches* in the forthcoming Volume VI.

<sup>2</sup> For a description of the guns and projectiles of *Ascobolus immersus*, *vide* these *Researches*, Vol. I, 1909, pp. 251–257.

<sup>3</sup> In most species of *Ascobolus*, *e.g.* *A. stercorarius*, *A. magnificus*, the eight spores of each ascus are relatively very small and they separate from one another as they pass out of the ascus mouth, so that they are carried away by the wind individually.

and the Hymenomycetes, but they trace out in the air a more or less parabolic trajectory and, within about one second of the moment of leaving their guns, fall upon the ground.

The projectiles of *Pilobolus* are shot horizontally a distance of less than 9 feet, those of *Ascobolus immersus* a distance of less than 2 feet, and those of *Sphaerobolus* a distance of less than 20 feet.

The projectile of *Pilobolus* consists of a sporangium containing tens of thousands of spores and of a large adherent drop of cell-sap derived from the sporangiophore (Fig. 172, *a*); that of *Ascobolus immersus* consists of eight very large coherent ascospores with thick gelatinous walls, together with an enveloping drop of ascus cell-sap (*b*); while that of *Sphaerobolus* is a glebal mass consisting of a gleba containing many thousands of basidiospores, gemmae, and rounded cells embedded in a solid fatty matrix, the whole surrounded on the exterior by a covering of cells derived from the innermost layer of the peridium (*c*). From the point of view of comparative morphology, therefore, the projectiles of the three fungi under discussion differ very considerably from one another.

(2) *Great Violence in the Discharge of the Projectile.* As compared with other fungi, the violence of discharge of the projectile in *Pilobolus*, *Ascobolus immersus*, and *Sphaerobolus* is very great. *Pilobolus longipes* and *P. Kleinii* can shoot their sporangia to a height just exceeding 6 feet and horizontally to a distance of about 8·5 feet<sup>1</sup>; *Ascobolus immersus* can shoot its spore-mass to a height of 1 foot 2 inches and, doubtless, horizontally to a distance of 1–2 feet<sup>2</sup>; while *Sphaerobolus stellatus* can shoot its glebal mass to a height of 14·5 feet and horizontally to a distance of 18·5 feet.<sup>3</sup> In the

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In *A. immersus*, as already described and illustrated in these *Researches* (*loc. cit.*), the eight spores of each ascus are relatively very large and are bound together by their thick outer gelatinous cell-walls so that, together with the drop of ascus-sap which surrounds them, they form a relatively very large projectile—too massive for the wind to carry away. *A. immersus*, therefore, in respect to the nature of its projectile, while resembling the species of *Saccobolus*, stands in striking contrast with most other *Ascoboli*.

<sup>1</sup> *Vide* these *Researches*, in the forthcoming Volume VI.

<sup>2</sup> These *Researches*, Vol. I, 1909, p. 253. The maximum height of projection measured was 35 cm. and the maximum horizontal distance measured was 30 cm. With further experiment, the latter distance no doubt could be considerably increased.

<sup>3</sup> *Vide supra*, p. 335.

Phycomycetes and the Basidiomycetes, respectively, there are no other guns to compare in violence with those of *Pilobolus* and *Sphaerobolus*; while, in the Discomycetes, *Ascobolus immersus* shoots its spore-masses higher and farther horizontally than any *Peziza* or other species shoots its isolated wind-dispersed spores.

(3) *Adhesiveness of the Projectile.* The *Pilobolus*, *Ascobolus*

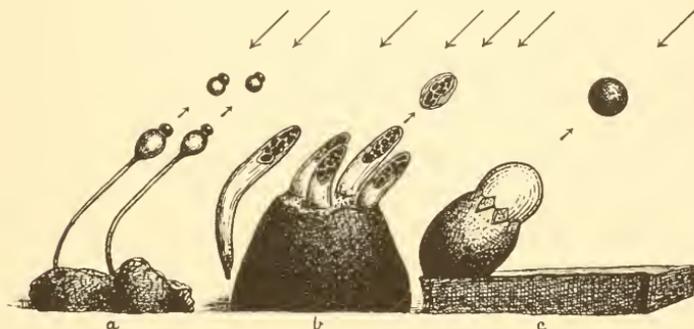


FIG. 172.—Diagram illustrating the fruit-bodies of three coprophilous fungi, similarly adapted for the dispersion of their spores, one from each of the three great fungus subdivisions: *a*, *Pilobolus longipes* (Phycomycetes); *b*, *Ascobolus immersus* (Ascomycetes); and *c*, *Sphaerobolus stellatus* (Basidiomycetes). The long arrows indicate the direction of the light and the short arrows the direction in which the projectiles are shot. The projectiles are shown between the two sets of arrows. The fungus gun of the *Pilobolus* is a sporangiophore and the projectile a sporangium to which a drop of cell-sap is attached. The gun of the *Ascobolus* is an ascus and the projectile is a group of eight ascospores clinging together and enveloped by a drop of cell-sap. The gun of the *Sphaerobolus* is a peridium with an everting inner layer (shown everted), and the projectile is a gleba surrounded by a thin coat of liquid. All the guns are heliotropic and all shoot away a massive projectile, containing many spores, on to herbage.

*immersus*, and *Sphaerobolus* projectiles are all remarkably glutinous, so that they adhere to herbage or to anything else that they may strike or fall upon after they have been shot from their guns, and so that, after drying, they cannot be dislodged by the wind.

The adhesive matter of the projectile of *Pilobolus* consists of a thick ring of jelly which is produced inside the sporangium and becomes exposed to the air by the dehiscence of the sporangial wall; that of *Ascobolus immersus* consists of eight very thick gelatinous outer spore-walls, which are fused together; while that of *Sphæro-*

bolus consists of the slimy products of the innermost layer of the peridium which partly deliquesces as the projectile is prepared for discharge. The adhesive matter of the projectiles of the three fungi under discussion, therefore, while having an identical function, comes into existence, from the developmental point of view, in three different ways.

(4) *Heliotropism of the Guns.* The sporangiophore of *Pilobolus* and the asci of *Ascobolus immersus* are positively heliotropic (Fig. 172, *a* and *b*). In unilateral light they execute a turning movement, so that in the end they come to point their apices in the direction of the source of strongest illumination. The fruit-body of *Sphaerobolus* also develops so that its apex faces the strongest incident rays of light (Fig. 172, *c*), but whether light stimulates the young fruit-body morphogenically or heliotropically remains to be decided by further investigation. Since the *Pilobolus*, *Ascobolus immersus*, and *Sphaerobolus* guns all point in the direction of the source of the strongest light, they all shoot their projectiles in the direction of the source of the strongest light.

The turning of the gun toward the light is accomplished: in *Pilobolus* by the bending of the sporangiophore under the influence of a light stimulus received by the subsporangial swelling; in *Ascobolus immersus* by the bending of the sporangium, *i.e.* of the ascus; and in *Sphaerobolus* by the fruit-body as a whole either morphogenically or heliotropically. Thus, in the three fungi under discussion, the structures which turn toward the light and so enable the projectiles to be shot toward the light, are very different morphologically.

(5) *Multisporous Nature of the Projectile and the Non-separation of the Spores whilst the Projectile is attached to Herbage.* The *Pilobolus*, *Ascobolus immersus*, and *Sphaerobolus* projectiles are all multisporous, and the spores (spores and gemmae in *Sphaerobolus*) cannot separate from one another without the help of some special external agent. Neither the wind nor the rain nor both combined can set free the spores from the projectile of which they form a part.

The numerous spores in the projectile of *Pilobolus* are prevented from escaping owing to their being enclosed in part by a tough and persistent sporangial wall, in part by a thick and insoluble gela-

tinous ring, and in part by the wall of the columella. The eight spores of the projectile of *Ascobolus immersus* are held together by their insoluble gelatinous outer cell-walls. The numerous spores and gemmae in the projectile of *Sphaerobolus* cannot gain their freedom because they lie embedded and immobilised in a dense fatty matrix upon which rain-water has no action.

(6) *Occurrence of the Gun-developing Fruit-bodies on the Dung of Herbivorous Animals.* The fruit-bodies of most species of *Pilobolus*, e.g. *P. longipes* and *P. Kleinii*, occur exclusively on the dung of herbivorous animals, such as the horse, the cow, and the rabbit. *Ascobolus immersus* occurs on the dung of the cow, the horse, the stag, and the sheep.<sup>1</sup> *Sphaerobolus*, as already recorded, is known to occur on the dung of the elephant, the horse, the cow, the hare, and the rabbit, so that there is plenty of evidence to show that *Sphaerobolus*, like *Pilobolus* and *Ascobolus immersus*, is well fitted for a coprophilous mode of existence.

(7) *Correlation of Facts.* In *Pilobolus*, in *Ascobolus immersus*, and in *Sphaerobolus* we can correlate: (1) the massiveness and parabolic trajectory of the projectile, (2) the great violence in the discharge of the projectile, (3) the adhesiveness of the projectile, (4) the positive heliotropism of the fungus gun, (5) the multispore nature of the projectile and the non-separation of the spores whilst the projectile is attached to herbage, and (6) the occurrence of the gun-developing fruit-bodies on the dung of herbivorous animals; for they are all factors associated with the dissemination of the spores by herbivorous animals. (1) The large size and considerable weight of the projectile is advantageous in that it permits of the projectile being cast away by a relatively large and powerful gun and receiving from the gun sufficient momentum to carry it against the resistance of the air and by a parabolic trajectory to the grass and other herbage surrounding the dung on which the fruit-body has developed. (2) The great violence of projectile-discharge ensures that the projectile will be shot clear of the dung on which the fruit-bodies grow

<sup>1</sup> H. Rehm, in Rabenhorst's *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*, Aufl. II, Bd. I, Abteil. 3, 1896, p. 1127. At Winnipeg, *Ascobolus immersus* occurs commonly on fresh horse dung kept moist for about a month in the laboratory.

and will be deposited on the surrounding grass and other plants upon which herbivorous animals feed. The greater the violence of discharge, the more widely are the projectiles spread in pastures and the more chance there is that, eventually, they will be swallowed by herbivorous animals. (3) The adhesiveness of the exterior surfaces of the projectiles enables the projectiles to remain attached to the leaves and stems of grass-plants, etc., despite the weather, for long periods of time until eventually herbivorous animals swallow them with their substratum. (4) The positive heliotropism of the fungus guns causes the guns to direct their apices toward the source of the strongest incident rays of light and, therefore, in the end, to shoot away their projectiles in directions least obstructed by obstacles. The result of this is that the projectiles are distributed more widely over the surrounding herbage than they otherwise would be and therefore have a better chance of being swallowed by herbivorous animals. (5) The multispore nature of each projectile serves to increase the mass of the projectile and thus to increase the distance to which the projectile can be ejaculated.<sup>1</sup> The non-separation of the spores in a projectile whilst the projectile is attached to grass, etc., is in no way disadvantageous; for the individual spores in a projectile readily separate from one another and become dispersed when the projectile is passing down the alimentary canal of a herbivorous animal. (6) Finally, the occurrence of the gun-developing fruit-bodies on dung is due to the fact that the projectiles, after being shot away from the dung on to grass, etc., are eventually swallowed by herbivorous animals. The spores pass unharmed down the alimentary canal of horses, cattle, rabbits, etc., separate from one another in the course of their journey, and so are deposited in the solid excreta which the animals drop to the ground. In these excreta the spores germinate and the mycelium so produced ultimately gives rise to new gun-producing fruit-bodies.

We therefore see that the guns and projectiles of *Pilobolus*, *Ascobolus immersus*, and *Sphaerobolus*, although diverse in origin, are beautifully adapted both by their structure and functions to bring about dispersion of the spores through the agency of (1) *flowering plants*, which are used as lodging places for the adhesive multi-

<sup>1</sup> Cf. these *Researches*, Vol. I, 1909, p. 253.

sporous projectiles, and of (2) *herbivorous animals*, which swallow the projectiles, disperse the spores in the contents of their alimentary canals, and deposit the spores in their excreta at various places in pastures.

**Sphaerobolus as a Member of the More Specialised of Two Groups of Coprophilous Fungi.**—So far as the arrangements for securing the dissemination of the spores are concerned, coprophilous fungi may be divided into two groups: (1) a more primitive group which successively makes use of three external agents—the *wind*, *flowering plants*, and *herbivorous animals*—and (2) a more highly specialised group which dispenses with the wind and which successively makes use of only two external agents—*flowering plants* and *herbivorous animals*. *Sphaerobolus* belongs to the more specialised group. Examples of fungi belonging to each of the two groups are given in the accompanying Table.

*Coprophilous Fungi*

Group I	Group II
Wind an essential factor in spore-dispersion	Wind not employed in spore-dispersion
Aleuria vesiculosa Ascobolus stercorarius A. magnificus Galera bulbifera (Figs. 173 and 174) <sup>1</sup> Stropharia semiglobata Panaeolus campanulatus Coprinus sterquilinus	Pilobolus longipes P. Kleinii Thelebolus stercoreus <sup>2</sup> Ascobolus immersus Saccobolus Kerverni Sphaerobolus stellatus S. iowensis

The fungi of Group I shoot away their ascospores, basidiospores, etc., singly and to a relatively short distance—a distance just sufficient to enable the wind to envelop the spores and carry them

<sup>1</sup> *Galera bulbifera* is a species described by C. H. Kauffman (*The Agaricaceae of Michigan*, Lansing, Mich., U.S.A., Vol. I, 1918, p. 496) and is known by its bulbous base. It occurs commonly on old horse dung in central Canada and has often come up on that substratum in my laboratory.

<sup>2</sup> I have not yet investigated *Thelebolus stercoreus*. However, Brefeld (*Untersuchungen über Pilze*, Münster i. W., Heft IX, 1891, p. 114) states that its great spore-mass is shot with much force on to the cover of the culture dish. It is therefore clear that the projectile of *T. stercoreus* resembles the projectiles of the other fungi of Group II.

away. Eventually, of the spores thus carried off by the wind, some settle on herbage in pastures, etc., *i.e.* so far as their ultimate fate is concerned in *favourable* places, while others settle in pools, lakes,

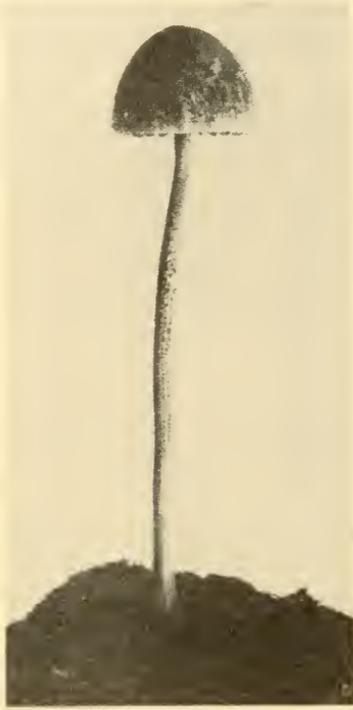


FIG. 173.—*Galera bulbifera*, a North American coprophilous fungus the spores of which are dispersed first by the wind which blows them on to grass and then by horses which eat the grass. The fruit-body here shown came up on an old horse-dung culture in the laboratory at Winnipeg. Natural size.

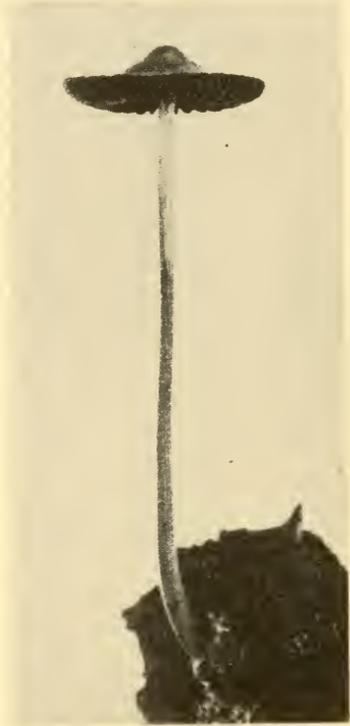


FIG. 174.—Another fruit-body of *Galera bulbifera* which came up on an old horse-dung culture in the laboratory at Winnipeg. The turning up of the edge of the pileus may have been due to the very moist conditions under which the fruit-body grew. Natural size.

streams, seas, on rocky ground, the bare earth, the leaves of trees, etc., *i.e.* so far as their ultimate fate is concerned in *unfavourable* places. Those spores which happen to lodge on herbage in pastures are likely, in the end, to be swallowed by herbivorous animals and thus to be deposited in fresh dung where they can germinate; while

those spores which do not settle on herbage are almost certain to perish without ever producing a mycelium.

The fungi of Group II have dispensed with the wind as an agent in the dispersal of their spores. Instead of shooting away their spores singly to a short distance and entrusting them to the uncertain operation of the wind which would deposit them indiscriminately in places favourable or unfavourable so far as their ultimate fate is concerned, they shoot them away in the form of large spore-masses and very violently, so that they at once strike and stick to, or fall upon, the surrounding vegetation. The spore-masses are so heavy that, despite the wind, their trajectory is a parabolic one and they come to earth within about one second after being discharged. Hence the spore-masses must settle on herbage within a few feet of the fruit-bodies which have discharged them. For the ultimate fate of the spores such a place of settlement is highly favourable; for herbivorous animals must have been there before and are therefore likely to come again and, when they come, they are likely to swallow the spore-masses with grass, etc., as they feed.

Thus, in the fungi of Group II, the deposition of the spores on herbage is secured directly through the activity of the fruit-bodies, and not indirectly by the wind as in the fungi of Group I, with the result that the chances of a high percentage of the spores being swallowed by herbivorous animals and finding their way into dung are greatly increased. A comparison of the arrangements for depositing spores on herbage exhibited by the two groups of coprophilous fungi leads to the conclusion that the fungi of Group II are far more highly specialised and more closely adapted for a coprophilous mode of existence than are those of Group I.

**Sphaerobolus as a Lignicolous Fungus and the Problem of its Mode of Infecting Wood.**—As already pointed out, *Sphaerobolus* very frequently occurs on wood. It may therefore be asked: if *Sphaerobolus* is primarily a coprophilous fungus, how does it enter sticks, stumps, and pieces of worked wood on which it is so often found? Since under natural conditions no one has yet observed the exact means by which a specific piece of wood has become infected with *Sphaerobolus*, for the present we are obliged to answer our question in a tentative and somewhat speculative manner.

First of all, it must be realised that, since the spores and gemmae in a discharged glebal mass cannot escape into the air owing to their being imprisoned in the gleba's glutinous matrix, a piece of wood cannot be infected by means of wind-blown spores or gemmae.

It must also be realised that, since a glebal mass, after discharge, sticks so tightly to whatever it has struck or fallen upon that it cannot be dislodged by the wind, a piece of wood cannot be infected by means of wind-blown glebal masses.

There appear to be at least four possible ways in which a piece of wood may become infected with *Sphaerobolus* : (1) by one or more glebal masses being shot on to it from fruit-bodies growing on another piece of *wood*, (2) by one or more glebal masses being shot on to it from fruit-bodies growing on a mass of *dung*, (3) by mycelium growing on to it from infected dung, and (4) by spores or possibly gemmae which have passed through the alimentary canal of some herbivorous animal. Let us consider these possibilities *seriatim*.

(1) *Infection of wood by glebal masses shot on to it from fruit-bodies growing on another piece of wood.* When a piece of wood happens to lie within about 20 feet of another piece of wood which bears *Sphaerobolus* fruit-bodies, it is within range of *Sphaerobolus* guns and glebal masses may fall upon it. We know that a glebal mass placed in water readily germinates as a whole owing to the fact that its gemmae send out radially a vigorous clamp-connexion-bearing mycelium. Therefore, if a piece of wood, not already occupied by another wood-destroying fungus and otherwise suitable as a substratum for *Sphaerobolus*, has one or more glebal masses shot on to it from fruit-bodies situated on a neighbouring piece of wood, there seems no reason why it should not become infected with *Sphaerobolus* as soon as ever the weather becomes sufficiently moist for the glebal mass or masses to germinate. In forests, probably, sticks and other masses of wood often become infected with *Sphaerobolus* by this very means. However, while the discharge of glebal masses from one motionless piece of wood to another may well provide for a slow and local spread of the fungus in particular woods, it does not account for the transportation of the fungus from one piece of wood to another piece of wood far distant from it nor for the spread of the fungus from one woodland area to another.

(2) *Infection of wood by glebal masses shot on to it from fruit-bodies growing on a mass of dung.* Here the mode of infection of a piece of wood is as in (1), except for the fact that the fruit-bodies from which the glebal masses are derived are situated not on another piece of wood but on a mass of dung.

Sphaerobolus, as already recorded, has been found on the dung of elephants, horses, cows, hares, and rabbits; and it is easily conceivable that such animals as these, so well endowed with powers of locomotion, serve to transport Sphaerobolus long distances and often from one woodland area to another. For the sake of illustration, let us consider rabbits as possible agents for the transportation of Sphaerobolus. Sphaerobolus fruit-bodies have been found on the dung of rabbits in Germany and England<sup>1</sup>; and there can be no doubt that these animals, whilst feeding on herbage in fields and woods, swallow glebal masses and that the spores or gemmae or both pass down their alimentary canals unharmed and germinate in their faeces. Rabbits are relatively small but active quadrupeds; and, where they occur, they are often very numerous. They leave the little balls which make up their solid excreta scattered far and wide upon the ground; and, in woodlands, one can often see these dung-balls lying in contact with fallen twigs and sticks, on logs, and at the foot, or even on the top, of stumps. When fruit-bodies are produced on rabbit dung, as they certainly are sometimes, it is conceivable that the glebal masses shot from the Sphaerobolus guns may land on adjacent sticks or stumps and that such discharged glebal masses may germinate and cause an infection of the underlying woody substratum.

If discharged glebal masses serve to transfer Sphaerobolus from rabbit dung to wood, they may also serve to transfer Sphaerobolus from cow dung, horse dung, elephant dung, etc., to wood.

(3) *Infection of wood by mycelium growing on to it from infected dung.* There is no difficulty in imagining that Sphaerobolus should begin its growth in the dung of hares, rabbits, cows, etc., from spores or gemmae derived from glebal masses swallowed by the animals concerned and that the mycelium so produced should spread from the dung to a stick or a stump lying beneath. The mycelium of

<sup>1</sup> *Vide supra*, pp. 350-351.

Sphaerobolus is, as a matter of fact, well adapted for spreading from one substratum to an adjoining one, for it forms mycelial strands as illustrated by Zopf in connexion with rabbit dung (Fig. 166, p. 350). This mycelial mode of infection which involves the agency of herbivorous animals, like the glebal mode of infection described under (2), would account for the spread of the fungus from one piece of wood to another far distant from it and for the transportation of the fungus from one woodland area to another.

(4) *Infection of wood by spores or possibly gemmae which have passed through the alimentary canal of some herbivorous animal.* After cows, horses, rabbits, etc., have swallowed one or more glebal masses of Sphaerobolus and when these glebal masses are passing down the alimentary canal, in all probability the fatty matrix of the masses is soon destroyed by the action of lipase or some other enzyme, with the result that the spores and gemmae are set free from the prisons which have held them. The spores or gemmae or both may therefore be extruded with the solid excrement of herbivorous animals and be present in large numbers in fresh dung just deposited on the ground. Now such fresh dung is much visited by dung-flies and other insects. These insects, in walking, feeding, or laying eggs, may either swallow some of the spores or gemmae or get their legs or proboscides fouled with dung containing them. It is conceivable that flies so contaminated may wing their way to sticks, logs, pieces of worked wood, etc., and then deposit the spores or gemmae of Sphaerobolus along with their excreta or with dung scraped off their legs, wings, or bodies, and it is further conceivable that such spores or gemmae might germinate subsequently and infect the mass of wood lying beneath them. If insect transportation of the kind just suggested actually takes place, it would account for the occurrence of Sphaerobolus on pieces of wood which, so far as one can judge, could not have been infected by any of the means suggested under (1), (2), or (3). At present, however, there are no experimental facts supporting the idea of fly-dispersion except perhaps one, namely, that according to both Pillay and Miss Walker, the spores of Sphaerobolus can be caused to germinate by placing them in a solution of pepsin.<sup>1</sup>

<sup>1</sup> *Vide supra*, p. 293.

## GENERAL SUMMARY

THE FOLLOWING IS A SUMMARY OF THE MORE IMPORTANT RESULTS OBTAINED DURING THE INVESTIGATIONS

### PART I

**Chapter I.**—By means of hyphal fusions (anastomoses) the mycelium in many species of Ascomycetes, Basidiomycetes, and Fungi Imperfecti becomes converted in an early state of development into a three-dimensional network. Hyphal fusions are also present in the fruit-bodies of these fungi. The advantages accruing to a mycelium and to a fruit-body through the existence of hyphal fusions have been briefly summarised.

It is important to distinguish between (1) a *hyphal contact*, (2) a *hyphal adhesion*, and (3) a *hyphal fusion*. Only in a hyphal fusion do the two masses of protoplasm of the two hyphae become confluent.

*Vegetative fusions* promote vegetative processes, *e.g.* the conduction of food materials, the transmission of stimuli, etc., while *sexual fusions* assist the coming together of nuclei destined to co-operate in a sexual process.

*Parasitic fusions*, *e.g.* those between *Chaetocladium* or *Parasitella* and certain Mucorineae, bring the protoplasm of the parasite and of the host into continuity and thereby promote the nutritive processes of the parasite.

In a single mycelium hyphal fusion sets in in the older parts where the culture medium is becoming exhausted and, in general, the process is promoted by conditions of starvation.

The mycelium as a whole strives to become a three-dimensional network, but a network of not too fine a mesh. The actual formation of hyphal fusions seems to alter the physiological condition of a mycelium in such a way that the mycelium in the end ceases to produce hyphal fusions. The formation of hyphal fusions in a mycelium as a whole is well regulated.

It is characteristic of both vegetative and sexual fusions that hyphae or sexual organs destined to fuse act upon one another *at a distance*. The observations made by previous workers on this phenomenon have been reviewed.

*Telemorphosis* is the phenomenon in which one hypha, acting at a distance, stimulates another hypha to alter its form by sending out an opposing fusion hypha.

*Zygotropism* is the phenomenon in which two hyphae, as a result of mutual stimulation, make growth curvatures toward one another and grow toward one another until they meet.

Naturally occurring hybrids between two species of fungi are unknown. However, a few hybrids have been produced artificially. Thus Burgeff obtained zygospores when he crossed *Phycomyces nitens* and *P. Blakesleeanus*, Dodge obtained fruit-bodies by crossing *Neurospora sitophila* and *N. tetrasperma*, Hanna and Popp obtained smut spores as a result of crossing *Ustilago avenae* and *U. levis*, and Flor obtained bunt balls as a result of crossing *Tilletia tritici* and *T. laevis*. In all these cases sexual fusions between two mycelia belonging to different species must have taken place.

In general, in Ascomycetes, Basidiomycetes, and Fungi Imperfecti, vegetative fusions are readily formed between adjacent hyphae of one and the same mycelium or of two mycelia of the same species; but, as a rule, they are *not* formed between two mycelia of two different species, even when the latter belong to the same genus.

In the Hymenomycetes the inability of the mycelia of distinct species of the same genus to fuse with one another throws light on the fact that in this great group of fungi (1) wild hybrids are unknown and (2) all attempts to hybridise two species of the same genus have so far failed.

In general, the occurrence or non-occurrence of hyphal fusions between hyphae of two mycelia of different origin may be applied as a criterion for identifying or distinguishing between species of Ascomycetes, Basidiomycetes, and Fungi Imperfecti whose affinities are uncertain.

The author has described the formation of hyphal fusions in Pyrenomycetes (*Pleurage curvicolla*, *P. anserina*, *Fimetaria fimicola*), Discomycetes (*Pyronema confluens*), Hymenomycetes (*Coprinus sterquilinus*, *C. lagopus*), and Gastromycetes (*Sphaerobolus stellatus*). The principle involved in the mode of observation was that of continuous watching of a particular part of a living mycelium with a view to witnessing all those stages in growth leading up to, and culminating in, the actual union of two hyphae of one and the same mycelium.

All *hyphal fusions are essentially end-to-end ones, i.e.*, when a hyphal fusion takes place, it takes place between the end of one hypha and the end of another hypha.

Four kinds of hyphal fusions have been distinguished: (1) *hypha-to-hypha* fusions, (2) *hypha-to-peg* fusions, (3) *peg-to-peg* fusions, and (4) *hook-to-peg* or *clamp-connexion* fusions. In this terminology: by the word *hypha* is meant an ordinary vegetative hypha of some length which has grown freely in the culture medium before becoming "attracted" by another hypha and undergoing growth changes leading to a hyphal fusion; by

the word *peg* is meant a very short special fusion hypha which has never grown freely in the medium by itself but has been stimulated to come into existence by another hypha, another peg, or a hook with which it is destined to fuse; and by the word *hook* is meant a very short curved hypha such as is always produced during the formation of a clamp-connexion. A peg may be regarded as a fusion organ.

In the formation of a clamp-connexion (hook-to-peg fusion) the stages of development follow a definite sequence as follows: (1) a hook grows outwards, backwards, and inwards towards the main hypha, (2) conjugate nuclear division takes place, (3) a septum is formed across the main hypha at the level of the middle of the hook, (4) a septum is formed across the top of the hook, (5) the main hypha sends out a peg opposite the end of the hook, (6) the end of the hook and the end of the peg flatten out against one another, (7) the double wall between the hook and the peg is dissolved away, and (8) the nucleus that was imprisoned temporarily in the hook-cell escapes from the hook into the main body of the penultimate cell of the hypha. In *Coprinus lagopus* this sequence of events, under favourable conditions of growth, may be accomplished in 23 minutes. The conjugate nuclear division takes place in less than 14 minutes and probably in about 12 minutes.

In Hymenomycetes and Gastromycetes, in both haploid and diploid mycelia, each septum has a small central open pore through which protoplasm can pass from cell to cell.

In Hymenomycetes and Gastromycetes, under natural conditions, the haplophase of a mycelium very soon passes into the diplophase and, as a rule, fruit-bodies are formed not on haploid but on diploid mycelia.

In Hymenomycetes and Gastromycetes the clamp-connexion may be regarded as a means for providing between any two adjacent cells of a diploid mycelium two septa instead of one and therefore two passageways for protoplasm instead of one. Thus, owing to the existence of clamp-connexions, the translocation of the protoplasm of a diploid mycelium into a fruit-body, when this is being formed, is facilitated.

The hooks of the ascogenous hyphae of *Pyronema confluens* and other similar Discomycetes are regarded by the author as differing from the clamp-connexions of the Hymenomycetes in (1) their physiological significance, (2) their mode of development, and (3) their evolutionary origin.

Throughout the Hymenomycetes and Gastromycetes, the hook of a clamp-connexion grows backwards and not forwards. If the hook of a clamp-connexion were to grow forwards instead of backwards, the terminal cell of each hypha, in which alone growth in length takes place, would periodically (during the formation of each clamp-connexion when a nucleus is temporarily a prisoner in the hook-cell) have its nucleoplasmic ratio upset to the extent of being reduced to one-half, thus affecting growth adversely; whereas, when the hook grows backwards,

as is actually the rule, there are always two nuclei in the terminal cell and the nucleoplasmic ratio is never greatly disturbed and remains relatively constant.

Mlle Bensaude's second mode of formation of a clamp-connexion, based on cytological evidence, finds no support in the author's observations on the stages in the development of living clamp-connexions and is therefore not accepted by him.

The evidence adduced (1) by Mlle Bensaude for regarding certain fusions in the mycelium of *Coprinus lagopus* as *end-to-side* ones and (2) by Hein for regarding the large vascular hyphae of the mycelial cords of *Psalliota campestris* as owing their origin to *side-to-side* fusions of a number of hyphae of smaller diameter is regarded by the author as unsatisfactory.

The problem of the means whereby hyphae taking part in hyphal fusions influence one another at a distance has been discussed but not solved.

Whether or not, in *Botrytis cinerea*, *Corticium solani* (= *Rhizoctonia solani*), etc., heterocaryosis actually comes into existence after hyphal fusions have been formed between the mycelia of two different strains has been left an open question.

**Chapter II.**—The history of our knowledge of protoplasmic streaming in fungi has been reviewed.

Arthur and Schröter stated that in *Rhizopus nigricans*, when streaming is actively taking place in the centre of a hypha in one direction, a thin peripheral sheath-like non-vacuolated layer of protoplasm can sometimes be seen moving in the opposite direction. Andrews held that the protoplasm in a hypha at any one time moves in one direction only and never in opposing directions. The author's observations confirm those of Arthur and Schröter.

The history of our knowledge of the existence of a central pore in each septum of the mycelia and fruit-bodies of Ascomycetes, Basidiomycetes, and Fungi Imperfecti has been reviewed; and, in particular, attention has been called to the work of Wahrlich (1893).

The author has observed protoplasmic bridges passing through the central pore of septa in mycelial hyphae of *Fimetaria fimicola* and *Rhizoctonia solani* (*Corticium solani*).

All the living cells which make up an individual fungus plant are connected together so as to form a single mass of protoplasm. A realisation of this important fact helps us to understand not merely the phenomenon of protoplasmic streaming in the mycelium of Discomycetes, Pyrenomycetes, and Hymenomycetes, but also how it is that a multicellular fungus can develop and react to external stimuli in a unitary manner.

Protoplasmic streaming in septate mycelia has been observed by the author in *Fimetaria fimicola*, *Gelasinospora tetrasperma*, *Pyronema con-*

*fluens*, *Ascophanus carneus*, *Ciboria* sp., and *Rhizoctonia solani* (*Corticium solani*).

The movement of vacuoles through the pores of the septa in the mycelium of *Fimetaria fimicola* has been described.

The maximum observed rate of flow of protoplasm along hyphae of *Fimetaria fimicola* was 6.0 cm. per hour.

In a mycelium of *Pyronema confluens* translocation of protoplasm takes place from hyphae which have ceased to grow toward hyphae which are rapidly growing. The translocation of protoplasm through a main central hypha was watched for 6.5 hours.

In both *Fimetaria fimicola* and *Pyronema confluens* it was observed that protoplasm often flows from one mycelium to another *via* passage-ways which have been formed by hyphal fusions.

In a mycelium of *Pyronema confluens* protoplasm was seen streaming through 161 successive cells, away from hyphae which were becoming exhausted of their protoplasm into a rapidly-growing branched system of hyphae. The length of the stream exceeded 1.6 cm.

In *Pyronema confluens*, the protoplasm, when flowing very rapidly through a highly vacuolated cell, deforms the vacuoles without detaching them from the lateral wall.

The causes of protoplasmic streaming in septate mycelia are (1) vacuolar pressure and (2) increase in the amount of protoplasm.

The evacuation of protoplasm from hyphae becoming exhausted is correlated with the formation of very large vacuoles in those hyphae. In the evacuation process vacuolar pressure plays the chief rôle.

The monoporous septum in the mycelium of a Higher Fungus is comparable with the polyporous septum or sieve-plate in a sieve-tube system of a Higher Plant; and it may well be that the discovery of the means by which colloidal matter is rapidly transported from one part of a mycelium to another may help us to elucidate the means whereby colloidal matter is rapidly transported from one part of a sieve-tube system to another.

The streaming of protoplasm is biologically significant in that it affords a simple means of transferring building materials from one part of a mycelium to another, or from a mycelium to a fruit-body.

In cleared dung-agar at room temperatures the spores of *Pyronema confluens* begin to germinate in about 4 hours, and on this medium the radial rate of growth was found to be 5.4 cm. (approx. 2.1 inches) per day. These facts help to explain how it is that *P. confluens* spreads so rapidly on burnt ground, sterilised soil, etc.

Brownian movements of the particles in the labile flowing protoplasm in cells of *Pyronema confluens* were observed with dark-field illumination.

In *Pyronema confluens*, certain ovoid bodies, called by the author *Woronin bodies*, have been described. They are attached to, and move

irregularly upon, the walls of the vacuoles, but do not enter the stream of labile protoplasm when this is flowing from cell to cell.

In *Pyronema confluens*, *Fimetaria fimicola*, etc., when a cell on one side of a septum is killed with a needle or when it dies, the pore in the centre of the septum is immediately closed by means of a plug of coagulated protoplasm, so that the escape of protoplasm from the living cell through the pore into the dead cell is prevented. At the same time, the septum is bulged outwards from the living cell into the dead cell by osmotic pressure.

In *Pyronema confluens*, one or more cells in a hypha were killed with a needle, with the result that intrahyphal hyphae grew out into them from the septa of the adjacent living cells. Thus intrahyphal hyphae have been obtained under experimental conditions.

Intrahyphal hyphae originating at septa often fuse together in such a way as to repair wounds made in a mycelium.

Protoplasmic streaming in the Hymenomycetes has been observed for the first time in the mycelium of *Rhizoctonia solani* (= *Corticium solani*). Here the labile protoplasm is finely clouded with particles which the eye can detect. Possibly in some Hymenomycetes, e.g. *Coprinus sterquilinus* and *C. lagopus*, in which the protoplasm is very homogeneous, streaming may never be detected.

The biological significance of protoplasmic streaming in the Hymenomycetes is the same as in the Pyrenomycetes and Discomycetes, i.e. it serves to supply protoplasm to rapidly growing hyphae wherever these may be situated.

With the discovery that, in the Hymenomycetes, protoplasm can flow rapidly from cell to cell through the pores of the septa, light is thrown on the means whereby hymenomycetous fruit-bodies in general obtain from the mycelium the nutriment required for their upbuilding and obtain it so rapidly.

Protoplasmic streaming in the Phycomycetes has been compared with that in the Ascomycetes and Basidiomycetes.

The septa formed in the mycelium of *Rhizopus nigricans* are, when fully developed, imperforate. They are never formed across a hypha which is full of protoplasm or in any position where they might be a hindrance to protoplasmic streaming. A septum is formed at the base of a terminal hypha or a system of terminal hyphae only after these have evacuated their massive protoplasm; and the columella-septum is formed only after the sporangium has attained full size and has received all the protoplasm required for the formation of the spores.

Septa in the mycelia of the Higher Fungi and Fungi Imperfecti serve as limits to the adverse influence of injuries to a mycelium. When a cell is killed or dies, the septa protect the adjacent living cells from the effects of the disturbance.

The advantage in each septum of a Higher Fungus or Fungus

Imperfectus being provided with a small pore seems to lie in this: that (1) the pore permits of protoplasm passing readily out of one cell into the next and thus being moved to places where it is needed, while (2) the pore, being very small, can be closed instantaneously when one of the cells adjacent to it is killed or dies.

The time taken for the formation of a septum as an annular ingrowth from the lateral wall of a hypha was found to be: in *Rhizopus nigricans*, 20–25 minutes; in *Rhizoctonia solani*, about 10 minutes; and in a *Ciboria* which grows on male Birch catkins, about 6 minutes.

Newly-formed septa in the mycelium of *Rhizoctonia solani* and of the *Ciboria* that grows on male Birch catkins bulge forward temporarily toward the growing-point, and sometimes, in *R. solani*, in a single hypha, a series of 2–5 of the last-formed septa bulge forward. Through the pore of a bulged septum protoplasm is streaming toward the growing-point of the hypha. The phenomenon of bulged septa affords evidence that, in a hypha, several cells behind the growing-point are manufacturing protoplasm and are constantly pressing it forwards toward the growing-point, thus enabling the latter to extend the hypha rapidly in length.

It is suggested that, in the Hymenomycetes, e.g. *Coprinus lagopus*, when a haploid mycelium is being diploidised by nuclei derived from a mycelium of the opposite sex, the invading nuclei travel through the mycelium from cell to cell *via* the open pores in the middle of the septa and that, contrary to the views of Lehfeldt, the cross-walls do not become broken down.

## PART II

**Chapter I.**—The history of our knowledge of the Sporobolomycetes has been reviewed. The group, according to Derx, contains two genera, *Sporobolomyces* and *Bullera*.

The species of *Sporobolomyces* chosen for study by the author was *S. roseus*.

In their asymmetrical development at the end of an aerial conical sterigma and in their drop-excretion mode of discharge, the spores of *Sporobolomyces roseus* exactly resemble (1) the basidiospores of the Hymenomycetes and the Uredineae, and (2) the secondary conidia, regarded by the author as the true basidiospores, of such Tilletiaceae as *Tilletia* and *Entyloma*.

In *Sporobolomyces roseus* growing on a culture medium, as was determined by continuous watching, a single sterigma may develop two, three, or possibly more spores in succession. This does not occur in the Hymenomycetes, the Uredineae, or the Tilletiaceae.

A yeast cell of *Sporobolomyces roseus*, when growing in a culture medium, may produce (1) a single sterigma or (2) two or three sterigmata in succession. When a single sterigma is produced, two, three, or possibly

more spores may be developed on the end of it in succession. When two sterigmata are produced, each sterigma gives rise to at least one spore, and one of the sterigmata (possibly both) may give rise to two spores in succession. When three sterigmata are produced, each sterigma gives rise to at least one spore.

A yeast cell of *Sporobolomyces roseus*, between the production of two successive spores, may bud off one or two daughter yeast cells.

Sometimes a sterigma of *Sporobolomyces roseus* becomes branched and then, presumably, a spore is produced on each branch.

In *Sporobolomyces roseus*, whenever a yeast cell produces several spores, the spores are developed not simultaneously but in succession.

In *Sporobolomyces roseus*, the yeast cells and the spores which the yeast cells develop on their sterigmata each contain a single nucleus and, up to the present, no stage in the life-history of *S. roseus* has been observed in which two nuclei come together to form a conjugate pair or in which two nuclei fuse with one another. It is possible that *S. roseus* is heterothallic, that so far it has been grown only in the haploid condition, and that the mating of (+) and (−) strains might result in a sexual reaction. It is also possible that *S. roseus* has lost all traces of sexuality.

From the point of view of phylogeny, the most important characteristics of *Sporobolomyces* are: (1) the peculiar shape of the conidium which is due to its possessing an excretory hilum that is developed on one side of the top of the sterigma, (2) the presence of a sterigma of typical conical shape beneath each conidium, and (3) the discharge of the conidium by the drop-excretion mechanism.

The possession of the characteristics just enumerated, which are identical with those concerned with the production and liberation of individual basidiospores in all Hymenomycetes and Uredineae, clearly indicates that *Sporobolomyces* belongs to the Basidiomycetes.

The behaviour of the nuclei in *Sporobolomyces*, as ascertained up to the present, cannot be used as an argument either for supporting or for rejecting the conclusion that *Sporobolomyces* belongs to the Basidiomycetes.

**Chapter II.**—The germination of a chlamydospore of *Tilletia tritici* on culture media, the formation of the promycelium, the development of the so-called primary and secondary conidia, and the development of a saprophytic mycelium have been redescribed and illustrated.

Six variations in the development of the primary and secondary conidia of *Tilletia tritici* have been recorded and discussed.

The germination of the chlamydospores of *Tilletia tritici* is dependent, in a high degree, on the proper ventilation of the air above the substratum upon which they are sown. Among the factors involved in ventilation are the movement of the air and the prevention of saturation of the air with water-vapour. The non-germination or delayed germination of chlamydospores in small closed chambers is not due to insufficient oxygen.

The development and violent discharge of the so-called secondary conidia of *Tilletia tritici* are described in detail. A secondary conidium takes 1·25–1·50 hours to develop from a tiny rudiment to maturity. As soon as it is mature, it is shot away from its sterigma along with a drop of liquid excreted at its hilum. The development and discharge of the secondary conidia of *Tilletia laevis* take place in exactly the same manner as in *T. tritici*.

The authors' theoretical conclusions in regard to the nature of the basidium of *Tilletia tritici*, which differ from those of Brefeld, are stated in the next two paragraphs.

The sickle-shaped so-called secondary conidia of Brefeld and others are the true basidiospores of *Tilletia tritici*.

The long slender so-called primary conidia of *Tilletia tritici* of Brefeld and others are sterigmata of a highly specialised type.

Conjugation in *Tilletia tritici* takes place at an earlier stage than in the heterothallic Hymenomycetes, for the conjugating elements are two haploid sterigmata instead of two haploid mycelia developed from basidiospores.

The *primary basidiospores* of the authors correspond to those sickle-shaped *secondary conidia* of Brefeld which are produced on the H-shaped pairs of his primary conidia; and the *secondary basidiospores* of the authors correspond to those sickle-shaped *secondary conidia* of Brefeld which are produced on a mycelium. A comparison of the authors' newly proposed terminology and of the terminology of Brefeld, as applied to the basidium of *Tilletia tritici* and its products, has been set out in a Table.

The nuclear condition and sexual processes of *Tilletia tritici* have been discussed. The primary basidiospores contain two haploid nuclei and the secondary basidiospores one haploid nucleus. The fungus may be regarded as homothallic in one stage of its life-history and heterothallic in another stage.

The asymmetrical development of the basidiospores of *Tilletia tritici* and their violent discharge with drop-excretion is in exact conformity with what is found in the Hymenomycetes and the Uredineae. This fact strongly supports the view, held by most botanists, that the Tilletiaceae belong to the great group of the Basidiomycetes.

The abnormalities in the discharge of the basidiospores of *Tilletia tritici*, particularly in respect to excessive drop-excretion, are similar to those which have been observed by other workers in the Hymenomycetes, the Uredineae, and the Sporobolomycetes.

The authors' view that in *Tilletia tritici* the secondary conidia of Brefeld are in reality the true basidiospores is supported not only by the mode of development, asymmetrical shape, and mode of discharge of the secondary conidia, but also by a comparison of the basidial apparatus of *T. tritici* with that of other Tilletiaceae, namely, *Urocystis violae*, *Tuber-cinia trientalis*, and *Neovossia molinia*.

A spore-fall method of making pure cultures of *Tilletia tritici* and other Tilletiaceae from basidiospores is described. By means of this method extensive mycelial mats of *T. tritici* may be obtained on agar media within three weeks of inoculation.

The mycelium of *Tilletia tritici*, when growing on an agar medium, if exposed to very dry air, continues to produce and discharge basidiospores whilst the agar is drying up.

Freshly-discharged basidiospores, which have not been allowed to dry, germinate in a film of water or on a nutrient medium to the extent of 100 per cent., and germination begins within about an hour of the discharge of the basidiospores from their sterigmata.

In falling through dry air, basidiospores dry up and in so doing lose their sickle shape, double up, and become pyriform. Basidiospores which have once dried up never germinate.

In the course of three months, the mycelium of *Tilletia tritici*, grown on agar, gave rise to a number of typically rounded and reticulated chlamydospores.

The fall of the basidiospores of *Tilletia tritici* was observed by the beam-of-light method.

The maximum vertical height of basidiospore-discharge was found to be about 1.0 mm. and the maximum horizontal distance 1.4 mm. *Tilletia tritici* discharges its basidiospores to a greater distance than either the Hymenomyces or the Uredineae.

The spore-fall method was employed for inoculating germinating wheat grains with the secondary basidiospores of *Tilletia tritici* and *T. laevis*. A considerable proportion of the inoculated plants yielded bunted heads at maturity. It was thus proved that secondary basidiospores by themselves may cause infection of the host-plants.

The promycelium of *Tilletia tritici* is negatively hydrotropic, but it is neither heliotropic nor geotropic.

The phenomenon of the migration of the protoplasm and the formation of septa in the promycelium of *Tilletia tritici* has been investigated. Each new septum, when first formed, separates a highly vacuolated subterminal cell from the terminal cell which is densely packed with protoplasm. The subterminal cell, however, soon dies and collapses.

**Chapter III.**—The history of our knowledge of *Sphaerobolus* has been reviewed. Descriptions of *S. stellatus*, *S. stellatus* var. *giganteus*, and *S. iowensis*, based on Miss Leva Walker's investigations, have been given. *S. iowensis*, as determined by Miss Walker, has chambered glebae.

Dry discharged glebal masses of *Sphaerobolus stellatus* may retain their vitality for upwards of ten years. When placed in water, their gemmae send out a profuse, radiating, clamp-bearing mycelium.

The glebal mass of *Sphaerobolus stellatus*, at the time of its discharge, consists of a thin brown adhesive outer wall and of a core made up of (1) many cystidia (large rounded or oval cells), (2) tens of thousands of

thick-walled spores, (3) thousands of thin-walled gemmae, and (4) a very tough fatty matrix.

The projectile of *Sphaerobolus stellatus* is very adhesive and its spores and gemmae are not dispersed by either wind or rain.

The structure of the *Sphaerobolus stellatus* fruit-body has been redescribed with the aid of photomicrographs and drawings. The peridium, as found by Miss Walker, consists of six layers: (1) mycelial hyphae surrounding the exterior of the sporocarp, (2) a gelatinous layer, (3) a pseudoparenchymatous layer, (4) a fibrous layer made up of interwoven largely tangential hyphae, (5) a palisade layer becoming pseudoparenchymatous over the top of the sporocarp, and (6) a thin layer of pseudoparenchyma surrounding the gleba.

The fruit-body of *Sphaerobolus stellatus* opens stellately. The rim of the cup, owing to continual expansion of the highly turgid palisade layer, gradually increases in diameter and bends outwards. When a certain stage in the process has been reached, the palisade layer and the fibrous layer, which act as everting membranes, yield to the strain which has been set up and suddenly turn inside out, thus slinging the glebal mass away. After discharge of the glebal mass, the everted membranes have a balloon-like appearance; and they are usually left standing over the cup, attached to its teeth, and covering the orifice.

The force which brings about the discharge of the *Sphaerobolus* gun is located in the inner everting membranes; for, if these are removed from the outer non-everting membranes and are placed on a moist surface in a damp-chamber or are submerged in water, they evert and cast away the projectile.

The expansion of the palisade layer, which leads eventually to the eversion of this layer and of the fibrous layer, is caused by the lateral swelling of the individual palisade cells. During this swelling, the glycogen in the cells is converted into sugar.

When from an opened fruit-body the glebal mass is removed and in its place is substituted a small pebble, a lead shot, a ball of paraffin wax, or a drop of mercury, these objects are eventually cast out of the fruit-body in the same way as the glebal mass would have been.

The tooth-tip mode of attachment of the inner everting set of membranes to the outer non-everting set of membranes is such that the inner set of membranes, when everting, is given the maximum possible *working distance* for pressing against the projectile. It is this long working distance—about 3 mm.—which in a large degree is responsible for the high efficiency of the *Sphaerobolus* gun.

The diamond-shaped openings which are formed laterally between the everting and the non-everting sets of membranes as eversion takes place allow air to rush into the central cavity and so prevent an internal vacuum coming into existence and interfering with the efficient working of the gun.

The outer non-everting set of membranes is of importance for the working of the *Sphaerobolus* gun in that it holds fast the inner everting set of membranes by its teeth as eversion takes place and so prevents the inner set of membranes following after the projectile and interfering with its flight.

When discharge of the *Sphaerobolus* gun takes place, along with the projectile there are shot away a few drops of slimy liquid derived from the deliquescence of the peridial layer immediately surrounding the gleba.

A projectile, on striking a hard object near the gun from which it has been discharged, flattens out like a leaden bullet and becomes plano-convex.

The *Sphaerobolus* gun emits a distinct sound as it shoots away its projectile. It is the largest, the most powerful, and the loudest of all fungus guns.

The maximum height and the maximum horizontal distance to which projectiles of *Sphaerobolus stellatus* were expelled were : (1) for wild fruit-bodies growing on a board, 7 feet 9 inches and 15 feet respectively ; and (2) for wild fruit-bodies growing on cow dung, 7 feet and upwards (limit not determined) and 18 feet 7 inches respectively. Miss Leva Walker, who worked with a pure horse-dung culture of *S. stellatus*, observed that the projectiles were shot to a maximum height of 14 feet 5 inches and to a maximum horizontal distance of 17 feet 3 inches.

The fruit-body of *Sphaerobolus stellatus* easily outranges any phanero-gamic fruit of its own size.

The kinetics of the *Sphaerobolus* gun have been investigated and the following calculations have been made.

If a projectile is shot 7 feet high, the projectile has an initial velocity of upwards of 21 feet per second and takes upwards of 0.65 second to reach its highest point.

If a projectile is shot 14.5 feet high, the projectile has an initial velocity of upwards of 30 feet per second and takes upwards of 0.95 second to reach its highest point.

The time taken in the discharge of the gun is : (1) if the gun shoots the projectile 7 feet high, about  $\frac{1}{10.66}$  second ; and (2) if the gun shoots the projectile 14.5 feet high, about  $\frac{1}{15.24}$  second.

The momentum of a projectile shot 14.5 feet high, when leaving the gun, is about 0.00008 inch.

The kinetic energy of a projectile shot 14.5 feet high, when leaving the gun, is about 0.000037 foot pounds.

The average force of the propelling peridium, when acting on the projectile, is 0.13 poundals or 0.004 pounds.

The rate of developing energy in a *Sphaerobolus* gun which shoots a projectile 14.5 feet high is  $\frac{1}{10.000}$  horse power.

Without a sufficient supply of water, the *Sphaerobolus* gun can neither open stellately nor, if opened stellately, discharge its projectile.

Unopened fruit-bodies can withstand desiccation for several months. The projectile is resistant to the disintegrating action of rain-water.

Light acts on *Sphaerobolus* in several different ways : (1) it is essential for fruit-body formation, (2) it causes the fruit-body to develop so that its apex faces the strongest incident rays of light, (3) it causes the fruit-body to open in the day instead of at night, and (4) it increases the vigour with which a fruit-body discharges its projectile.

*Sphaerobolus* has been found not only on wood but also on the dung of elephants, horses, cows, hares, and rabbits. The fungus therefore is not only xylophilous but also coprophilous.

At Winnipeg *Sphaerobolus stellatus* has been found in the months of October and November growing and fruiting freely on old cow-dung plats in pastures.

It is a remarkable fact and an illustration of parallelism in the course of organic evolution that in *Pilobolus*, *Ascobolus immersus*, and *Sphaerobolus*, which belong respectively to the Phycomycetes, the Ascomycetes, and the Basidiomycetes—the three great divisions of the Fungi proper—the sporocarps are either converted into guns directly (*Pilobolus*, *Sphaerobolus*) or else produce guns (*Ascobolus immersus*), which have many characteristics in common, namely, (1) massiveness and parabolic trajectory of the projectile, (2) great violence in the discharge of the projectile, (3) adhesiveness of the projectile, (4) heliotropism of the guns, (5) multispore nature of the projectile and non-separation of the spores whilst the projectile is attached to herbage, and (6) occurrence of the gun-developing fruit-bodies on the dung of herbivorous animals. These characteristics are correlated, for they are all associated with the dispersion of the spores by herbivorous animals.

So far as the arrangements for securing the dissemination of the spores are concerned, coprophilous fungi may be divided into two groups : (1) a more primitive group which successively makes use of three external agents—the wind, flowering plants, and herbivorous animals—and (2) a more highly specialised group which dispenses with the wind and which successively makes use of only two external agents—flowering plants and herbivorous animals. To the first group belong *Aleuria vesiculosa*, *Ascobolus stercorarius*, *Stropharia semiglobata*, and *Coprinus sterquilinus* ; while to the second more specialised group belong *Pilobolus*, *Ascobolus immersus* and *Sphaerobolus*.

The manner in which *Sphaerobolus* comes to infect wood has been discussed. Wood may become infected (1) by means of glebal masses shot on to it from fruit-bodies growing on another piece of wood or on dung or (2) by mycelium growing on to it from infected dung. It also seems possible that wood may become infected by spores, or possibly gemmae, which have passed through the alimentary canal of some herbivorous animal and have then been transferred to wood by dung-flies.



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