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# TRANSACTIONS

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# WISCONSIN ACADEMY

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## SCIENCES, ARTS, AND LETTERS

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# CONTENTS

# STUDIES ON THE TREMELLINEAE OF WISCONSIN.

E. M. Gilbert.

The *Tremellineae* have so far been little studied in any of the Mississippi Valley States. In the following list I have brought together the material collected by myself and others in Wisconsin as a preliminary contribution to our knowledge of the species of this region. The list is of course far from complete and without a doubt many additions to it may be expected in the near future. The identification of the species is especially difficult, but it is a necessary preliminary to a further physiological and cytological study of the group. The classification used is based upon that of Fries as somewhat rearranged by Winter.

Fries's technical description of the group is as follows: "Entire fungus homogenous, gelatinous, collapsing when dry, regaining its form when moistened, traversed internally by branched hyphae which terminate in basidia at the periphery; basidia variable in form, elongate or fusoid; transversely septate or continuous, undivided or with the apex forked, or sub-globose and cruciately divided and bearing two or four sterigmata; spores hyaline, globose, ovoid or kidney shaped, continuous or septate, often becoming variously septate on germination and producing sporidiola (conidia) of various forms.

The basidia which of course are the characteristic feature of the group, are, as is well known, of several quite distinct types.

In *Auricularia,* the basidia are essentially like the promycelium of a rust. They are transversely septate, each cell producing near or at its apex a single sterigma. A second type occurs in *Dacrymces* and *Guepinia* where the basidium is more

or less cylindrical and forked at the apex, each branch ending in a sterigma bearing a single spore. A third type occurs in *Tremella* where the basidium at first appears as a sub-globose or pear shaped body terminating a hypha. This oval cell divides into four by longitudinal septa placed at right angles to each other. Each of these cells produces an apical sterigma bearing a spore. The spores are always hyaline or colorless, and this is the only family included among the *basidiomycetes* in which some of the species are regarded as having septate spores.

The spores in most cases, according to Brefeld produce in germinating characteristic secondary spores, sporidioles. Brefeld has also shown that the production of these conidia is not at all uncommon among various members of the family. In *tremella lutescens* for example, these are produced on peculiar branches in the substratum of the carpophore, while in *Guepinia* they are produced on the side opposite the hymenium.

The *Tremellineae* are also characterized by their more or less gelatinous consistency. In *Tremella* the main genus, the substance is in some species so tender as to lose its form and almost deliquesce on being handled, while such forms as *Auricularia,* have a coriaceous texture and many of them retain their shape even when dry. This peculiarity is due to the fact that the external portion of the walls of the very much interwoven hyphae are diffluent, forming a soft quaking mass when moist but hard and horny when dry, but again becoming softened upon being moistened.

In some forms such as *Dacrymyces,* the hymenium may cover the entire exposed surface of the fungus while in the higher forms it is often confined to a portion only of the surface and in some this portion has a tendency to turn away from the light.

The group was early observed but the evanescent and fluctuating character of the forms made it impossible for any of the earlier authors to group them satisfactorily.

Vaillant (1), Micheli (2), Dillenius (3), and Gleditsch (5), all mention the *Tremellineae* in their classifications, but it is a matter of uncertainty after all whether any of their identifications can be recognized now.

Linnaeus in his *Species Plantarum* (8) names seven species for the group of which only one is now included in it.

Schaeffer (14) does not mention the group as such, but in his group *Elvella*, he describes a form seemingly a tremella, possibly *Tr. sarcoides.* This form and a few others described by him seem to be tremellas, but their specific identity is not to be determined with certainty.

Persoon (23) in his *Synopsis Fungorum* may be said to be the first to make a fairly practicable classification of the group. He includes the *Tremellineae* in his second class of Fungi (*Gymnocarpii*) and the fifth order, the *Hymenothecii* which he divides into six suborders the last of which the *Helvelloidei*, include *Spathularia, Leotia, Helvella, Morchella, Tremella, Peziza, Ascobolus, Helotium, Stilbum* and *Aegerita.*

Persoon is the first to exclude all aquatic forms and in this he is far in advance of his contemporaries, who all included *Nostoc* forms with the *Tremellas*, but on the other hand, his inclusion of the group with the ascomycetous genera noted shows how little he had on which to base a natural classification. In his *Mycologia Europaea* (33a), he rearranges and modifies his earlier grouping. The Tremellas are here put in the second order and co-equal with *Mycoderma, Thelephora* and *Clavaria.* He separates the *Auricularias* from the *Tremellas* and puts them into a genus by themselves. The descriptions are still very meager, but he gives more references to the literature of the group. No figures are given.

*Albertini et Schweinitz* (28), base their classification upon the earlier work of Persoon, and describe additional varieties of certain of Persoon's species. The descriptions are, however, fragmentary and their figure of a *Tremella,* (Tr. saligna nobis Tab. IX. f. 7) is of no value for identification.

Bulliard (30), makes the *Clavarias* and Tremellas the second of his four orders of Fungi, but errs in including some aquatic forms with them.

Bulliard's colored figures of many of the forms are most excellent. *Tremella mesenteriformis* (*Tr. frondosa* Fr.) is infinitely more easily identified from his figures than from the

descriptions given by Fries.   Our specimens correspond both in structure and color very closely to those figured by Bulliard. There seems to me to have been no adequate reason why Fries should not have retained the name mesenteriformis for the forms to which he gave the name frondosa.   Our specimens are strikingly characterized by the name mesenteriformis.

His figure of *Tr. deliquescens* is a fairly good picture of our Wisconsin specimens of *Dacrymyces deliquescens* and the same may be said of his figure of *Tr. glandulosa* as representing our *Exidia glandulosa* (Bulliard) Fr.

Bulliard's *Tr. cerebrina* (*Tr. albida,* Huds.) if not so tall would fairly well represent the forms found here which I have identified as *Exidia albida* Bref.   The figure of *Tr. auricula* Judae is also very good for our specimens.   His figure of *Tr. mesenteriformis* var. *violacea,* (*Tr. foliacea-violacea* Fr.) looks much more like smaller, denser forms which I have included with *Tr. frondosa* Fr., than like the forms I have included under *Tr. foliacea* var. *purpurascens* which are still much more compact and have thicker folds.   They more nearly resemble light colored forms which I have included under *Tr. frondosa,* (though doubtless the same as those identified by Fries as *Tr. lutescens* Pers.).

Here again Bulliard's conception of the specific delimination of the main species which he calls *Tr. mesenterica* seems much better than that of Fries.

In the *Systema Mycologicum* (30) of the elder Fries, we find the classification which has been used as the basis for all later work.   We find the *Tremellas* for the first time brought together in these genera; *Tremella, Exidia, Femsjonia, Hirneola, Naematelia, Guepinia* and *Dacrymyces.*

Later authors have largely accepted these genera as Fries delimited them.   It is to be noted, however, that even in this later work the *Caloceras,* are not included among the *Tremellineae.*

The American students of the fungi have done little with the *Tremellineae,* but the commoner species have been reported in most of the local floras which deal with fungi.   It is most unfortunate that in most cases, neither specimens nor figures of

the forms collected by Schweinitz (34) are available for study. It is to be remembered that the group was imperfectly under-stood at that time, and it seems doubtful whether the Schwein-itzian species will be identifiable in many cases.

Tulasne, (53) studied further the internal structure of the carpophore and the hymenium, and it is to him that we are in-debted for the earliest data as to the germination of the spores. His figures have been widely copied. He figures *Tr. mesen-terica, Ex. spiculosa, Tr. violacea,* and *D. deliquescens* in his first papers (53), and in his second series (62) he figures fur-ther *Guepinia peziza* and gives three sketches of *Tr. cerasi* and one of Pilacre.

Paulet et Leveille (54a) give a few colored figures, but they are difficult to identify. Their figure of *Tremellodon* may pos-sibly be our species *gelatinosum.* Their *Tr. mesenterica,* Mur-ray. Pl. III, fig. 5 can hardly be a *tremella.* Their *Tr. hyd-noides* Jacquin Pl. III, fig. 6–7 is probably our *Tremellodon gelatinosum.* Their *Tr. undulata* Pl. CLXXXVI, fig. 3, they identify with *Tr. mesenterica* Pers. They also figure *Tr. sar-coides* Fr. Pl. CLXXXVII. fig. 5.

Gillet (68) gives excellent colored figures of *Tr. mesenterica* Pers. and *Tr. lutescens* Retz. They represent, however, much larger forms than any I have found in Wisconsin.

Winter (90) reproduces for the most part Tulasne's figures for the types of the genera. A good proportion of the species reported from America are described in Winter's flora.

Brefeld, (91) spent years of careful study on the germina-tion of the spores and the formation of the mycellia and conidia of the group, and his work has since been authoritative on these points. Brefeld makes the *Tremellineae* of Fries and De Bary the basis of his group of *Protobasidiomycetes,* but includes *Dacrymyces* with the *Autobasidiomycetes.* Still in my opinion the similarity of their fruiting bodies in form and consistency and their evident relationship to the other members of the group are sufficient justification for keeping *Dacrymyces* with the *Tremellineae.*

Bresadola, (110) gives a colored figure of *Tr. foliacea* Pers.

Tab. 209, Fig. I, which, however, is very much like forms I have included with *Tr. frondosa* and very much more leaf like and larger than the forms of foliacea found by me.

Möller, (118) accepts Brefeld's classification and adds to the group, as a result of his work on tropical forms, a large number of new genera. His figures of *Tr. fuciformis* well represent our specimens of this species. His figure of *Tr. undulata,* however, is very much like loose, open forms of what I have called *Tr. frondosa.*

Arthur (136) has proposed to use the name *Tremella* for the *Gymnosporangia,* but it remains to be seen whether a name so well established in its present usage can be thus transferred.

Atkinson's (142) figure of *Tr. frondosa* is a good representation of forms which I have found here. He describes the specimens as pinkish yellow in color, but our forms are purplish to deep wine color. Our yellowish forms are much more compact, relatively taller, and much smaller. Atkinson's figure of *Tr. fuciformis* is also a fair representation of the coarser, bulkier, forms found here which I shall follow Farlow in identifying as *Tr. reticulata* (Berk) Farlow. Atkinson (150) describes the basidia of *Thelephora Schweinitzii* as of the *Tremella* type and proposes to make for it the new genus *Tremellodendron* to include also *Thelephora pallida* Bres.

*Thelephora Schweinitzii* is a very common fungus in this region. It frequently shows a yellowish incrusting hymenial layer about the base with the basidia of a *Sebacina* or *Exidiopsis.* Whether this hymenium does not belong to a parasitic Exidiopsis form is a question which in my opinion needs further investigation. For the present I have not included this form with the *Tremellineae.* In this connection *Ceracea vernicosa Cragin,* must also be further studied. This is reported as forming incrusting masses on immature specimens of *Polyporus.*

Massee (160), makes a curious statement that he has not been able to find a true septum in the sense of a plate separating the substance of the apex of the basidium in *Tremella,* but considers that the cross marking present at the apex of the basidium

is simply due to the first bulging out of the stout bases of the four sterigmata. This off-hand and careless method of disputing facts, established by so many earlier authors and which as I have myself observed can be confirmed with the greatest readiness, is very surprising.

My collections were made in the region of Madison, Blair and Lake Superior, from 1906 to 1909. Considerable material, already in the herbarium of the University of Wisconsin was also available.

With the list of the species, I have included rather copious notes since the forms are so variable and evanescent that their identification is a matter of great difficulty. The technical descriptions of genera and species are largely those of Fries as modified by Winter.

## Lists of Species by States.

I find the number of Tremellineae reported from the United States is small and from a number of states I do not find any at all in the literature that I have examined.

The following lists are the most complete I have found:

Farlow (80) Mass.
   *Dacrymyces stillatus* Fr.
   *Calocera cornea* Fr.
   *Exidia glandulosa* Fr.
   *Guepinia spathularia* Fr.
   *Tremella aurantia* Schw.
   *Tremella foliacea* Pers.
Farlow (86) Vt.
   *Tremella aurantia* Schw.
   *Calocera viscosa* (S) Peck.
   *Hirneola auricula-Judae* (L.) Berk.
Bundy (67) Wis.
   *Tremella albida* Huds.
   *Tremella fimbriata* Pers.
   *Tremella lutescens* Fr.
   *Tremella mesenterica* Retz.
   *Dacrymyces stillatus* Nees.
   *Exidia glandulosa* Fr.
   *Auricularia mesenterica* Bull.

Johnson (77a) Minn.

 *Tremella fimbriata* Pers.
  "  *frondosa* Fr.
  "  *foliacea* Pers.
  "  *lutescens* Fr.
  "  *mesenterica* Retz.
  "  *vesicaria* Bull.
  "  *albida* Huds.
  "  *intumescens* Sow.
  "  *indecorata* Somm.
  "  *tubercularia* Berk.
  "  *torta* Willd.
  "  *epigaea* B & Br.
 *Exidia glandulosa* Fr.
 *Hirneola Auricularia-Judae* Berk.
 *Dacrymyces stillatus* Nees.

Morgan (119) Ohio.

 *Tremella foliacea* Pers.
  "  *lutescens* Fr.
  "  *mesenterica* Retz.
  "  *intumescens* Sow.
  "  *vesicaria* Bull.
  "  *albida* Huds.
 *Exidia truncata* Fr.
  "  *glandulosa* Fr.
 *Naematelia nucleata* Fr.
 *Guepinia spathularia* Schw.
  "  *elegans* B & C.
  "  *peziza* Tul.
 *Dacrymyces fragiformis* Nees.
  "  *deliquescens* Bull.
  "  *stillatus* Nees.
  "  *chrysocomus* Bull.
  "  *pellucidus* Schw.
 *Hirneola aur.-Judae* Linn.
  "  *auriformis* Schw.
 *Calocera palmata* Schum.
  "  *cornea* Batsch.
  "  *stricta* Fr.

Herbst (133) Pa.

 *Tremella lutescens* Fr.
  "  *mesenterica* Retz.
  "  *vesicaria* Bull.
  "  *violacea.*

*Exidia glandulosa* Fr.
*Hirneola aur.-Judae* Berk.
*Guepinia spathularia* Schw.

By far the most complete list of the Tremellineae for any state is that by Peck for New York. Peck figures *Tr. myce-tophila* and *Tr. stipitata.* He has recognized only seven of the species of *Tremella* reported by Schw. and none of these are Schweinitz' new species. Of the *Exidias* Peck lists three; one of which is a *Schweinitizian* species. Peck describes three new species of *Dacrymyces* but finds none of the new species of *Schw.* Peck lists three species of *Naematelia,* one of them new; *N. atrata.* Of *Ditiola* he lists the common species *D. radicata.* Peck has three species of *Calocera,* one species of *Pilacre* and three of *Guepinia.* It is of interest to note the large number of *Schweinitzian* new species which Peck has failed to find.

Peck's complete list is as follows:

*Calocera cornea* Fr.  Vol. 24–82.
  ”    *palmata* Schum.  Vol. 24–82.
  ”    *viscosa.*  Vol. 24–82.
*Dacrymyces tortus* Fr.  Vol. 22–88.
  ”    *stillatus* Fr.  Vol. 22–88.
  ”    *fragiformis* Nees.  Vol. 27–101.
  ”    *conglobatus* Peck.  Vol. 32–37.
*Ditiola radicata* Fr.  Vol. 27–101.
  ”    *conformis* Karst.  Vol. 43–70.
*Exidia auricula-Judae* Fr.  Vol. 22–88.
  ”    *glandulosa* Fr.  Vol. 22–88.
  ”    *truncata* Fr.  Vol. 22–88.
  ”    *cinnabarina* B & C.  Vol. 22–88.
  ”    *repanda* Fr.  Vol. 24–83.
*Guepinia spathularia* Fr.  Vol. 24–80.
  ”    *helvelloides* D. C.  Vol. 29–45.
  ”    *peziza* Tul.  Vol. 31–39.
*Naematelia nucleata* Fr.  Vol. 24–83.
  ”    *atrata* Peck.  Vol. 24–83.
  ”    *cerebriformis* Ellis.  Vol. 30–49.
*Pilacre faginea* Fr.  Vol. 26–79.
*Tremella aurantia* Schw.  Vol. 22–88.
  ”    *mesenterica* Retz.  Vol. 22–88.

    "     *sarcoides* With.  Vol. 22–88.
    "     *foliacea* Pers.  Vol. 24–80.
    "     *albida* Huds.  Vol. 25–83.
    "     *colorata* Peck.  Vol. 25–83.
    "     *frondosa* Fr.  Vol. 26–72.
    "     *enata* B & B.  Vol. 27–100.
    "     *stipitata* Peck.  Vol. 27–100.
    "     *vesicaria* Bull.  Vol. 28–53.
    "     *mycetophila* Peck.  Vol. 28–53.
    "     *intumescens* Sow.  Vol. 30–49.
    "     *lutescens* Pers.  Vol. 31–39.
    "     *subcarnosa* Peck.  Vol. 32–36.
    "     *epigea* B. & Br.  Vol. 34–43.
    "     *subochracea* Peck.  Vol. 34–43.
    "     *pinicola* Peck.  Vol. 39–44.

## TREMELLINEAE OF WISCONSIN.

*Auricularia*—Cartilaginous, gelatinous, soft, tremulous, when wet, but not distended with jelly; when dry, coriacious, horny, reviving again when moistened but scarcely swelling. Hymenium of basidia longitudinally four parted, spores continuous, oblong, curved.

   *Auricularia sambucina* Martius (Flora erlang. p. 459).
   *Tremella Auricula-Judae* Linn. Sp. 1625. p. 1.
   *Peziza Auricula* Linne (Syst. veg. Ed. XV. p. 1018).
   *Merulius Auricula* Roth (Tentanem florae Germ. I, p. 535).
   *Exidia Auricula-Judae* Fries (Systema. II, p. 221).
   *Hirneola Auriculae-Judae* Berk.  (Outlines p. 289).
   *Hirneola Auricula-Judae* Fries (Hym. Eur. p. 695).
   *Auricularia sambucina* Bref. (Unters. VII. t. IV, f. 3–9).

1. *Auricularia auricula*—Judae Linn.  Sp. 1625.  Pl. I. fig. 1.

Carpophore cartilaginous-gelatinous, soft and tremelloid when moist, cup shaped, rigid when dry, reviving when moist.  Basidia rod shaped, or fusoid, transversely septate, cells each bearing a single monosporous sterigma; spores oblong, curved.  Hymenium venoso-plicate: pileus greyish olive brown, minutely tomentose, spores 20–25$\mu$ by 7–9$\mu$, Carpophore up to 12 cm. in diameter.  Common.

Specimens have been found differing somewhat in the color of the pileus and also in size of the spores. Many are very light brown when moistened and dry down to a much darker brown. Quite often they are found growing in tufts but usually singly scattered along the length of the branches of oaks and other deciduous trees.

2. Var.? Pl. 1. Fig. 2: A form with very much the same general shape as above, but about half the size, having a deep rich wine colored upper surface and an almost black velvety under surface and the whole very much thickened throughout. I was unable to secure spores from this form and so cannot say positively whether it is a new variety, although I feel confident that it is not an immature form of the common Jews ear.

Found on decaying branches of oak. Madison.

*Tachaphantium* Bref. (Unters. VII p. 78)

3. *Tachaphantium* sp? Figs. 3-4. This is a form which seems to belong in Brefelds genus *Tachaphantium* but I find no species to which it can be assigned. It may be described as follows: Small, ½ cm. by 1/3 cm. of a crystalline yellowish appearance when moist and shrinking only slightly upon drying. Stipe about ½ the diameter of the upper portion and slightly darker in color. The hymenial layer is rounded hemispherical and glistening powdery. Basidia like those of *Auricularia Auricula–judae* and spores of about the same shape but slightly smaller. On decaying stump of larch. Madison.

*Exidia.* Fries (Systema II. p. 220).

Fungus distended with jelly, tremulous, somewhat marginate, papillose; a colored gelatinous stratum encloses the basidia the sterignata of which are erumpent only at the apex; spores curved.

4. *Exidia glandulosa* Bulliard. Pl. Figs. 5-6.

Flattened, thick, gelatinous, becoming blackish, disc, covered with minute papillae, below greyish and subtomentose; spores reniform 12 x 14μ by 4 x 5.

This form is found forming very irregular patches on decaying stems and branches of many of our more common deciduous

trees, the patches varying in size from 3 cm to 20 cm in diameter. When moistened they have a dirty blackish color and the papillae are very prominent; when fully mature it dries down to a hard shining black mass, forming a thin crust and now shows nothing of its previous form.

Most of the specimens collected have been found on hickory, although specimens have also been found on elm, poplar, and cherry. Common, Madison and vicinity.

### 5. *Exidia saccharina fries* Systema II p. 225.

*Ulocolla saccharina* Bref. (Unters. VII p. 95).
*Ulocolla saccharina* Massee (Fungus Flora p. 59).
*Ulocolla saccharina* Sacc. (Syl. Fung. VI p. 777).
Tuberculose, effused, thick, gyrose and undulate, fulvous, cinnamon, here and there papillose, spores reniform $10-12\mu$ by $5-5\mu$. Conidia, equal in size to the spores.

Brefeld gives the following description of this species:

Carpophore convex, pulvinate, gyrose, cerebriform, gelatinous, basidia, globose, soon longitudinally or obliquely cruciately partite, sterigmata elongated, thick; spores for a long time continuous, then once septate, reniform, each locule on germination giving origin to a very short promycelium, bearing at its apex a crown of straight, rod-like sporidiola.

Specimens in collection in such poor condition that the identification is doubtful. On fallen pine trunk, Madison.

### 6. *Exidia albida* (Huds.) Bref. Fig. 7.

*Tremella albida* Huds. (Flora anglica II. p. 565.)
  *Tremella hyalina* Pers. (Myc. Eur. I. p. 105.)
  *Tremella albida* Fr. (Hym. Eur. p. 691.)
  *Tremella albida* Engl. Bot. t. 2117.)
  *Tremella albida* Kickx (Flora p. 102.)
  *Tremella albida* Winter (Krypt. Flora p. 287.)
  *Tremella cerebrina* alba Bull. (Champ. t. 386.)
  Hard (158) gives a figure of this form.

Gelatinous, expanded, undulate, white, becoming brownish with age and pruinose with the white spores. Spores oblong, slightly curved, apiculus at end 12–14$\mu$ long and 5–7$\mu$ in diam.

The form found in great abundance with us, and which answers very well to this description is one of the largest and most beautiful of the group. I have found it making a creamy white mass covering a log in patches 30 cm. and more in length by 10–15 cm. in width and reaching a height of from 3–6 cm. Woods near Blue Mounds, Wis.

### 7. *Tremella lutescens* Pers. Fig. 8.

*Tremella lutescens* Pers. (Syn. p. 622.)
*Tremella mesenteriformis* Bull. (Champ. t. 406 Band D.)
Very soft and gelatinous, lobes crowded, entire, undulately gyrose, pale then yellowish, spores sub-globose 12–16$\mu$, conidia. 1.5–2$\mu$ diam.

*Tremella lutescens* and *mesenterica,* are in many instances very much alike, but can be distinguished by the fact that *mesenterica* becomes paler as it grows older while *lutescens* becomes more yellowish. *Tr. lutescens* is not as firm as *Tr. mesenterica* as a usual thing, but specimens may be found of *lutescens* which dry down into quite a firm mass retaining the folds to the last.

*Mesenterica* is more brainlike in its folds and *lutescens* more mesentery like.

Common Madison and vicinity.

### 8. *Tremella mesenterica* Retz. Fig. 9–10.

*Elvella mesenterica* Schaef. (Ic. Fung. taf. 168.)
*Tremella chrysocoma* Bull. (Champ. taf. 174.)
*Tremella auriformis* Hoffm. Veg. Crypt. I p. 51.)
Gelatinous but firm, bright orange yellow, variously contorted; lobes short, smooth, pruinose with the white spores at maturity; spores broadly elliptical 6–9$\mu$ diameter, conidia 1–1.5$\mu$.

Found usually on dead poplar, and varies greatly in form but is usually distinguishable by its bright orange color, and dries down into irregular patches which become more transparent and watery in appearance. Specimens vary in size from 1 cm to 4 cm across and 1–2 cm in height.

Common, Madison and vicinity.

### 9. *Tremella foliacea* Pers. Fig. 11.

*Tremella succina* Pers. (Myc. Eur. I. p. 101.)
*Tremella ferruginea* (Engl. Bot. taf. 1452.)
*Tremella mesenteriformis* Bull. (Champ. tab. 406 A.)
*Tremella violascens* Alb. et Schw. (Sonsp. Fung. p. 305.)

Tufted and much lobed and waved, segments thin, springing from a plicate base; color variable, diaphanous, pinkish cinnamon, rarely deep brown or even violet; spores reniform 10–12 by 5–6$\mu$; conidia like spores. Substance soft gelatinous, at first rounded but gradually becoming very irregular.

It is to be noted that this differs from the true tremellas as arranged by Brefeld in that the spores are different in shape and the conidia much larger. This species with *Tr. saccharina* is so variable in color and in appearance at various times that there is no doubt in my mind that these two forms with *Tremella frondosa* are often mistaken for one another.

Specimens are found on many large trees reaching in diameter from 2–10 cm and from 2–5 cm in height. The form is described as being thin but that is not always the case as some specimens have the lobes quite thickened. Many of the specimens approach more nearly the variety *violescens of Alb. et Schw.* in being smaller, thicker and of a general purplish red and even violet color.

This form is more often found on oak, alder and birch. Common. I do not think that the term *foliacea* can be regarded as descriptive since a specimen is rarely found which can be said to be foliaceous in appearance.

### 10. *Tremella Frondosa* Fr. Figs. 12-13-14.

*Tremella frondosa* Fries (Systema II p. 212.)

    *Tremella mesenteriformis* Bull.  (Champ. t. 499, f. T.)

    *Tremella quercina Pollin.*  (Pl. nov. p. 31.)

    *Tremella undulata* Hoffm.  (Veg. Crypt. I t. 7, f. 1.)

    *Naematelia* Bonorden (Handb. Myk. p. 152.)

Gelatinous, tufted, large; lobes undulate and contorted, smooth, (not corrugated); base firmer, plicate; pale pinkish yellow; spores sub-globose, apiculate, $7-9\mu$.

The above description is hardly adequate in that it does not make enough allowance for the variations in this the finest of all *Tremellas*.  Its color variation is more marked than that of other *Tremellas*.  I have found specimens of a beautiful yellow untinged by any other color, again specimens are found with a pinkish tinge to yellow, some vinaceous pink and others creamy buff; still others are a rich wine color running almost into a reddish brown.

The yellow forms are nearly always smaller, but this may be because I have found them on smaller branches.  One deep wine colored specimen formed a massive tuft at least 25 cm in length and at least 10-12 cm high.  *Tremella frondosa* seems to prefer the oak although found on other trees.

The various types of *frondosa* dry down in many cases retaining their shape, but becoming very much smaller and of a hard, tough consistency.  The term *frondosa* is no more description for this form than the term *foliacea* for the last as very few if any forms are *frondose* in nature.

Not very common.

### 11. *Tremella*—species undetermined Fig. 15.

Small, soft, cartilaginous-gelatinous, irregularly ear shaped, pure white when wet, turning to a light yellow color and shrinking greatly when dry.  Hymenial layer on both surfaces, spores ovoid, small; carpophore when expanded from 1.5-2.5 cm.

Few specimens found on oak, Blue Mounds, Wis.

12. *Tremella reticulata* (Berk) Farlow, notes on Fungi, Rhodora, '08, p. 10.

*Tremella fuciformis* Berk. (Outl.) Berkeley's description is too brief to be of value. Atkinson has described and figured what he identifies as *Tr. fuciformis* Berk. as follows: "This is a very beautiful white *tremella* growing in woods on leaf mold close to the ground. It forms a large white tubercular mass resting on the ground, from the upper surface of which numerous stout, short, white processes arise which branch a few times in a dichotomous manner."

This description agrees very well with what is perhaps the commonest form of our specimens. Our forms are, however, quite variable as to size, degree of branching and character of the tips. They may be thrown roughly into the following groups:

Form a. fig. 17. This is the typical and most abundant form and it agrees well with the description given by Atkinson. The masses are 10–15 cm. in diameter and nearly or quite as high. The flesh is very soft, and the parts are more or less hollow. The basidia are like those of the genus, globose, sunk in the substance of the plant, and terminate with four long slender sterigmata which rise to the surface and bear the spores. The spores are nearly ovoid, but inequilateral and somewhat reniform, continuous 7–9 by 5–6$\mu$.

Atkinson says the fungus is not very common, but I have found this form rather common about Madison and Blue Mounds.

Form b. Figs. 19–20–21 show what seem to me to be more complex forms, but still having the essential characteristics of the type.

These specimens were found in the same localities as form a. It is to be noted that these forms branch repeatedly and that the branches or nearly all of them seem to end in very short fimbriate tips. They do not show any of the yellowish or brownish color sometimes found in mature specimens of form a. All the specimens are of a rich creamy whiteness. These forms seem

to be most like those described by Farlow. Common in the woods of Madison and vicinity.

Form c. fig. 22 forms an urn like mass on old stems of bracken. It has a gelatinous-cartilaginous consistency with basidia deeply imbedded in the substance and of the typical tremella type. The fungus was attached to the stipe of the fern at the base but was free at the top forming a distinct cup. Its color was a pure white much clearer than that of form a. It was unbranched and entire except for a few large wave like folds. The whole had a diameter of about 3 cm and a height of about 5 cm. Spores were white, oblong. Found in dense woods, Blair, Wis., August, 1906.

Form d, fig. 18. Pure white, club shaped, much thickened at the top and plicate folded, partly hollow, stipe slightly yellowish in color, 2 cm by 4 cm. Found on ground in dense woods. Hymenial layer over entire surface and basidia of the ordinary tremella type. I have been unable to secure any spores of this form. It was thought at first to be a young stage of Form a, but the fact that the basidia were found over its entire surface seems to indicate that the plant had reached its mature form. A single specimen from Madison.

Farlow reports the interesting fact that *Corticium tremellinum* B. and Rav. and *Cort. tremellinum* var. *reticulatum* are not *corticiums* but *Tremellas* and that the latter is a distinct species *Tr. reticulata* (Berk) Farl. Farlow believes that Atkinson's specimens belong also with *Tr. reticulata* and that *Tr. fuciformis* from South America and the West Indies, is a distinct species. Specimens in our collections seem to agree well with both Atkinson's and Möller's figures. The fungus is certainly quite variable in form. It is to be noted that our forms are found on the ground as well as those of Atkinson. The South American forms are reported on rotten wood and Farlow's specimens were found growing over the ground and fallen branches. The spreading habit referred to by Farlow and mentioned in the note appended to the description by Berkeley is not characteristic of our Wisconsin forms which are even when

2—S. & A.

quite rankly developed rather definitely limited plants with a tendency to a narrowed base.

*Naematelia*—Firm, fleshy nucleus enclosed by a thick gelatinous stratum, fibrous floccose within, the whole surface covered with hymenium.

### 13. *Naematelia encephala* (Wild) Fr. (Syst. Myc. II p. 227.)

Subsessile, pulvinate, variously plicate and contorted, firm; pale flesh color; nucleus large, white; spores pear shaped, 15–18$\mu$.   Fungus 1 to 3 cm in diameter and 1 cm high.

Rare.   The only typical specimen that I have seen was found by B. O. Dodge of Algoma, Wis.   It is much larger than the one described by Fries and not so distinctly flesh colored, but there seems to be no question as to its identity.   Not reported before for this country.

### 14. *Naematelia nucleata* Schw.   Fig. 27.

This form I have found on dead branches of oak, poplar and elm.   At first it forms a clear transparent body sessile or almost so as the stipe is hidden by the mass of the fungus which is much flattened out on the sub-stratum.   As soon as the fungus begins to dry the so-called nucleus or central body is plainly seen and the fungus takes on a reddish tinge and dries down into a reddish brown film in the center of which is the hard white nucleus which does not seem to have been affected by the drying out of the fungus.

Specimens of a *Naematelia* were found on birch which differed from the one just described in that they had a slight pinkish color from the first and formed a darker brownish crust when completely dried out.   The nuclei were the same in both cases.

Common.   Apparently not before reported for this country.

### 15 *Tremellodon gelatinosum* (Scop.) Pers. Fig. 35.

*Hydnum gelatinosum* Scop. (Flora carn. II p. 472.)
*Hydnum gelatinosum* (Flora dannica taf. 717.)
*Hydnum gelatinosum* Cooke (Handbook p. 289.)
Gelatinous, tremelloid, dimidiate or fan shaped, 3–10 cm

across, thick, expanded behind into lateral thick, stem-like base, pileus brownish with opalescent shades, very minutely granular; hymenium watery grey; teeth stout, acute, whitish; spores sub-globose 7–8μ diam.

This is the only true *tremelloid* fungus having spines and it has been classed for some time along with the *Hydnums* but Engler and Prantl place it where it doubtless belongs, among the *Tremellineae,* though its outward appearance would indicate that it belongs with the Hydnums.

No specimens have been found at Madison, but specimens have been received from the pine woods of Northern Wisconsin.

The form described as *Tremella mycetophila* Pk. (N. Y. Rep. Nat. Hist. 28 p. 53.) and *Exobasidium mycetophila* (Pk.) Burt. (B. T. C. XXVIII p. 283) is fairly common in Wisconsin and is so strangely developed at times that the entire body of the *Collybia* is completely hidden by the fungus. I am inclined to regard this fungus as in need of still further study for the determination of its proper relationship.

16. *Sebacina incrustans* (Tul.) Bref. Fig. 36–37.

*Sebacina incrustans* (Tul.) Ann. Sc. Nat. 1872 t. X f. 6–10.
*Sebacina incrustans* Bref. (Unters. VII p. 103.)
*Thelephora sebacea* Pers. (Synops. p. 577.)
*Merasma serratum* Pers. (Comment. p. 106.)
*Corticium incrustans* Pers. (Observ. I p. 39.)
*Thelephora incrustans* Pers. (Synops. p. 577.)

Whitish at first, fleshy, soft, then becoming rigid, incrusting; form very variable; hymenium collapsing when dry and often tinged with cinnamon brown; spores sub-globose, spinulose, vinous 6–10μ, basidia tetra-sporous.

Found on stumps, twigs and forming irregular growths in patches of grass. White, soft and fleshy when growing. 3–5 cm in diameter and about the same in height.

The researches of Tulasne and Brefeld have put this form among the *Tremellineae* where it belongs, removing it from the *Thelephoreae* where it has been put by many investigators. Rare.

## *Dacrymces.* Nees.

Gelatinous, roundish or irregular, convex; basal portion often root like and entering the matrix. Basidia forked, ending in two tapering sterigmata, terminated by a single spore cylindrical in shape and often septate.

### 17. *Dacrymces deliquescens* (Bull.) Fig. 23.

*Dacrymyces deliquescens* (Bull. Champ. p. 218.3
*Dacrymyces deliquescens* Duby (Bot. Gal. II p. 729.)
*Tremella deliquescens* Bull. (Champ. p. 219.)
*Tremella deliquescens* Grevillea V p. 88.)
*Tremella lacrymalis* Pers. (Synops. p. 628.)
*Dacrymyces tortus* Fries (Elench. II p. 36.)
*Calloria delinquescens* Fries (Summa Veg. p. 359.)
*Septocolla adpressa* Bonorden (Handb. p. 152.)

Gelatinous, roundish or irregular, convex, gyrose, yellow, hyaline, basal portion rootlike and entering the matrix; spores cylindrical, obtuse, curved, 3 septate, $15-17\mu$ by $6-7\mu$.

Found in winter and early spring in greatest abundance, though often found during the summer months. Usually found on pine wood, small 1-2 cm. in diameter. Quite often found deliquesced into a small viscid almost transparent mass. Dries to a yellow brown.

### 18. *Dacrymyces deliquescens.* Var. ? Fig. 24.

This is a form which answers to the description of D. deliquescens in color and in the fact that it deliquesces, but which is found on the wood of deciduous trees and grows to the size of 1-1½ cm. Found in dense deep woods during July and August. Madison and vicinity.

### 19. *Dacrymyces* (new species)? Figs. 25-26-28.

Gelatinous, irregular, forming tufts of a rich golden yellow color, usually 3-6 together separate at top but united at base, each from 2-3 cm. across, and of a height of from 2-3 cm. The rich hymenium covers the entire upper half and the spores form

a ring of a rich yellow color around the base of the fungus when dry. The fungus changes only slightly in color while drying, becoming slightly brownish. When dry the fungus becomes very irregular, sometimes prostrate and sometimes remaining erect. Spores golden orange 16–22 by 5–8$\mu$. Found in various localities in the state on decaying logs of larch.

### *Calocera.* Fries.

Gelatinous-cartilaginous, horny when dry, vertical, sub-cylindrical, simple or branched, viscid, without a distinct stem. Hymenium covering every part of the carpophore, basidia terete, apex furcate or bilobed, each lobe bearing a single one-spored sterigma. Spores oblong, curved, septate, on germination and producing heads of ellipsoid sporidiola.

### 20. *Calocera cornea* Batsch. Fig. 32.

*Clavaria cornea* Batsch (Elenchus Cont. I fig. 161.)
*Clavaria aculeiformis* Bull. (Champ. t. 463.)

Tufted, rooted, clubs smooth, viscid, subulate, simple or slightly branched, orange or paler yellow at times, several connate at base; spores cylindrical-oblong 7–8 by 5$\mu$. Carpophore 1–2 cm., one specimen 3–4 cm. high. On stumps and trunks of oak.

### 21. *Calocera cornea* var. ? Fig. 33–34.

Color same as *calocera cornea* except that it is a lighter yellow; carpophore much shorter and thicker. Grows in closely compacted tufts and the tips are seldom branched. Rare specimens on oak. Blair and Madison, Wis.

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## EXPLANATION OF FIGURES.

Photographs were in almost every instance made from freshly collected material.   The photos of *Tr. fuciforms* figs. 19, 20 and 21 were made from alcoholic specimens in the collection of the University of Wisconsin.   All are life size with the exception of fig. 14, *Tr. frondosa* which is only one-fourth the actual size, and fig. 35, *Tremellodon gelatinosum* which is two and one-half times the actual size.

EXPLANATION OF PLATE LXXXII.

Fig. 1. *Hirneola auricula Judae.*   Large specimen from dead oak, rich brownish color, showing the upper surface, with faint wave-like folds which become more prominent upon drying.

Fig. 2. Cluster of form described as Aur. var.?   This was found to be very numerous on dead branches of oak and was much thicker in proportion to size than fig. 1, and was rich deep wine color.

Fig. 3–4. *Tachaphantium* Bref. fig. 3 side view showing the elevation of fungus above substratum, and fig. 4 view of upper surface showing the form of the fungus.   The figure of course does not bring out the clear glistening surface of this beautiful little fungus.

Fig. 5–6 show typical forms of *Ex. glandulosa* the first being on poplar where it spreads out quite considerably and the second on oak where it is much more compact.   It is impossible to bring out the minute papillae which project in large numbers from the upper surface.

Fig. 7. A young and very small specimen of *Ex. albida.*   The fungus grows in height and at the same time spreads out to cover large portions of the dead stems of basswood.

Fig. 8. *Tr. lutescens.*   Not a perfect specimen in that it does not show the two types of folds so common to this form.

Figs. 9–10. *Tr. mesenterica.*   The first being a very compact form growing from a break in oak bark while the other shows a more spreading type.

Fig. 11. *Tr. foliacea.*   Small forms of this fungus found on stem of alder.   Does not show the foliaceous nature described by many observers.

Fig. 12. *Tr. frondosa.*   A brownish form found on dead elm, quite fleshy and soft as compared with the more firm and erect specimens of fig. 14.   A very small specimen.

Fig. 13. *Tr. frondosa.*   A form characterized by the very thin folds. This was quite firm and changed only slightly upon drying.   It was wine colored and the surface was not glistening as in the form just described.

Fig. 14. *Tr. frondosa.*   Reduced three-fourths.   The right lower portion was exposed by removing the bark of the oak upon which it grew and was a very dark brown and shows the compressed folds while the left upper portion shows the fully developed fungus which was of a beautiful glistening brown, with a tinge of wine color in the lower portion.

Fig. 15. Tr. sp.?   A specimen of a beautiful form which in earlier stages and when fully moistened is rich creamy white, but changes to a beautiful yellow, darker at the base and gradually becoming paler toward the outer edge.
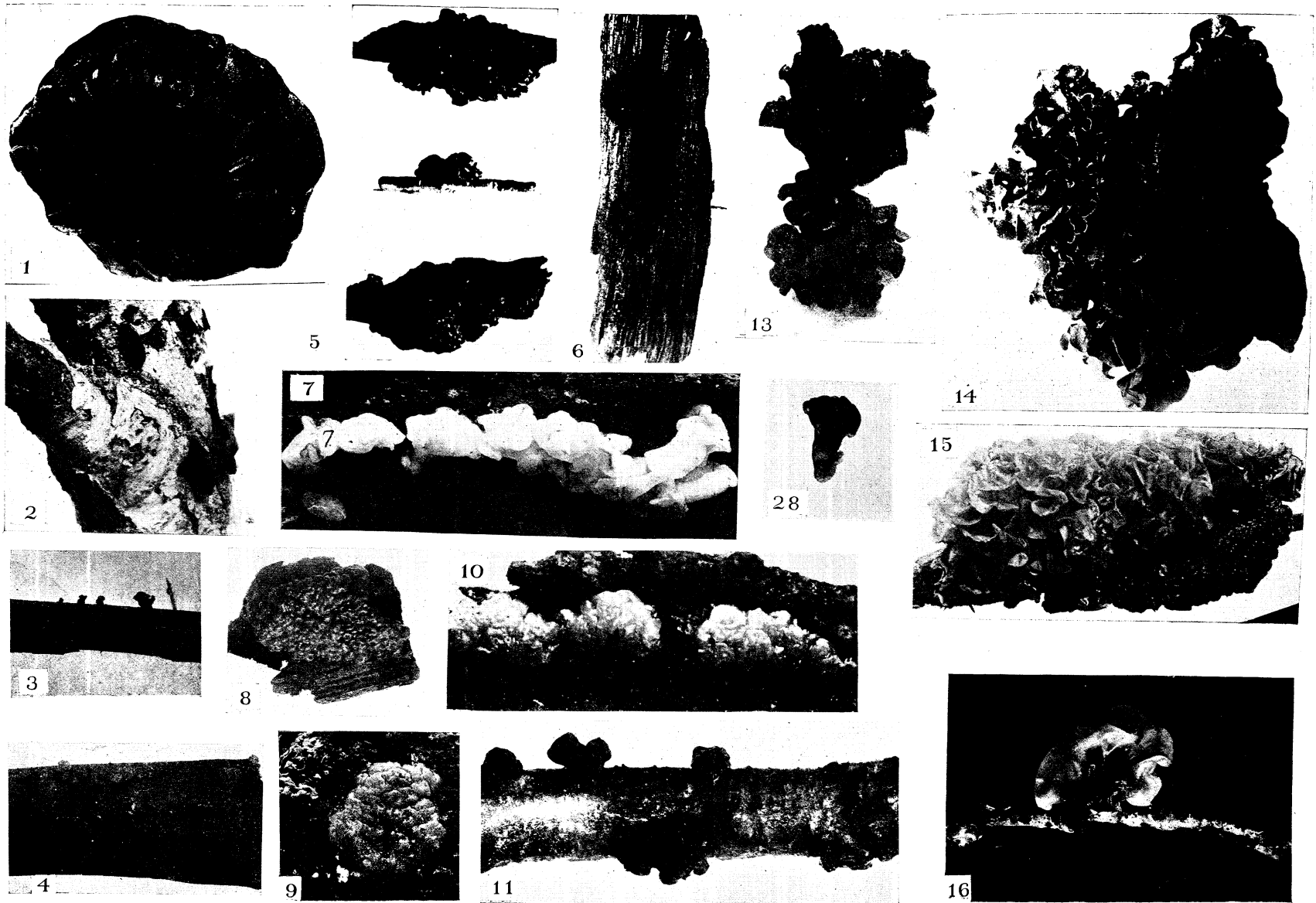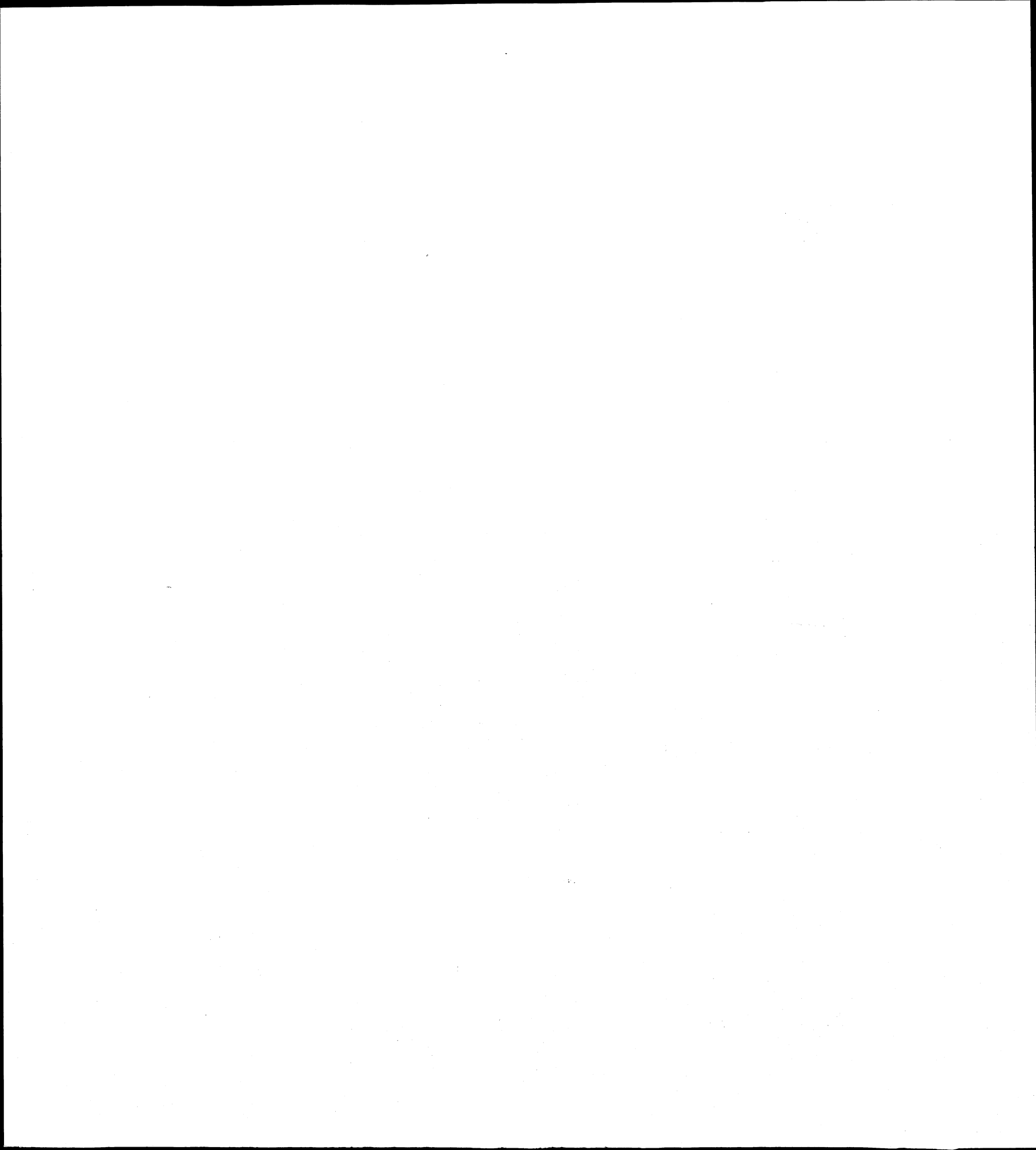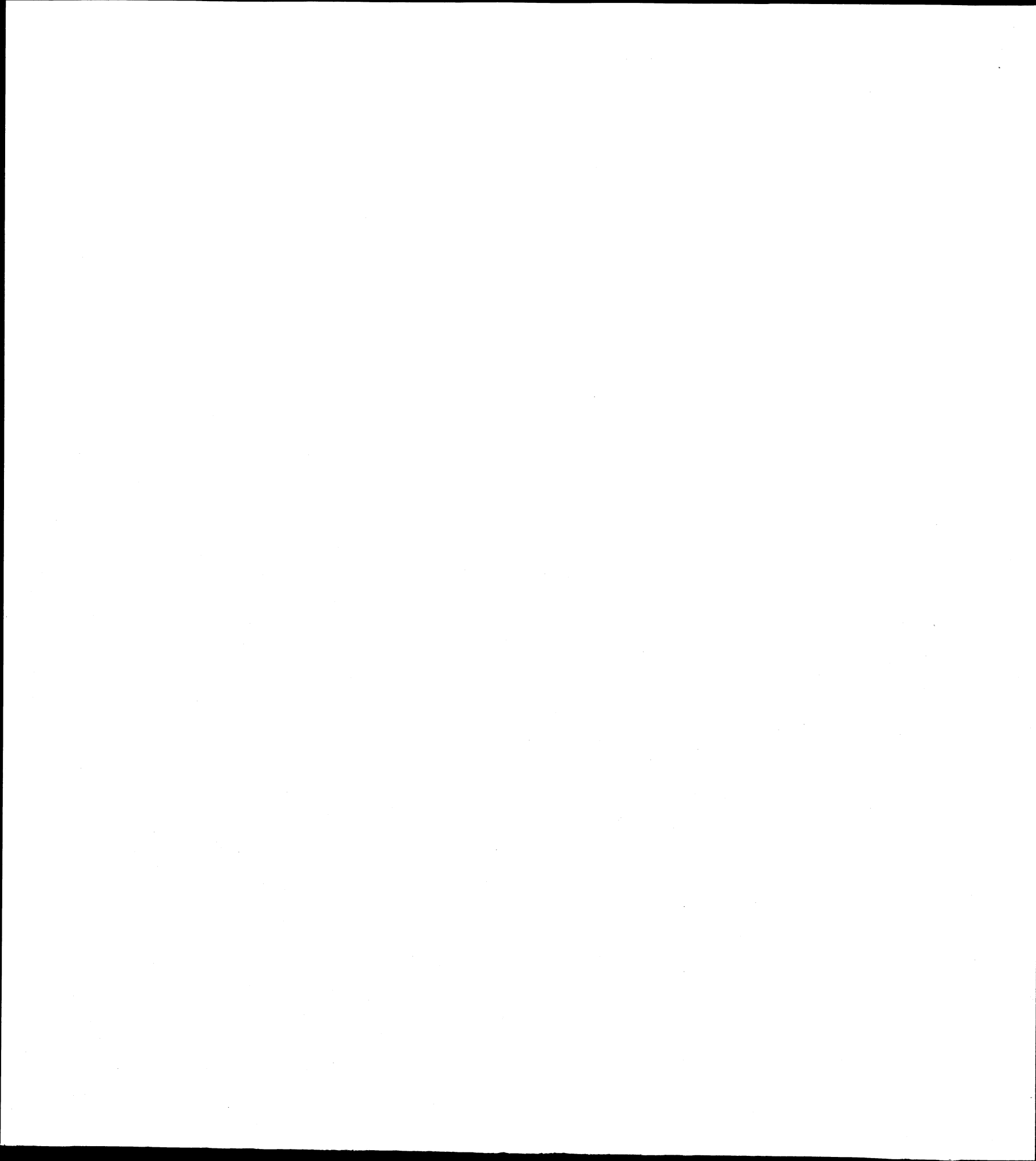
PLATE LXXXII

PLATE LXXXII.

## EXPLANATION OF PLATE LXXXIII.

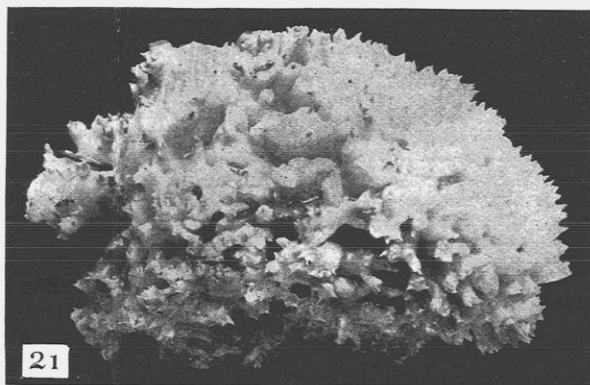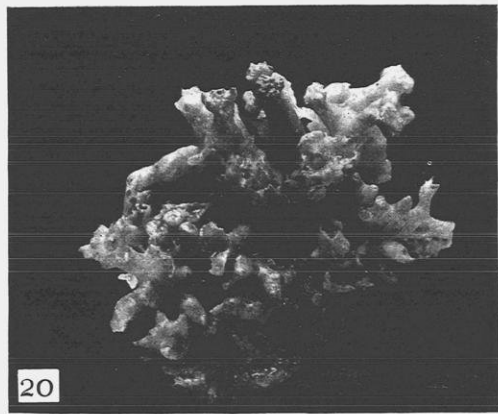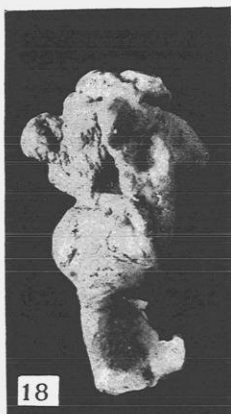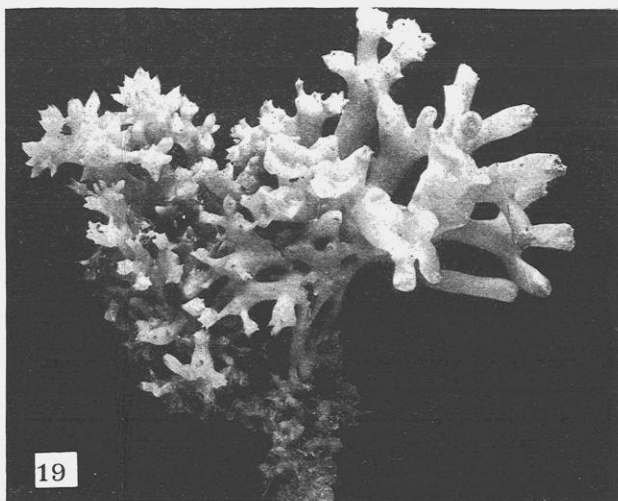Fig. 17. *Tr. fuciformis.* The typical form of the species as described by Atkinson. Somewhat reduced, but specimens have been found 2 to 3 times as large. The whole fungus is a rich creamy white turning slightly yellowish with age.

Fig. 18. *Tr. fuciformis.* Club shaped form found on ground and described as form d.

Figs. 19, 20, 21. *Tr. fuciformis.* Much branched type described as form b. The figures show the variation in the branching and in the tips.

Fig. 22. *Tr. fuciformis.* Form c.

Fig. 23. *Dacrymyces* sp.? A small form found on larch and cedar. Yellowish color, slightly tinged with brown when mature.

Fig. 24. *Dacrymyces deliquescens* var.?

Figs. 25–26 and fig. 28 of Plate I. Dacrymyces sp. found on larch in almost every instance. Possibly the form described as *D. chrysocomus* by many collectors.

Fig. 27. *Naematelia nucleata.* Mature form, no nuclei showing.

Fig. 29. Tr. species? Rich brownish color, almost sessile with lower surface much darker than upper and in some specimens seemingly roughened with minute papillae.

Fig. 30. Tr. species.? Rich glistening greyish black, with upper surface broken by papillae of rather large size. Stipe central, very thick and short. Basidia of the typical tremella type, very large and lying deep in sub-stratum.

Fig. 31. Tr. species? Fungus of 4-5 large lobes of a glistening dull yellow: the entire substance almost crystalline in appearance when broken open. Quite firm when fresh but very soon softening and almost deliquescing.

Fig. 32. *Calocera cornea.* Shows a type which is quite common. The fungus at once divides into several finger like branches, but in some other respects is not typical.

Fig. 33–34. *Calocera cornea* var.? Tufted types but the branches do not radiate. The fungus may form a mass several inches in length and only half an inch in width.

Fig. 35. *Tremellodon gelatinosum,* two and one-half times actual size. Typical of all specimens in collection. The whole had a pale greyish blue color and in parts was almost transparent.
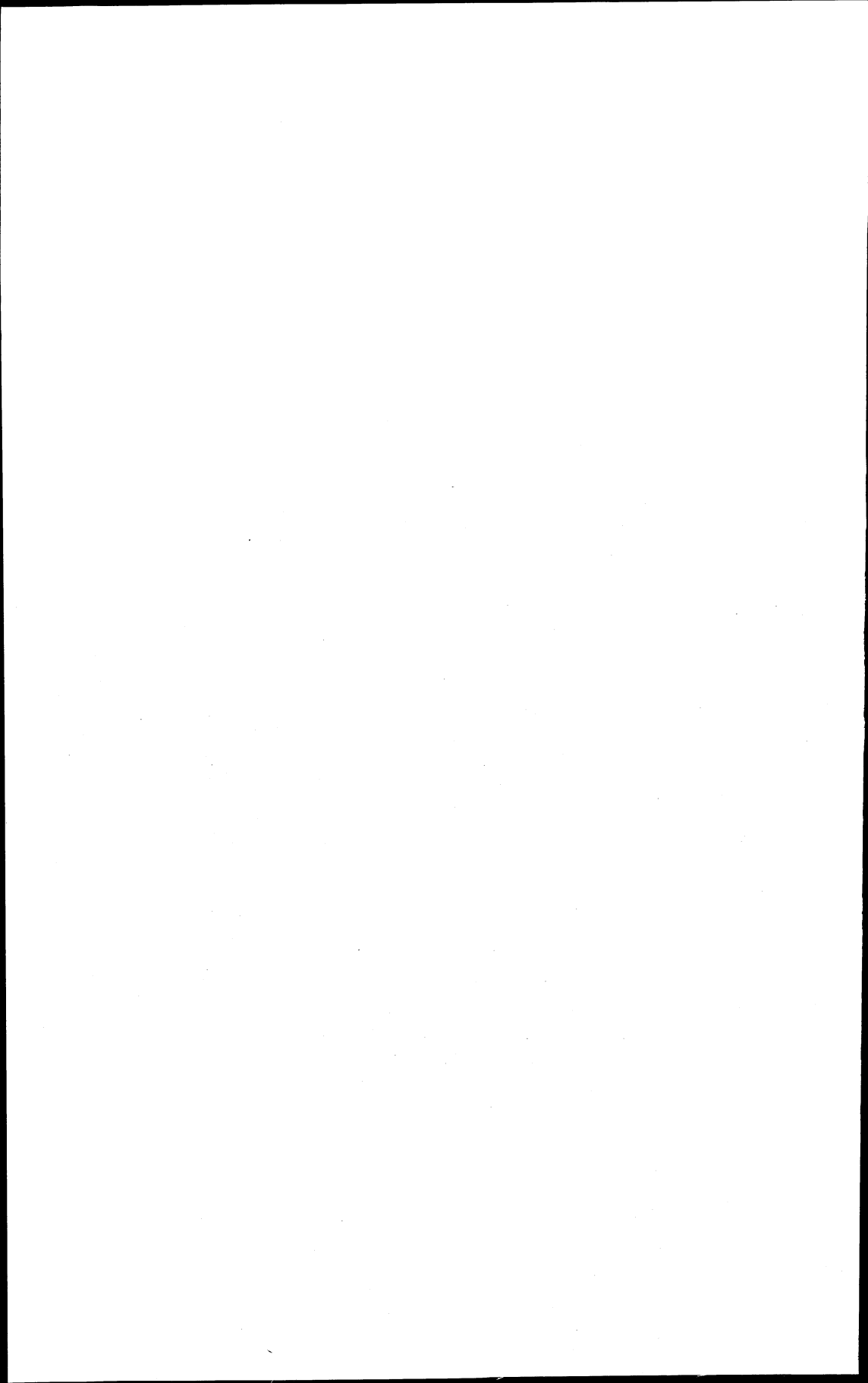
Fig. 36–37. *Sebacina incrustans.*
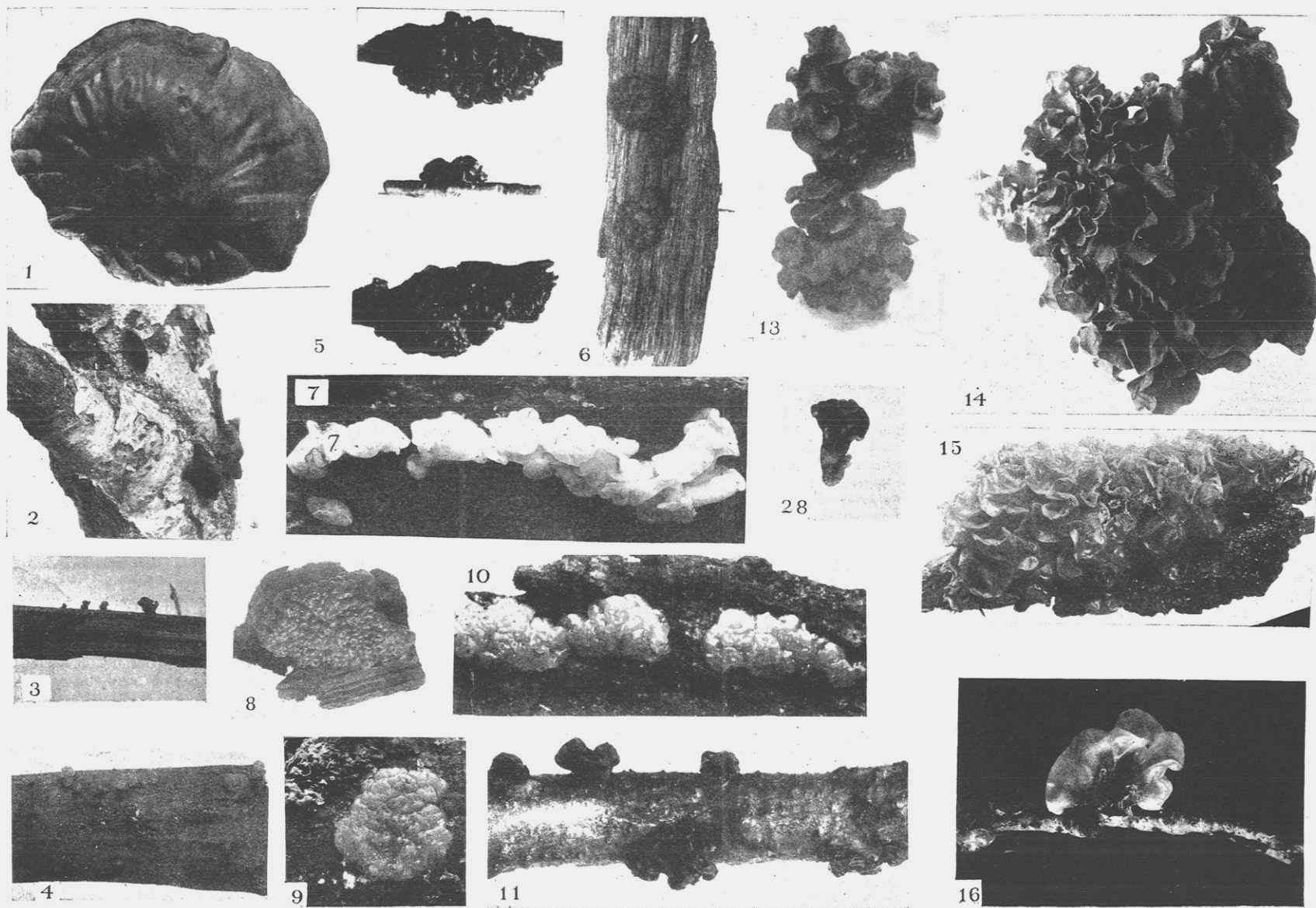
GILBERT:—

TREMELLINEAE

# SPORE FORMATION IN GEOGLOSUM GLABRUM PERS.

HALLY D. M. JOLIVETTE.

The method of delimitation of the ascospores in free cell formation has been found to be remarkably uniform in all the asci which have so far been carefully studied. We have here a problem of cell division, in which the protoplasm of the ascus or mother-cell, is separated into the protoplasm of the spores,— the spore-plasm,—and the enveloping epiplasm in which they are embedded.

De Bary (10) as early as 1863 observed in certain species of Peziza, Helvella and Morchella that the eight nuclei of the ascus at the close of the third division come to lie about equidistant from each other. Ultimately each nucleus becomes surrounded by a mass of protoplasm, which, as he noted in living material, is distinguished from the remainder of the protoplasm by its greater transparency. These portions are the young spores and they soon become invested with cell membranes. De Bary gave the name epiplasm to the portion of the protoplasm not included in the spores.

An essential feature in the process of spore delimitation, as described by Harper (25), is the transformation of the aster by the folding back of its rays to form an ellipsoidal cell plate. This implies an activity of the rays comparable, as Harper has shown, to that of cilia, and it is interesting to note the accumulation of evidence of resemblance between astral rays and cilia. It is generally agreed that the axile threads of spermatozoa and the cilia of atherozoids arise from a centrosome or a centrosome-like blepharoplast. The same is true for the cilia of the swarmspores of Vaucheria and perhaps of Oedogonium.

Meves (32) has further described and figured in the divisions of the spermatocytes of *Pygaera bucephala,* one of the Lepidoptera, a system of astral rays about a center at either pole of the spindle, a number of which extend into the pseudopodia which arise from the surface of the cell at this stage. Running out from each of the centers, are also two cilia in the primary spermatocyte and one in the secondary spermatocyte, which are plainly nothing but thicker and longer rays of the polar asters. At the close of the second division the radial pseudopodia with the exception of one disappear. This one contains the axile thread of the future sperm, which again is not to be distinguished from the cilia and astral rays. The axile thread extends beyond the tip of the pseudopod for some distance. Meves himself regards cilia and rays alike as for the most part outgrowths from the centers.

The details of the process of ascospore formation as described by Harper (23, 24, 25, 26, 27, 28) and in the papers of Guilliermond, (20) Maire (30), and Faull (13), dealing with this subject, have been recently summarized by Sands (37) and others.

Berlese (3) figures a beaked nucleus attached to the plasma-membrane of the spore of *Tuber brumale* and agrees with Harper as to the general method of spore formation.

Sands (37) describes the bending backward of the astral rays of the third division figure and their fusion to form the plasma membrane of the spore very much as described by Harper (25). She criticizes Faull's interpretation of certain of his own figures and suggests that those with the beaked nucleus with its aster within the plasma membrane of the spore, which Faull (13) regards as conclusive evidence that the rays take no part in forming the spore membrane, may be explained as polar views of spore formation by astral rays.

Fraser (15, 16), as a result of her observations of spore formation in *Humaria rutilans, Peziza vesiculosa,* and *Otidea aurantia,* holds to the view that the spore is delimited by astral rays. These rays, she maintains, are not of the nature of cilia as suggested by Harper (25) but are currents set up in the neighborhood of the centrosome as it pushes into the dense cyto-

plasm near the pole. According to the hypothesis that the centrosome is the seat of fermentative activities, as suggested by Harper (25 page 274) Fraser (16) holds that the centrosome as it pushes outward through the cytoplasm at the end of the third division might be regarded as constantly generating a ferment. This ferment would flow back in the wake of the centrosome and would produce a chemical change in the area through which it was distributed. Its effect would be limited in certain directions by the presence of vacuoles and the ascus wall. Fraser (16) concludes that, whether the changes which take place in the cytoplasm are or are not due to enzyme activity, the main factor in the delimitation of the spore is an alteration in the cytoplasm originating at the centrosome and essentially similar in character to that which produces the aster.

The asci in *Geoglossum glabrum* Pers. have been found to be very favorable material for the study of spore formation and the following description is based on an extended study of the asci of this species. The material was fixed in the field in Fleming's weak and Fleming's medium solutions. The sections were cut five microns thick and stained with the triple stain. A large number of stages in spore formation can of course be obtained from a single ascocarp. I shall not now describe the formation of the ascus but proceed at once to the stages of nuclear division and spore formation. When about mature the ascus is of the ordinary type but the wall at the apex is a conspicuous lens shaped mass. In the one-nucleated stage the nucleus usually lies near one side of the ascus and about forty to fifty microns from the top. The cytoplasm is rather dense in the wall layer surrounding the nucleus while that in the remainder of the ascus, is greatly vacuolated consisting largely of strands running in from the periphery in various directions and often to the mass about the nucleus. The denser spore plasm at this stage does not fill the complete cross section of the ascus in the neighborhood of the nucleus as it does in so many other forms.

While in this position the nucleus divides. Examples of the equatorial plate stages are comparatively abundant. Figure 1

represents a longitudinal section through the ascus at this stage. The spindle runs parallel with the long axis of the ascus and is very near the plasma membrane. The chromosomes are rather small and scattered along the spindle. The number of the chromosomes appears to be eight. The spindle is long and narrow, the fibres being attached at the poles to broad central bodies so that the poles of the spindle are very broad in comparison with its diameter. The astral rays are strongly developed in this stage. The portions of the fibres near the central body stain almost as deeply as the central body itself does so that the latter is not easily distinguished from the fibres.

Figure 2 represents the same stage as figure 1. Here the ascus has been cut somewhat obliquely through the side containing the spindle and parallel with the spindle which runs at an oblique angle to the long axis. The spindle is not so near the plasma membrane as it is in figure 1. The cytoplasm is conspicuously more dense in the vicinity of the spindle and becomes more vacuolate as the distance from the spindle increases. The spindle and astral rays appear very similar to those described for figure 1, being very distinct in both cases. The chromosome number again appears to be eight. In the cytoplasm are two large red-staining bodies, one to the right, the other above the spindle. The former appears as if it might be the remnant of the nucleole.

At the close of this division the fibres disappear and the daughter nuclei round up. These nuclei are much smaller than the primary nucleus. When in the resting condition, they are to be found near the lower edge of the mass of spore plasm which now fills entirely the tip of the ascus. This spore plasm now shows a finely reticulated structure. Below the nuclei there appear to be a few rays running into the cytoplasm, but this condition is probably due to an irregular streaming of the cytoplasm since these rays are not oriented on a centrosome as are the kinoplasmic fibres of the division figures and since furthermore the rays often pass into the strands of cytoplasm which run down into the lower portions of the ascus.

The spindles in the second division are similar to those of

the first with the exception that the central bodies appear to be more flattened.   The asters are also well developed here.   They disappear at the close of the anaphase stage.   When the four daughter nuclei are completely reconstructed, they are to be found near the lower part of the dense mass of spore plasm occupying the upper portion of the ascus.   They are so arranged that in longitudinal sections of the ascus only two can be seen in one plane.   As you focus down below these two, the other two come into view so that a cross section through the ascus in the region of the nuclei shows all four in one plane.   Of course in some cases their position may vary more or less.   The spore plasm here as during the second division is finely reticulated.

Figures 3 and 4 show the equatorial plate stage of the third division and were drawn from one section of an ascus but in different planes.   The spindle is narrow with a broad distinct center at each end.   The polar asters are extraordinarily well developed.   The fibres are very long and distinct and they run out into the cytoplasm in all directions, some of them appearing to terminate in the plasma membrane.   The fibres of the polar aster are longer than those of the central spindle.   In these figures 3 and 4 there is a great deal of variation as regards the course of the fibres although in all cases they are extremely well developed.   The fibres from the centers to the left in figure 3 bend away from the plasma membrane, while those to the right for the greater part run towards the plasma membrane.   The astral rays of the center nearest the tip of the ascus extend out in all directions, many of them running as far as the plasma membrane.   In figure 4 the rays of the aster nearest the base of the ascus run to the edge of the spore plasm.   The fibres of the aster nearest the tip spread out in all directions and could be traced to a considerable distance from their origin.   In the two remaining asters most of the rays appear to end in the plasma membrane.   The fibres in all these asters are extremely numerous, as can be seen from the figures.   It is a conspicuous fact at this stage that the spindles are so arranged that the asters interfere as little as possible with each other.   This condition is general at this stage.   In the particular ascus here described the

spindles in figure 3 run almost at right angles to those in figure 4 so that the four spindles form a four sided figure.   The chromosomes are very small in this stage.   In the late anaphase the chromosomes appears to be massed against the central bodies. The spindles elongate and the fibres of the spindle gradually disappear, while those of the polar aster become even more pronounced than during division, so that a section through the ascus shows the large conspicuous red staining centers with the astral rays streaming out in all directions very prominently. The chromosomes are somewhat obscured by the centers.

A little later the nuclear membrane forms around the chromosomes and the nucleus becomes beaked.   The nucleus at this time is still very small.   The contents stain very densely.   Subsequently the nucleus grows considerably and then the chromatin and the nucleole can easily be distinguished.   The astral rays develop still further.   Figure 5 shows a longitudinal section of the ascus after the new daughter nuclei have reached a considerable size.   The beak is conspicuous.   None of the central bodies are turned toward the center of the ascus, but they lie irregularly arranged in the cytoplasm, as can be seen from the figure in which the beaks of the nuclei extend either toward the base, the apex or sides of the ascus.   The cytoplasm is still of the same consistency as in the division stages.   The asters are still larger.   The rays of each aster extend out until they meet each other.   This is especially conspicuous in figure 6. The fibres are distinct and those of one aster can easily be traced until they meet those of another.   The cytoplasm is somewhat more darkly stained in the zone where the fibres meet.   One might at first glance believe that the spores were already delimited.   In poorly stained sections where the asters are indistinct and the fibres cannot be traced very far from the centers it might appear that the spores are delimited by this zone formed by the meeting of the astral rays.   But on careful observation it is plainly seen that this apparently limiting layer is nothing else than the region in which the rays of the different asters meet.   These stages are comparatively frequent in my material and could very easily be mistaken for stages of spore delimitation.   That the delimitation has not even yet begun is quite

plain from the stages which follow. Figure 7 shows another section through the same ascus from which figure 6 was drawn and shows the same conditions.

A longitudinal section through another ascus at the same stage as that just described is shown in figure 8. Only two nuclei were in the plane in which the figure was drawn. In one the beak points toward the apex, in the other towards the base of the ascus. The zones in which the asters come in contact are very obvious.

This zone shows also very clearly in the oblique section, (figure 9). Figure 10 represents a cross section of the ascus at this period. At *a* the center is lacking, the rays having been cut across immediately below it. A small part of the central body is shown at *b*. The nucleus at point *c* was cut through in the neighborhood of the nucleole. The effect produced by the rays resembles closely that in the longitudinal section. We see thus that there may be an apparent differentiation of regions in the spore plasm at this early stage which have nothing to do with the spore outlines to be formed later. We may call these regions of the meeting of the rays the interastral zones.

The rays now begin to bend down over the nuclei to cut out the spores. Figure 11 represents the ascus when the rays are just beginning to turn back. The plasma membrane of the ascus is pulled in slightly near the central body. We see conspicuously here the curving in of the rays at their ends. They lie very close to the plasma membrane for a considerable distance if they have not actually fused with it. The rays curve in and delimit the spore at points *a* and *b*. At these points, if the plasma membrane of the ascus entered into the formation of the plasma membrane of the spore, we would expect to see the plasma just outside the spore rounded up against the spore membrane—due to surface tension. This, however, is not the case. The cytoplasm runs out to rather sharp points at *a* and *b*. There is no indication of a rounding up due to change in surface tension caused by a break in the ascus membrane. The fact that the spore plasm is not rounded up against the forming spore membrane at these points suggests that the ascus mem-

brane extends entirely around the spore plasm independently of the forming spore membranes.

In later stages the fibres continue their growth backward and around the nucleus and delimit the spore. In figure 12 the membrane is not yet completed. The membrane of the spore can be plainly seen near the central body before that at the opposite end is visible. The spore near the tip of the ascus has been cut across at some distance from the center where the rays have not yet fused to form the membrane. The cut ends of the rays show plainly as a darker region. At this time the beaks of the nuclei still point in all directions in the ascus.

Figure 13 is a little later stage in which the spore is completely delimited. Some of the fibres of the plasma membrane as well as some inside the spore could still be distinguished. The nuclei and the centers are still connected with the spore membranes. The figure shows the beaks of the nuclei pointing in various directions as in the earlier stages just described. No membranes such as Faull described could be seen on the epiplasm adjacent to the spore membrane.

The beaks of the nuclei which up to the time when the spore membrane is completely formed were pointing in all directions in the ascus all come now to point in the same direction—toward the base of the ascus (figure 14) so that the central body in all cases is found on the inner end of the spore. That the membrane of the spore was formed independently of the ascus membrane is further proved by the facts shown in this figure since the spores have changed their position and the plasma membrane is wholly intact and not torn in places as we would expect to find it if it entered into the formation of the spore membrane.

The spores now begin to elongate (figure 15) and come to lie more nearly side by side. They are found about fifteen to twenty microns from the tip of the ascus. The epiplasm is still finely reticulated and occupies for the most part the portion of the ascus between the spores and the apex. The center is at the part of the spore pointing toward the base of the ascus. It is still connected with the plasma membrane. The structure of the

nucleus with its beak connected with the central body is still to be made out. The nuclei are considerably longer than broad in this stage. The nucleole is distinct. The chromatin is scattered in small masses in the nuclear cavity. The traces of the fibres have not entirely disappeared at this stage.

The spores now become pointed at the end toward the base of the ascus (figure 16). It is apparent that this condition is due to growth. The persistence of the central body and nucleus in their position at the inner end of the spore would seem to indicate that they may be concerned in the growth of the spore at this point. The spores at this time lie side by side resembling in form a bunch of cigars. Although this growth process has taken a comparatively long time, which is shown by the numerous different stages that can easily be found, the nucleus is still beaked and the central body is in contact with the spore membrane. The astral rays have entirely disappeared by this time. The epiplasm is still in the same condition as before. Finally, however, after the spore has reached a considerable length the nucleus with the central body is drawn back from the wall.

Figure 17 was drawn from a cross section of the ascus which cuts through the spores in the region of the nuclei after the latter have moved back to the middle of the spores. The cytoplasm here appears a little more coarsely reticulated than in the earlier stages. The nucleus is usually slightly elongated in the long axis of the spore at this time, becoming more spherical in shape a little later.

The spores increase in length, the cytoplasm takes on a spongy appearance, the nuclei divide karyokinetically and cross walls are put in so that the spores become several celled. The walls of the spores become thicker. The spores now vary in length and are less regularly arranged than they were at an earlier stage, although they still occupy the distal end of the ascus.

### General Discussion.

As described above the polar asters of the third division in the ascus of *Geoglossum glabrum* Pers. grow until they come into contact with each other and form a definite region,

which I have called the interastral zone. The persistence and growth of the polar rays after the disappearance of the spindle is unquestionable in Geoglossum. The centers are distinct and for the most part lie near the plasma-membrane of the ascus and widely separated from each other. There is, however, no regularity in their orientation as has already been noted. The growth of the fibres continues and the asters thus come into contact with each other. The practically radial arrangement of the fibres is conspicuous up to the interastral zone (figures 5, 6, 7, 8, 9 and 10). In this zone there is a break. The cytoplasm in this region stains slightly darker and is very conspicuous, thus appearing to divide the cytoplasmic mass into definite portions which, however, have nothing whatever to do with the spores which are to be cut out later.

The interastral zone has nothing whatever to do with the future boundary of the spore. When the fibres begin to curve about the nucleus, the ends of the fibres, which up to this time formed the interastral zone, change their position and bend in toward the nucleus. The rays soon begin to fuse in the neighborhood of the central body (figure 12). At this stage when the aster is cut across at some distance from the central body and beyond the region where the rays have already fused, the cut ends of the numerous fibres form a dense circular region as has already been described and shown in figure 12, *a*.

In a slightly later stage these rays fuse to complete the formation of the plasma membrane already started near the center (figure 13).

Not all of the rays of the aster enter into the formation of the plasma membrane of the spore. Some of them remain inside the membrane and ultimately fade out in the cytoplasm of the spore. This condition Harper has already described and figured. It is entirely different from that figured by Faull. It appears plainly from my figures that the number of the rays enclosed in the spore is much smaller than that of the entire polar aster shown in the figures of stages previous to that of the completion of the spore membrane. My figures 13, 14, 15 and 16 in Geoglossum do not correspond at all to Faull's figures 27, 28,

29, 34 and 35 Plate 8. Perhaps as Sands suggests Faull has drawn polar views of these stages and interpreted them wrongly.

Further in the early stages in the formation of the membrane the astral fibres of which it is composed can be distinctly made out. This same condition has been figured by Harper.

There can be no question that the central body in all these stages is in contact with, or a part of the plasma membrane of the spore. Faull agrees that the delimitation of the spore begins at the center and proceeds backward.

My figures show that the central body stays in contact with the plasma membrane during a comparatively long period, i. e., while the spore changes the direction of its axis of growth, its position in the ascus and its shape. The nucleus with its central body which at the time of the delimitation may be pointing in any direction comes to point toward the base of the ascus. The length of the spore is considerably increased and the end nearest the base of the ascus is drawn out into a blunt point. The spores have come to lie side by side in the ascus resembling in their arrangement and shape a bunch of cigars. The numerous stages in this process that can be found show that this connection between the central body and the membrane obtains for a proportionately long time. At the time when the nucleus with its central body is drawn back from the plasma membrane into the spore the fibres are no longer visible. That the nucleus with the central body has taken an extremely active part not only in the formation of the spore membrane but in the changes of the position and shape of the spore is evident from these figures, and all these phenomena here are in harmony with the theory that the membrane was formed out of the fibres.

The plasma membrane of the ascus in Geoglossum does not enter into the formation of the spore membrane as can be seen from my figures (figs. 11, 12, 13, 14, 15, 16 and 17). Although it is impossible in some cases to trace the plasma membrane of the ascus entirely past the spores during the formation of their membranes the evidence is in favor of the complete independence of the two membranes. If the ascus membrane was broken at any point the epiplasm at that point would tend to

round up due to surface tension.   But the ascus membrane where it is impossible to trace it past the spores, can be seen to thin out gradually to a delicate film on either side of the spore (fig. 11, point *b*).   In some cases these films on either side of the spore can be traced almost to the point of union of the two. If the ascus membrane was cut into during the formation of the spore membrane these edges could not maintain themselves but would necessarily draw back and round off.   In still other cases the ascus membrane with a thin layer of epiplasm can just barely be traced outside the spore membrane.

A further proof of the formation of the spore membrane independently of the ascus membrane is that, in all stages where the spores are completely delimited and have started to change their position, the ascus membrane is still entire.   Figures 13, 14, 15, 16 and 17 showing completely delimited spores show no cases of incomplete ascus membranes.

In *Geoglossum glabrum* Pers. the nuclei with their central bodies do not always point toward the median line of the ascus. The axes of the spindles of the third division show no tendency to lie in the transverse axes of the ascus; they may lie at any angle.   Figures 3 and 4, examples of this stage, show this plainly.   In this case two of the spindles are practically at right angles to the other two.   The arrangement appears to be such that the asters interfere very little with each other.   Accordingly the nucleus with its central body may point toward the distal or proximal end of the ascus or at almost any angle with the wall.   Figures 5, 6, 7 and 8 afford striking examples of this condition.   The nuclei are not arranged regularly with their beaks at right angles to the ascus wall but are pointed in almost any direction except toward the center of the ascus.

In these stages the interastral zones are quite conspicuous as described and it is quite possible Faull (13) took this zone for a limiting layer of the spores.   As noted above, in poorly stained preparations where the asters were not well differentiated the interastral zone might at first glance appear to be a limiting layer.   Such preparations may have misled Faull (13).   The meeting of the fibres in the zones above described might be in-

terpreted as having some such general appearance as Faull (13) ascribes to his limiting layer. Such stages as Faull shows in his figure 21 (Pl. 8) in my preparations showed an interastral zone. In a slightly later stage, when the fibres have increased in length and just before they begin to bend downward toward the nucleus, the interastral zone is still more pronounced, and this later stage Faull (13) probably took for his limiting layer. Faull's misinterpretation of the stages when the interastral zone is present may also account for the large number of preparations that he found showing what he calls the limiting layer.

Sands (37) in her paper on nuclear structure and spore-formation in *Microsphaeri alni* points out that Faull's figures (figs. 27, 28, 29, 34 and 35 pl. 8) of the young completely delimited spore with a beaked nucleus and an aster whose rays end in the plasma membrane may have been drawn from polar views of the spore. This possibility with the facts described above indicate that Faull's contention that the astral rays have nothing to do with the delimitation of the spores is not in accord with the facts shown by his own preparations.

In his study of *Peziza Stevensoniana* Harper (23) observed that at the close of the third division of the nucleus of the ascus each of the eight nuclei was surrounded by a rounded plasma mass, which is densest near the nucleus and gradually becomes less dense as the distance from the nucleus increases. This condition is represented in his Figure 23. There can be little doubt both from the description and figure that this is the stage where the asters are in contact and that the bounding zones separating the masses about the eight nuclei are the interastral zones described above. The asci of *Peziza Stevensoniana* are not favorable for the differentiation of the asters at this stage.

There can be no question from the figures described above for Geoglossum that the rays actually bend in toward the nucleus just before they form the spore membrane. There is a comparatively long period after the third division when the polar rays run out radially from the central bodies and meet in the interastral zone after the central spindles have entirely disappeared (figs. 5, 6, 7, 8, 9 and 10). The nuclei have become re-

constructed and have increased considerably in size. The long beaks on the nuclei are very distinct. The centers at this stage are as near the plasma membrane as at a later stage when the rays are bent downward, some of them appearing to be very near. Figure 11 shows the fibres when they are just beginning to bend toward the nucleus. Here some of the centers are very near the wall and the rays have still to curve about the nucleus. The series of stages here makes it evident that in Geoglossum the rays must bend downward through the cytoplasm and that they are not, as Fraser (16) has suggested, bent backward over the nucleus as the central body pushes its way outward through the cytoplasm at the end of the third division.

Harper's (25) hypothesis, that the movement of the astral rays in free cell formation can be compared to that of cilia, receives strong support from the conditions found in Geoglossum. The rays here move through the cytoplasm in toward the nucleus when there is no indication of the movement of the central bodies outward.

That the movement of the astral rays should resemble that of cilia is quite in harmony with the phenomena found in the development of cilia and flagella in other cases. In the formation of the antherozoids in the ferns and cycads the cilia are formed inside of the cell by growth outward from the blepharoplast, and only later do they make their way through the plasma membrane to function as motile organs.

As noted above we have a further striking example of the similarity between cilia and rays of the polar aster in the case of the spermatocytes of *Pygaera bucephala* as described by Meves (32). His figures suggest a very close resemblance between the astral rays and the cilia or flagella. According to Meves both the cilia and astral rays grow out from the centers. The ray, which runs out to the very tip of the pseudopod can be imagined to grow longer and longer while the pseudopod becomes thinner and thinner until only the ray can be made out when we have a cilium. At the close of the second division but one pseudopod remains and that one contains the axile thread of the sperm. The appearance here is strikingly

like that in the lesser pseudopodia. Here we have an example of the origin of rays and cilia by growth from a common center. The strong resemblance in appearance of the two as well as the numerous stages of transition point to a strong similarity between the polar rays and the cilia, and this is further confirmed by the generally accepted origin of the axile thread of the flagella of the spermatozoa from the centrosome in amphibia and other animals.

\*     \*     \*     \*     \*     \*     \*     \*

This work was done at the suggestion of Dr. R. A. Harper to whom I am indebted for many valuable suggestions and criticisms.

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PLATES LXXXIII, LXXXIV, LXXXV.

## EXPLANATION OF FIGURES.

All figures were drawn with the aid of the Camera lucida and with the 1-16 Leitz objective, ocular 4 and tube length 17 cm. The drawings were made with a magnification of about 1975 diameters and are reduced somewhat in reproduction.

Figs. 1, 2. Longitudinal sections through ascus. Equatorial plate stage of first division of the nucleus.

Figs. 3, 4. Spindles of third division. Both figures drawn from the same ascus but in different planes.

Figs. 5, 6, 7, 8: Longitudinal section through the ascus at stage of interastral zones.

Fig. 9. Oblique section through the ascus showing interastral zones.

Fig. 10. Cross section throug ascus showing interastral zones.

Fig. 11. Longitudinal section through ascus when rays are just beginning to curve downward about the nucleus.

Fig. 12. Longitudinal section through ascus when fibres are fused near the central body.

Fig. 13. Longitudinal section of ascus. Spores completely delimited. Centers still pointing in all directions.

Fig. 14. Spores little later. Centers all pointing downward.

Fig. 15. Spores partly elongated.

Fig. 16. Spores much elongated. Centers still attached.

Fig. 17. Cross section of elongated spores after nucleus is drawn back to center of ascus.

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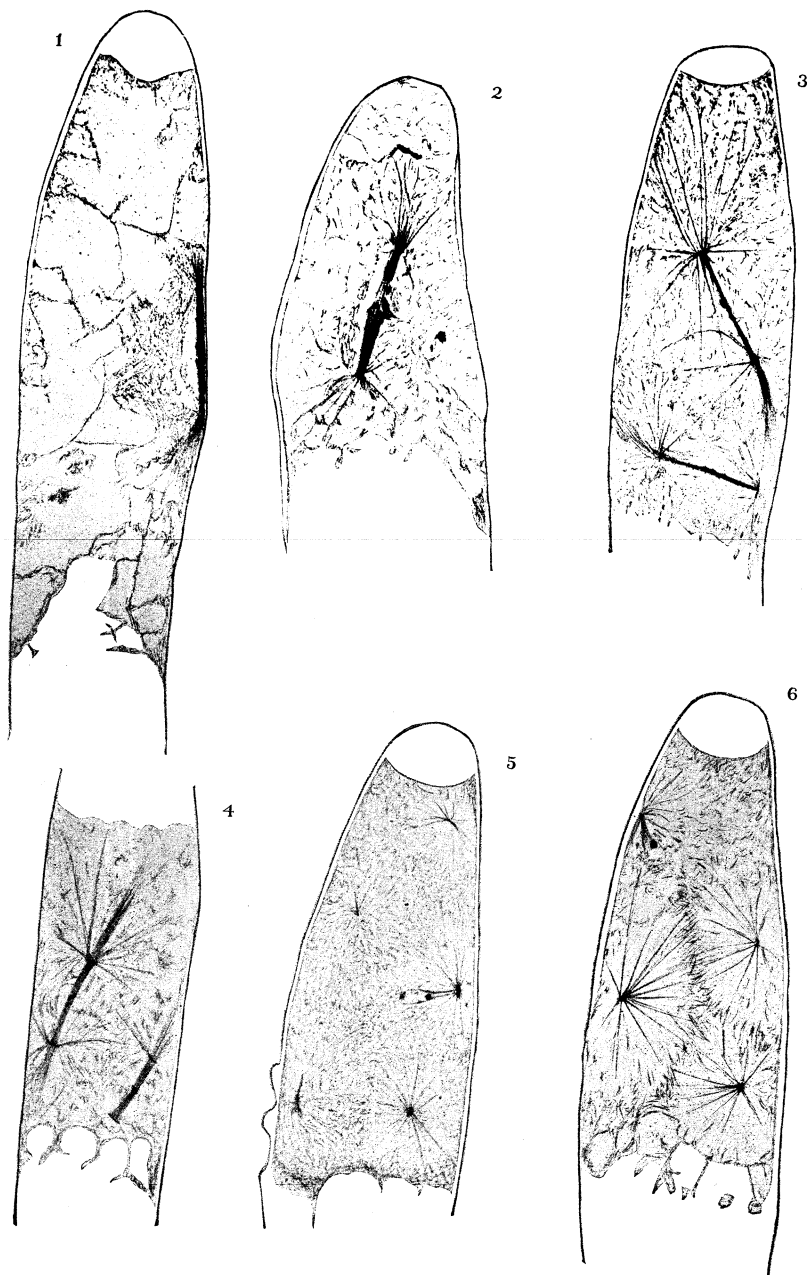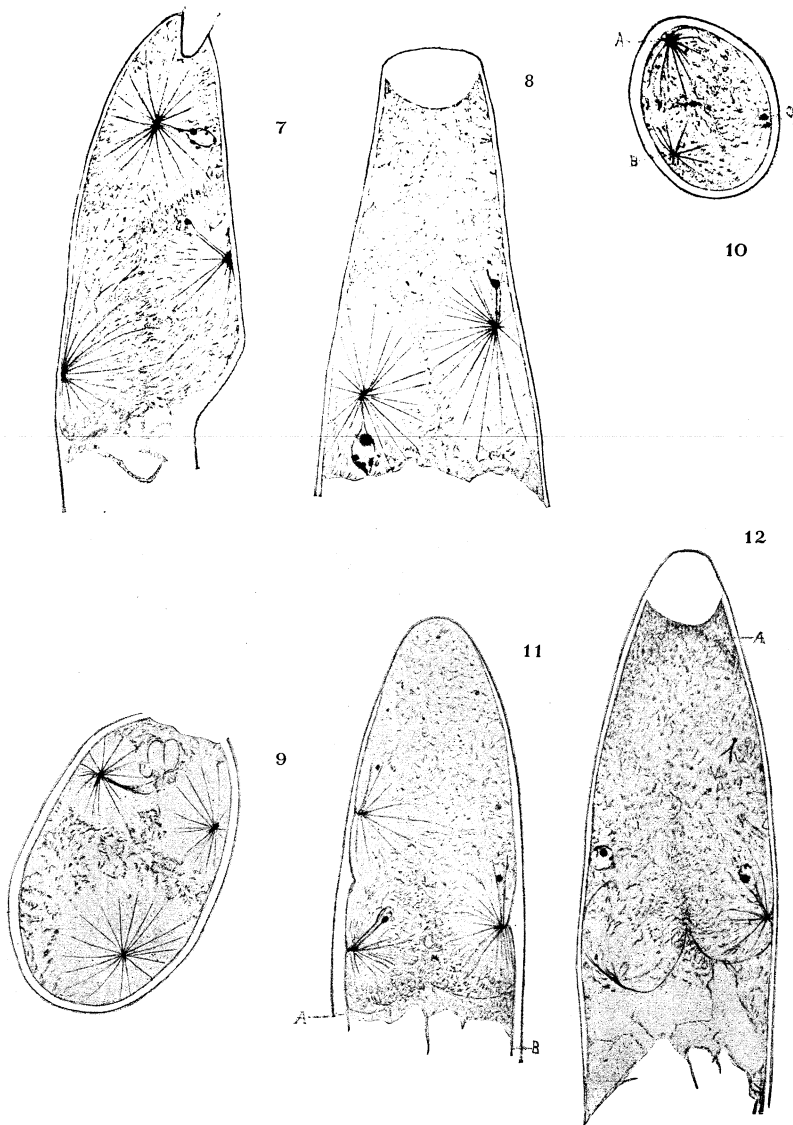
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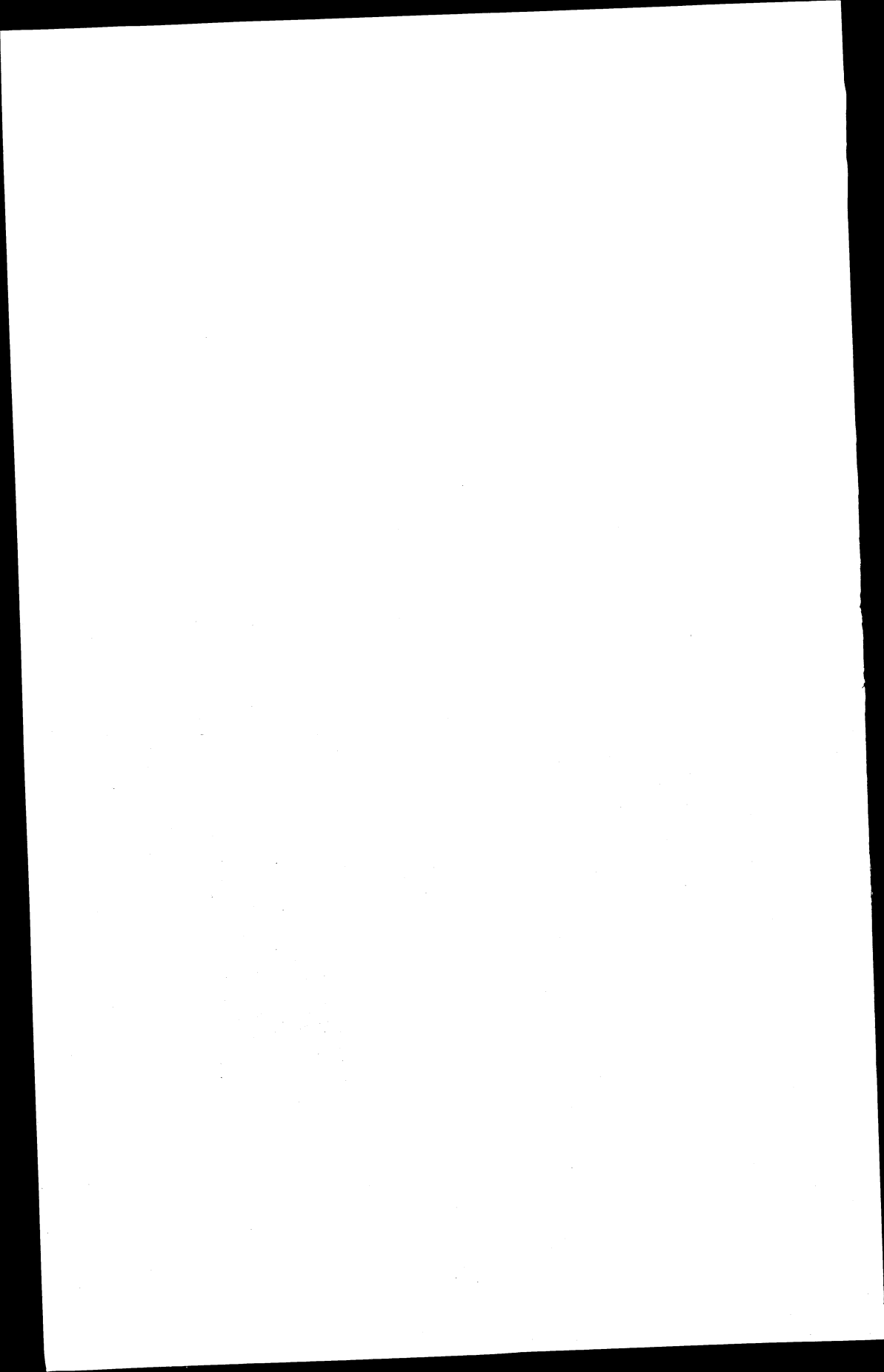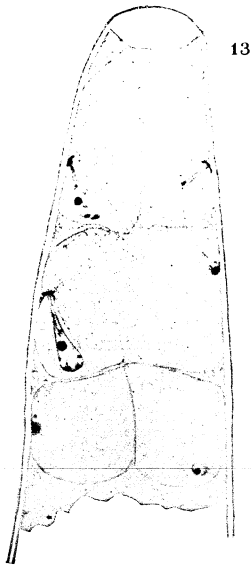
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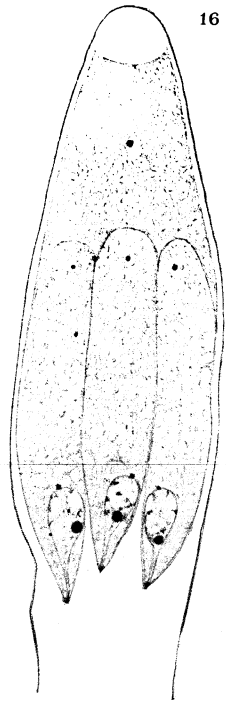
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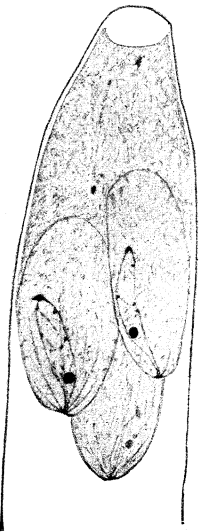
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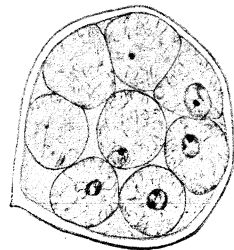
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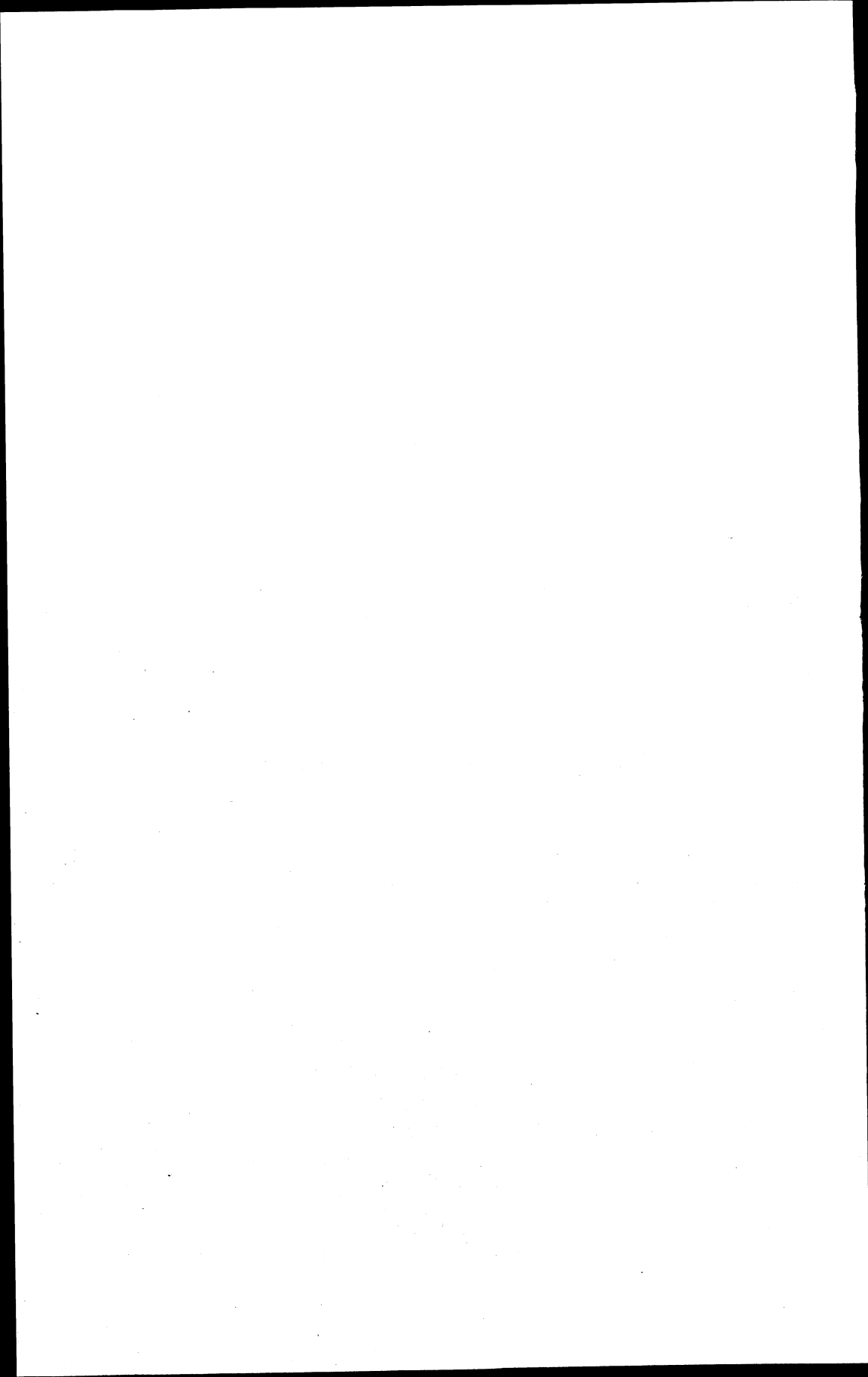
# SOME CONTRIBUTIONS TO THE LIFE HISTORY AND CYTOLOGY OF THE SMUTS.

B. F. LUTMAN.

Our knowledge of the smuts, in spite of their economic and theoretical importance and the immense amount of work that has been done on them, is deficient at many very critical points in their life history. There is much evidence to show as Brefeld (6) has especially argued that they form the lowest group of those fungi that have a basidium. The nuclear phenomena in the two higher groups, the Uredineae and the Basidiomycetes, have received a great deal of attention recently both as to the cells of the mycelium and as to the nuclear behavior in spore formation and germination while little has been done on the Ustilagineae.

The later investigations of the rusts and Basidiomycetes have lead to the general conclusion that they have typically, binucleated cells during a large part of their life-histories and that this binucleated condition is terminated in the teleutospore or basidium by the fusion of the two nuclei. Neither the origin of the binucleated condition of the cells nor the length of the uninucleated phase is the same in the Uredineae and Basidiomycetes.

In the Ustilagineae Dangeard's descriptions of spore formation (12) show that in its early stages the spore is binucleated but later becomes uninucleated by the fusion of the two nuclei. This observation of the binucleated condition of the young spore is of the utmost importance and must be confirmed on as many forms as possible. No successful attempt has been made to go back of the spore to find the number of nuclei in the mycelial cells. All that we have in the literature concerning the num-

ber of nuclei in the cells of the mycelium is scattering and fragmentary.

The earlier papers on the smuts described, of course, only the external features of the mycelium and of spore-formation and germination. Prevost (33) in 1807 by germinating the spores of Tilletia Caries was able for the first time to recognize without a doubt that this disease of wheat was due to a vegetable parasite. He was able also to observe the germination of the spores of Ustilago Carbo from oats. His work was confirmed by Corda and Tulasne (39–40) who by using his methods were able to germinate the spores of additional species. Tulasne published his results in quite an extensive monograph of the group which covered not only classification but also many points in the life history of a number of species.

Fischer von Waldheim (41) described and figured spore formation and the external appearance of the mycelium in a number of species of Ustilago, Tilletia, and in one species of Sorosporium. Winter (42) figured the mycelium and spore formation in Geminella Delastrina and Urocystis Colchici as well as in several species of Ustilago. Cornu (11) in 1883 described the structure of the sori and spore formation of Doassansia Alismatis and D. Farlowii, and in 1892 Setchell (38) monographed the genus Doassansia adding many new points as to their spore-formation and life-history.

Some of the more recent work especially that of a semi-popular nature contains descriptions of the mycelium and spore-formation of some of the species that are destructive to cultivated plants. Miss Knowles (26) has shown the mycelium and spore-formation of the corn smut in her paper on the abnormal structures produced by it. Clinton (10) worked out the life-history and spore-formation in Cintractia Sorghi-vulgaris of the broomcorn.

On account of the ease with which the spores germinate this was one of the earliest facts observed in the life-history of the smuts. Prevost (33), Cornu (11), Tulasne (39–40), De Bary (14), Fischer von Waldheim (41) and a great number of recent investigators have germinated the spores until now we know this

stage of development in nearly all the forms. Brefeld (4 & 6) in particular has paid attention to spore-germination and the saprophytic growth of these forms in various media and has described and figured the germination of 56 Ustilagos, 4 Tilletias, 4 Urocystis, 4 Doassansias, and 4 Tolyposporiums in 20 plates containing over 50 figures. This work stands as a monument of industry and thoroughness. He has also described (5) the methods of infection and figured the beginnings of the parasitic mycelium of the oat, corn and sorghum smut.

Apparently the first cytological work that was done on the group was that of Fisch (16) and Schmitz (37). Schmitz described the mycelial cells of U. longissima as multinucleated and stated that when spore formation takes place the hyphae break up into segments each containing a single nucleus. The spores are formed from these segments and are uninucleated. Fisch investigated species of Tilletia, Urocystis, and Ustilago. He found a single nucleus in the spore but multinucleated cells in the mycelium. He states that the conidia become multinucleated before they cause infection.

Dangeard (12) was the next to study the group in its cytological phases and it is to him that we owe practically all that we know concerning nuclear behavior in the formation and germination of the spores.

Dangeard's (12) first work was a description of spore-formation or germination in the following species: Ustilago Tragopogi, U. Carbo, U. violacea, Doassansia Alismatis, Entyloma Glaucii, Urocystis Violae, and Tilletia Caries.

He finds that the spores of U. Tragopogi arise as short terminal branches of the mycelium, which, by rounding off, form the spores. The young spore contains two nuclei. These fuse in turn and the mature spore is uninucleated. The germination of this spore and that of U. Carbo are practically the same. The nucleus of the spore passes into the promycelium and there divides into two and then into four. The promycelium cuts up into four cells each of which produced a conidium containing a single nucleus. The formation of the spores was not worked out in U. Carbo but was in U. violacea. The mycelial walls of this species at the stage of spore-formation become thick and

gelatinous and the hyphae break up into an indefinite number of segments. Dangeard found it impossible at his time to determine the number of nuclei in the segments and it was only with difficulty after the walls were put in that the two nuclei could be seen in each young spore. From his observations on U. Tragopogi he feels justified in assuming that these two nuclei fuse. The germination of the spores of this species is the same as that for the other Ustilagos he had already described.

In Doassansia Alismatis, Dangeard describes the spores as usually produced as short branches on the principal filaments although some may be intercalary. These branches then become rounded or elliptical. The first ones produced are at the interior of the spore mass while the younger ones are always on the outside. Each spore encloses two nuclei which later fuse. Spore germination was not followed any further than to find, that it was similar to that of Tilletia and Urocystis.

Entyloma Glaucii, he found, forms its spores either apically or perhaps intercalary. The two nuclei of each young spore later fuse.

The spore balls of Urocystis Violae are difficult to follow in their origin but after their formation the fertile cells in the centre each have two nuclei while the sterile cells on the outside are described as not having any. The two nuclei of the fertile cells fuse. In germination a non-septate promycelium is formed containing eight nuclei derived by the three fold division of the original spore nucleus. There are eight uninucleated sporidia as a rule, although there may occasionally be fewer.

Dangeard describes only the cytology of the germination of the spores in Tilletia Caries. A non-septate promycelium is formed containing eight nuclei derived as in Urocystis Violae by three divisions from the original spore nucleus. Eight slender conidia are borne in a crown at the apex of the promycelium. While the conidia fuse in pairs the nuclei do not seem to do so. In the secondary conidia there are always two nuclei, produced by the division of the nucleus of the primary conidium. As will be seen from the above, Dangeard's work relates almost entirely to spore formation or germination. In his general discussion of the group, however, he makes the statement

that the mycelium has multinucleated cells up until the time that spores are to be formed. The statement is made as a general one and so may be assumed to refer to all the genera studied.

Dangeard has certainly brought together a mass of evidence for the regular appearance of two nuclei in the young spore which by their fusion produce the single nucleus of the mature spore. One of these nuclei is regarded by Dangeard as male, the other as female and the whole structure is interpreted as an oogone. The fusion results in an uninucleated oospore; this spore being the equivalent of the uninucleated stage in the ascus, to the same stage in the basidium, or to the teleutospore of the rusts, according to Dangeard's views on the morphology of those structures.

In a later paper Dangeard (13) goes into further details on the spore-formation in Entyloma Glaucii but nothing new of essential importance was brought out.

Raciborski (34) made a detailed study of Entyloma nymphaeae especially as to spore-formation and the peculiar haustoria that this species has developed. He describes the young spores as binucleated but states that later by the fusion of the two nuclei they become uninucleated.

Harper (22) has described nuclear phenomena in the germination of the spores and the formation of the promycelium and conidia in Ustilago scabiosae and U. antherarum. He finds the divisions of the spore nucleus to be of the typical mitotic form but was unable to distinguish whether they were reduction divisions or not. The nuclei of the promycelial cells undergo a similar mitotic division in the formation of the nuclei for the conidia; each conidium receiving one nucleus from the uninucleated promycelial cell. A similar process occurs too in the budding of the conidia. As has long been known the conidia of U. antherarum fuse in pairs when kept in cultures from which the nutrient substances have been largely used up. He found that no fusion of the nuclei was to be observed in these cases, the fused pairs were always much larger than those that had not fused; and usually stood the unfavorable conditions better. He

regards the fusion as purely cytoplasmic "that leads to a growth in the size of the fused cells, perhaps also making them more resistant to unfavorable conditions."

Maire (30) in a short note on the spore formation of Tilletia Tritici states that not only the spore but also the mycelial hypha from which it arises is binucleated. The nuclei in the spore fuse before its maturity.

Istvanffi (25) thought the conidia of Ustilago Zeae contained three nuclei; one in the middle and one at either end. Maire was able to show, however, that Istvanffi had mistaken accumulations of cyplasm in the two ends for nuclei and that the spores were really uninucleated.

Federly (15) working on the fusion of the conidia in Ustilago Tragopogi finds that a nuclear fusion occurs also. The nucleus passes through the connecting tube from one conidium over to the nucleus of the other one of the conjugating pair and the two fuse. The fusion of the conidia involving thus both nuclear and cytoplasmic unions is regarded as a true sexual act and Federly opposes the view of De Bary and Harper that it is only a vegetative union.

The rusts are now by many believed to be nearly related to the smuts and they present so many similarities to them, that in any discussion of the cytology of the smuts a consideration of the important work that has recently been done on the rusts is essential.

It was observed by Rosen (35) in 1892 that the rust spores were all binucleated and that the so-called "basidium" from which the aecidiospores arose was uninucleate. He did not observe the fusion of the two nuclei in the teleutospore but believed that it occurred, as he saw them lying closely pressed together.

In 1896 Seppin-Trouffy's (36) general work on the histological structure of about 35 species from 10 genera of rusts appeared. In this he was able to show that the two nuclei fuse in the teleutospore, and that the mycelium that produces uredospores and teleutospores is binucleated. He was able to observe too that the mycelium was not binucleated at all stages but that it originated as uninucleated and later became binucleated.

Dangeard and Sappin-Trouffy regarded the fusion of nuclei in the teleutospore as the sexual process in the rusts and paid little attention to the origin of the binucleated condition further than to discover that there were pairs of nuclei of different origin far back in the mycelium.

Maire (28) was able to show that the vegetative cells of the hyphae of Endophyllum are uninucleated up to the base of the aecidium. This condition was considered general by him for the aecidium bearing mycelium. The end cells are binucleated and from these binucleated end cells the spores are produced. To Maire is due the credit of recognizing the general significance of this discovery in his suggestion of the alternation of generations in the rusts. The change from sporophyte to gametophyte takes place in the reduction divisions to form the four promycelial cell nuclei.

Holden and Harper (24) showed that the nuclear divisions in the promycelium of Coleosporium sonchi-arvensis are of the ordinary mitotic type with from six to ten chromosomes present instead of two as had been claimed by Maire.

When the origin of the sporophyte had been pointed out by Maire as lying at the beginning of the binucleated condition instead of in the fusion of the two nuclei in the teleutospore, as Dangeard had suggested, the importance of finding the first cells having the two nuclei and the method of their origin was much increased.

Blackman (2) in 1904 and Christman (7) in 1905 found the origin of the binucleated condition in rusts with aecidia of the caeoma type. Blackman in Phragmidium violaceum discovered that the binucleated condition arises by the entrance of the nucleus of a vegetative cell into a large cell, the so-called basidum, above it. Christman in Phragmidium speciosum found a fusion of two such cells giving rise to a binucleated cell from which arises the row of two nucleated aecidiospores.

Blackman and Fraser (3) found in the aecidia of Uromyces poae and Puccinia poarum that the fusion was of the type previously described by Blackman, while in Melampsora Rostrupi there were evidences of a fusion like those described by Christman in Phrag. speciosum. Christman (8) later described

fusions similar to those in Phrag. speciosum in the formation of
the primary uredospores of Phragmidium potentillae-canadensis
and further (9) published a general account of the spore forms
in the rusts in which he advances the view that the aecidio–,
uredo–, and teleutospores are only a series of asexual repio-
ductive stages arising from morphologically equivalent basal
cells in the sporophyte generation and that one form of spore
may replace the other; if there are no aecidiospores, the sporo-
phyte generation begins with the uredospores, etc.

It will be seen from the above that while our knowledge of
the number of nuclei in the mycelium and the origin of the bi-
nucleated condition of the cells of the rusts does not as yet,
cover nearly all the species, still we are sure of enough to make
some generalizations.   It has now been fairly definitely estab-
lished that all forms of spores in the rusts are binucleated begin-
ning with the aecidiospores, or if they are suppressed, with the
uredospores, and that the mycelium that is produced from these
spores has binucleated cells up until the formation of the teleu-
tospores when the nuclei fuse.   There is further, considerable
evidence that the reduction divisions occur in the formation of
the four cells of the promycelium and that from these come un-
inucleated sporidia which in their turn develop into a my-
celium with uninucleated cells.

For an understanding of the life cycle of the smuts it will al-
so be necessary to review the cytological work that has been done
on the nuclear phenomena and on the spore formation of the
Basidiomycetes.   This group while not so nearly related to the
Ustilagineae still has much in common with them in the homol-
ogous structure of basidium and promycelium and of basidio-
spores and conidia.

It was established during the '90's by the work of Rosen,
Wager, and Dangeard that the cells of the carpophore are fre-
quently multinucleated while the basidia are at first binucleated
but later become uninucleated by the fusion of the two nuclei.

Maire (29) in 1902 found that in a number of species the
cells of the sub-hymenial layer are binucleated while many of
those of the stipe and pileus were multinucleated.   He did not,

however, trace the origin of the binucleated cells in the development of the carpophore.

The fusion of the two nuclei in the basidium and the later division of the fusion nucleus has been followed in great detail by Wager, Maire, Ruhland, Juel and others. The division to form the four nuclei which pass into the basidiospores is supposed to be the reduction division.

Harper (21) was able in Hypochnus to trace a series of binucleated cells from the hymenium back into the mycelium in the sub-stratum.

Neither Maire or Harper found the origin of the binucleated cells. Miss Nichols (32) working on this problem came to the conclusion that in some species the mycelium becomes multi-nucleated, very soon after the spore germinates. She found no definite point at which the multi-nucleated condition of the cells passed over into the binucleated condition.

The binucleated condition of the young ascus has been recently shown by Maire (31) to extend in Galactinia succosa into the ascogenous hyphae in the sub-hymenium for several cells at least. This would seem to indicate that the binucleated condition in this species at least is working back into the earlier cells of the sporophyte.

Several of the yeasts show conjugations similar to those of the conidia or promycelial cells of the smuts. Barker (1) has described two cells of Zygosaccharomyces Barkeri as fusing, the nuclei also fuse and then eight endospores are formed. Guillermond (17, 18) has further described such nuclear and cytoplasmic fusions in a number of species of yeasts in some of which it occurs prior to spore formation while in others the spores fuse on germinating.

The difficulty in working out the nuclear phenomena in the entire life cycle of the smut is very great. The saprophytic parts of it are in most cases easier to get than the parasitic stages, especially the earlier stages of parasitism. It is frequently difficult to detect the fungus in the host until it is too late to find out much about mycelial conditions especially in those species in which the mycelium simply breaks up into the

spores. Added to these difficulties is the fact that the mycelium is very much twisted and branched and that the nuclei are very small and not at all easy to differentiate.

I have studied at various stages of development the following species: Ustilago levis, U. Zeae ,U. Triaici, U. nuda, Urocystis Anemones, Doassansia Alismatis, D. deformans, and Entyloma Nymphaeae. As my methods were different for almost every species I shall describe them as I take up the individual forms.

I desire to thank a number of mycologists for material, particularly Dr. J. J. Davis of Racine, Wis. My thanks are due also to Prof. R. A. Harper at whose suggestion the work was undertaken and on account of whose assistance and encouragement it was completed.

## Ustilago levis (Kell. & Sw.) Magn.

Ustilago levis has been our most common species of oat smut during the past three years. As it is available at any time during the summer and the spores are viable for a long time it is on the whole a very favorable smut for cytological study.

In order to obtain large quantities of the conidia for the purpose of infecting seedlings it was necessary to resort to bacteriological methods. A very high dilution of the spores in sterile water was plated out in 1% beerwort agar and kept at a temperature of about 12° C., a refrigerator being used. This temperature seemed to prevent the too rapid growth of other species of fungi such as moulds and Penicillium while it did not hinder so much the development of the smut conidia. After three or four days, small white colonies appear on the plates; these spread in the film of water that always covers agar plates and were allowed to grow until they had attained the diameter of a centimetre. These colonies can be obtained as practically pure growths of conidia and were used in this condition for infection purposes, or they could be still further increased in quantity by inoculating flasks of liquid beerwort and allowing them to stand for a few days. In most cases the colonies themselves were used for inoculations as they were much easier to handle and the location of the infected area on the seedling could be more readily

distinguished by the smear from the agar culture as described below. It required 5–10 days for the colonies to attain sufficient size for use when they were kept at these low temperatures.

In the meantime the oat seedlings were being grown in moist filter paper. Seedlings about 2–3 days old and whose leaf sheathe was not longer than 1 cm. were used for inoculation, those with leaf sheathe about 1/2 cm. long being preferred. The conidia from the colonies on the plates were now spread on the young leaf sheathes with a scalpel. The infected seedlings were then replaced in the damp filter paper and put back in the refrigerator at a temperature of about 12° C. After being kept there for 5–7 days they were planted out in soil in large pots.

By peeling off the epidermis under the infected area and examining it with the microscope the process of infection could be readily followed. The first penetration of the epidermis occurred after 2–3 days and after 5 days many of the epidermal cells were full of the smut mycelium. Keeping the seedlings at a low temperature greatly retards their growth without seeming to have nearly so great an effect on the fungus growth, thus giving the latter as nearly as possible the conditions that are said to favor its growth in nature and cause such high percentages of smutted heads, i. e., cold, damp spring weather. The infection of the growing point of the young plant cannot be seen from the outside but in sections it was found to be infected after about 10 days.

While this method of securing infected heads has been found very successful, the percentage of infection obtained is also dependent on the season when the inoculation is made and the rate of growth of the oat plants. Plants infected in this way about Sept. 1st and kept in the green house over winter did not come to maturity until about Feb. 1st when they showed about 95% of infected heads. Another lot infected April 1st, came to maturity about June 1st and only showed about 50% of infected heads. I am inclined to believe that this difference was not due to increased immunity from infection but to a higher percentage of recovery after infection. The winter conditions in the green

5—S. & A.

house apparently retarded and stunted the growth of the young oat plants so much that the fungus was able, in most cases, to keep pace with it and it was only the most rapidly growing individuals that were able to push ahead of it. When the plants were given a fairer chance under more normal conditions, as in the spring months, a larger number could outgrow the fungus and produce clean heads.

There has been considerable question as to whether U. levis is a true species or whether its characters of smooth walled spores, formed in rather definite pustules, are only the result of physiological conditions in the host plant, leading to these characteristics in the parasite. In the course of these experiments I have carried this smut through three generations and have always had the same kind of heads in which the smut is enclosed in the only partly destroyed glumes and the same smooth walled spores. It may be that my conditions were the very ones required to bring out these peculiarities but it would hardly seem that they would appear for three successive times and in plants grown both rapidly and slowly. So I am inclined to regard Ustilago levis as a stable species.

My principal object in the above experiments was to obtain an abundance of the fungus at all stages of its development for further study of the cell structure, spore formation, etc.

For the younger stages of the mycelium the parts of the leaf sheathe under the smear of conidia were sliced off and fixed in Fleming's weaker solution. For the older stages when the growing point of the plant had become infected (10 days–2 months) the older leaves were removed and the entire growing tip fixed, usually also in Fleming's weaker solution. Staining was largely with the triple stain although the iron-haematoxylin was also used.

In addition to the parasitic stages of the fungus it was possible also, from the cultures to get all stages in its Saprophytic development. The conidia, as has been frequently described before, reproduce abundantly by budding in the same manner as yeasts. Ordinarily the bud drops off almost as soon as formed but in rich media, long chains of these, produced by continued budding without separation of the cells may remain together.

These may produce finally the tree-like groups such as Brefeld (4 & 6) has figured. The conidia (Fig. 1) during all of this budding are usually uninucleated. This is the case in liquid media that are still rich in nutrient substances.

If, however, the conidia are taken from the dense masses on the plate cultures they show quite different nuclear conditions. Sometimes there appears only a single nucleus but there are more likely to be two or more in each cell (Fig. 2). The number does not seem to be definitely fixed though it does not seem to exceed four or five. It seems to be a general rule that the conidia when formed in the crowded masses in the plate cultures may become multinucleated but when growing free in liquid cultures full of nutrient substances they remain uninucleated. In addition to becoming multinucleated these conidia lose the oval shape typical of the oat smut and become swollen and irregular (Fig. 3). A germ tube may be pushed out (Fig. 2) and the material in the conidium travel out into it. As progress is made forward by this tube, successive walls are put in behind the advancing cytoplasm. The forward end loses the form of a conidium and also becomes multinucleated (Fig. 3). In very old cultures these germ tubes may be seen radiating out in all directions from the edges of the colonies on the agar, each of them with a bit of nucleated protoplasm in its tip and a long empty tube behind it. It appears that as the conidia become too crowded and are enfeebled they cease budding and spend their last efforts in putting out germ-tubes; if these do not find a host plant, of course, they die.

Sections made from parts of the leaf sheathe immediately under the smears will show the fungus making its way through the outer epidermal walls. The conidium apparently fastens itself to the wall and proceeds to penetrate it (Fig. 4–5) while the host in response to this stimulus builds the wall thicker. The fungus pierces further and further into the cell until finally the wall is passed and the conidial tube makes its way into the protoplast through a small opening. This continued dissolving and thickening of the walls produces the funnel-like openings of the conidial germ-tubes observed by Brefeld (5) in surface

view.   Once through the outer wall the hyphae grow straight across the cell and pierce the inner wall.

It is difficult to follow this process except at places where the conidia are rather scattered.   It was very unusual to find more than three or four tubes making their entrance in one section (Fig. 6) of an infection spot.   There seems little doubt, however, that the successful entrance of even one germ-tube into a plant might under favorable conditions be sufficient to smut the entire oat head.

In the material in which the early stages of infection were found the conidia usually had 2–3 nuclei; the majority having two (Fig. 6).   The conidia which had failed to make their way in, usually had about the same number and there was no evidence that the successful ones were in any way different so far as number of nuclei is concerned.

A few days later, and about the fifth day after infection, the cells of the leaf sheathe will be found to be full of much branched, sparingly septate, multinucleated fungus cells.   As the hyphae make their way through the leaf sheathe they are largely intracellular in position but they become almost entirely intercellular in their distribution in the young growing plant.

Young plants fixed and sectioned ten days to two weeks after the infection have an abundance of smut mycelium between the cells of the first node and of their tip (Fig. 7).   Young plants sectioned from this time up until pretty close to flowering show practically the same conditions as to distribution of the mycelium; it being most conspicuous at the nodes and the growing tips.   There is, as other authors have observed, apparently some kind of an equilibrium maintained between the host and its parasite.   In spite of the numerous hyphae that run all through the growing region the normal functions of the oat cells are apparently not interfered with.   The cells divide normally in the usual mitotic manner (Fig. 9) and the nuclei and cytoplasm give no evidence that they are abnormal in any way.

The hyphae of the smut are scattered throughout the growing tip of the young plant with apparently little reference to its structure.   The growing tip and the base of the leaves show the most abundant hyphae (Fig. 7); the central parts of the plant

in sections not showing quite as many fragments of mycelium. The hyphae (Figs. 11–12) at this time are very much contorted, of varying diameter, the cells multinucleated, and almost entirely intercellular (Fig. 9). The young leaves are full of the hyphae (Fig. 8) and even those that have lengthened may show the presence of the fungus up nearly to their tips. Many of the hyphae in the leaves are found to be intracellular in contrast to those nearer the axis of the plant. This is in plants six weeks to two months old in which the leaves are fairly well developed. Of course, no indication of spore formation appears in any part of the oat plant at this time.

At flowering time longitudinal sections (Fig. 10) through the young infloresence showed the fungus in the older flowers as young spore pustules. The younger flowers show all stages in the development of the smut spores.

The characteristic of this smut in addition to its smooth walled spores is that it does not convert the entire flower into a dusty mass but forms pustules of rather small size which, as they do not run together, do not destroy all the cells of the flowering glumes. The glumes thus retain the shape of the flower and keep the spore mass from becoming entirely diffuse. Spore formation can be traced in all its stages in a single panicle fixed at the proper time (Fig. 10). Sometimes the flowers near the tip remain immune while the ones below them are attacked indiscriminately especially the older ones near the base. At this time the rudiments of the glumes and sometimes of the stamens are still to be distinguished but the smut seems to make no discrimination as to which tissue is attacked first in forming spores. None of the floral parts ever come to maturity except the glumes; all the other are converted into spores before they are fully differentiated.

The first indication of spore formation in the fungal hyphae is a much branched and contorted condition of some of the hyphal tips. These are at the time intercellular and this knotting up of the hyphal tips frequently occurs at the angles of the host cells where they may be wedged apart considerably. These swollen ends of the hyphae (Fig. 13) are multinucleated, each one containing 10–15 nuclei.

The cell walls of the hyphae now begin to gelatinize from the inside (Fig. 14), a clear zone appearing between the protoplasm and the darker staining wall.   The nests or pustules of hyphae continue to grow and swell and their walls become so completely gelatinized at this stage (Fig. 15) that all that seems to be present is a tangle of hyphae of irregular shape and varying diameter without walls and lying in a clear matrix.   At the same time the walls of the host cells immediately adjacent lose the capacity to take up the stain, the gelatinization of the fungal walls having apparently extended to the walls of the host cells also.

The gelatinized walls do not stain at all in the iron haematoxylin and only take a faint blue in the triple so that all that appears in the sections are the darker staining protoplasts of the hyphae.   The protoplasm of the latter seems to be so dense at this time that it is almost impossible to distinguish nuclei. Bodies appear in them which may be nuclei (Fig. 16) but it is impossible to say with great certainty.   The hyphae continue to spin out more and more becoming still more finely attenuated in places until they are apparently pinched off into segments (Fig. 17).   At this time these little pieces of hyphae are very angular and the walls difficult to make out.   Some of these little pieces show two nuclei and some one.   Whether the two fuse to form the one cannot be ascertained with certainty as they are exceedingly small and about all that can be said about them is that they sometimes contain two dark staining bodies and sometimes one.   The irregular segments now change their shape, round up, and the spore wall begins to develop.

From this account it will be seen that the uninucleated conidia of U. levis frequently become multinucleated before they cause infection.   The mycelium is composed of multinucleated cells from its beginning and these are continued throughout the life of the fungus up to the time of spore formation.   At that stage the hyphae break up into segments, the number of nuclei of which is difficult to determine, but which is either one or two. The young spores contain one or two nuclei and the mature ones, one, but it is not possible to determine with certainty whether at some early stage all have two which later fuse to form the single one.

## Ustilago Zeae (Beckm.) Ung.

Pure cultures of the conidia of this smut were obtained by plating out the spores on 1% beer-wort agar. A transfer was then made to flasks of liquid beer-wort. When a thick white sediment of conidia had collected in the bottom of the flask the culture was shaken up and sprayed into young corn ears in which the silk had just appeared as recommended by Brefeld (5). After a week or ten days the young kernels were found hypertrophied usually on one side or one end. These affected parts were fixed in Flemming's Weaker Solution. Sections of such a part would show an abundance of smut hyphae between the cells. The very early stages when the smut was making its way into the ear could not be obtained in this way but all later stages up to and including spore formation were abundant.

The appearance of the mycelium (Fig. 18) in these hypertrophied parts of a kernel is practically the same as that of the mycelium of U. levis in the oat plant. It is largely intercellular, much twisted and branched, and the cells are multinucleated. The host cells are apparently in a fairly healthy condition but somewhat hypertrophied.

The process of spore formation is essentially similar to that in U. levis. De Bary (14) and Miss Knowles (26) have described the external features of it very accurately. The blunt and much branched ends of the hyphae form balls which fill the intercellular spaces between the host cells. There is great variation in the size and shape of these hyphal ends but they are always multinucleated. The same gelatinization of the hyphal walls occurs, making it impossible to distinguish anything accurately in the hyphae at just this stage. Some of the segments show, when stained with iron-haematoxylin, two dark staining bodies that are undoubtedly the nuclei. The segments (Fig. 19) at this time may be irregular or only long, but they all have very thick walls. A little later (Fig. 20) when these segments have changed their shape and become spherical, it is easier to distinguish the nuclei. Some of them show two but there are others with apparently only one. The same thing that was said of U. levis applies here; it is impossible to prove whether a

fusion really takes place or not, as in both species the nuclei are so exceedingly small. The mature spore, however, is uninucleated and its cytoplasm frequently contains very large vacuoles while the nucleus is pushed to one side.

In all essentials the mycelium and spore formation of U. Zeae will be seen to be the same as that of U. levis. The vegetative mycelium, composed of multinucleated cells, breaks up into short segments containing one or two nuclei which pass into the spores. In both species it is impossible to determine whether two nuclei are always present in these segments or whether they fuse to form the one nucleus which appears in the mature spores.

### Fusions in the Promycelial Cells of Some of the Smuts.

The spores were germinated in drop cultures on slides placed on racks in damp chambers. The material found best for this purpose was an exceedingly dilute beer-wort. An addition of even a few drops of beer-wort to a watch glass full of water was sufficient to help the germination of the smut spores and not sufficient to cause much immediate growth of moulds. The food material being so dilute was soon used up and the promycelial cells started almost immediately to fuse in pairs. These cultures could be kept without drying out or without so very much contamination for a week or ten days which was as long as it was necessary to obtain material at all stages of development. The spores of U. hordei, U. Avenae, and U. Tritici were used for this germination and all showed practically the same results.

The promycelia were stained on the slide directly by means of a modification of the method proposed by Harper (22): A slide was rubbed with albumen fixative as for sections, one of these cultures was pipetted on it and the Flemming's weaker solution added. The fixing fluid coagulated the white of egg and the spores and promycelia stick in it after the drop is allowed to dry out. After bleaching in hydrogen peroxide the slide was stained in iron-haematoxylin or the triple stain.

Under the above conditions the promycelial cells fuse in pairs almost as soon as formed; there is sometimes a fusion in threes

but this is exceptional. Each cell of the promycelium is uninucleated. If now some of the promycelia be taken from the cultures 3–5 days old it will be seen that conditions are changing. The cytoplasm of each cell of the fused pair is becoming highly vacuolated and the nucleus of one cell can be found at all stages of passage from one over into the other. This passage of the nucleus can be traced in great detail from one cell into the other (Figs. 30–32) through the conjugation tube. In the passage it often becomes drawn out or amoeboid in shape. This movement of the nucleus occurs before there is any flow of cytoplasm; seeming to be the result of some kind of an attractive stimulus exerted on one nucleus by the other. It is probable too that food supply has something to do with it as the movement seems to be toward the better nourished cell. The cytoplasm of the enucleated cell becomes more and more vacuolated and the material in it begins to withdraw into the one with the two nuclei (Fig. 33). As it retreats from the enucleated cell it builds successive walls behind it (Fig. 34). The enucleated cell finally appears very much shriveled as a result of losing its contents and in some cases with a number of cross-walls marking the successive retreats of the cytoplasm. In the last stage all the material from both cells is found in the one with the two nuclei. The two nuclei may now lie side by side closely pressed together or they may be at some distance apart. As to whether they actually fuse or not is rather difficult to decide but at any rate they become so closely pressed together that it is impossible to differentiate them as two in some cases (Figs. 33 and 34). It must be remembered too that at this time the cell is filled with large vacuoles in many cases and the nuclei are compressed between them so as to lose their shape. At this stage, the nucleole, which earlier is easily differentiated, cannot be readily distinguished. If the nuclear fusion occurs it is probably only in a part of the cases; in many, the two nuclei simply seem to lie separate in the cell until it dies.

In one case (Fig. 35) that was observed, three promycelial cells had fused and the nuclei of all three was in the middle one. Another interesting variation is that which occurs sometimes in U. Avenae where a long fusion tube is frequently formed, the

tube being much larger than the cells it connects.   In these it is usual to find all the greater bulk of the cytoplasm with the two nuclei has flowed out into this tube.   The two cells are left comparatively empty while the tube contains a dense cytoplasm and has the two nuclei either separate or apparently fusing.

I have not been able to determine what would become of these cells if they could be started growing again.   This was found impossible as by the time the two nuclei were in one cell the cultures were in such a condition from contamination with bacteria that it was impossible to give the smut cells a new start by adding fresh beer-wort.   It would be interesting also to determine whether these large fusion tubes could cause infection or whether even the fused promycelial cells themselves could do so. It has generally been assumed that the promycelial cells are the equivalents of the conidia but it has not been shown directly that they can produce infection.

## Doassansia Alismatis (Nees.) Cornu.

Infected spots on the leaves of Alisma plantago were fixed in Flemming's Weaker Solution, then imbedded, sectioned, and stained either with iron-haematoxylin or the triple stain.

In the main my work on this species confirms that of Dangeard (12) but I have been able to observe a number of further facts on this highly specialized smut.

A single section will usually show a number of stages in the development of the spore-balls; the older ones being in the middle and the younger ones on the edges of the infected area.   The spore-balls originate apparently as described by Dangeard as a tangle of hyphae in an intercellular space (Fig. 47).   From these hyphae are budded off the spores as short branches.   At first the mass of spores is undifferentiated, all containing two nuclei, but as they become more and more tightly pressed together, the outside ones lose their nuclei and become thick walled, forming the outer sterile layer of the spore-ball.   Later, the two nuclei in each fertile spore fuse.

The mycelium is difficult to follow but in the spaces formed between the spore-balls the hyphae frequently travel straight for

a considerable distance and in this position can be studied to advantage. They seem to have two nuclei which lie rather close together, and while the ends of the cells cannot in the majority of cases be made out; in some cases (Fig. 46) they can, and in these cells the binucleated condition can be determined with certainty. Further, the young hyphal branches from which the spores seem to arise seem to be binucleated in some of the few cases that I was able to trace. If the fungus does not have binucleated cells universally, at this stage of development, there is at least a strong tendency toward the binucleated condition.

An interesting fact with regard to the germination of the spore-balls was brought out in examination of sections of rather old infected spots of the leaf. Brefeld (6) has described and figured the germination of the spores in water after the spore-balls have been teased out or after the ball has been broken up but does not describe the germination in situ in the leaf. When the free spore-ball germinates after being freed from the leaf it pushes out its promycelia in a radial manner all over the surface of the ball between the sterile cells. In germination in the leaf (Fig. 48), however, and this may apparently occur as soon as the ball is mature, a break occurs at one point on the surface of the ball, usually on the side nearest the leaf epidermis, and is due to the first promycelia wedging the sterile layer apart at that point; the figure that is formed being almost an exact reproduction of the one given by Magnus (27) for Setchellia punctiformis (Niessl) Magn. The promycelia are always long enough to reach the exterior and protrude from this opening so that they may bear their tufts of conidia at the apex, exteriorly to the surface of the leaf. This confirms Setchell's (38) statement that the balls germinate immediately after formation.

#### *Doassania deformans* (Setch).

The material for this work was fixed and sent to me by Dr. J. J. Davis of Racine, Wis., where it occurred on Sagittaria variabilis. It was imbedded, sectioned, and stained in iron-haematoxylin or the triple stain.

The hypertrophied petioles were full of smut balls in all stages of development.   This is one of the forms of smut balls in which the fertile cells are on the outside and the sterile cells form a pseudoparenchyma in the interior; the exact reverse of the conditions in D. Alismatis.   Setchell (38) has described the morphology of the smut balls of this species very thoroughly.

The ball begins as a tangled mass of hyphae in one of the intercellular spaces of the hypertrophied host tissue (Fig. 40). It is not possible to make very much out of the nuclear structures at this time as the cytoplasm is very dense.   Careful staining, however, with the iron-haematoxylin will bring out the fact that many of these cells are binucleated; whether all are so is difficult to say.   I should say from my preparations that the binucleated condition is probably constant, for while in some cases the cytoplasm is too dense for the nuclei to be seen, in all cases where they can be seen there are always two and only two present.

At the beginning, the cells are all alike, but those on the interior soon begin to lose their contents and become transparent (Fig. 41).   The material in the cell seems to all go to increase the size of the cell.   The outer cells remain dark-staining and apparently furnish new cells to the central region as the original number of cells there is not a fourth of that which is found in the mature balls and I do not believe that these central cells divide.   It may be possible that the hyphae from the outside push in between the fertile cells on the outside and add further pseudoparenchymatous cells to the interior and there seems to be some indication that this occurs.   In the last stage, that of the nearly mature spore-ball, the external cells are dense with cytoplasm and contain two nuclei that are in various stages of fusion.   Surrounding these are the protective hyphae, wound around the exterior of the ball, making a felted layer outside it (Fig. 42).

The nuclei of the cells of the mycelium are rather difficult to differentiate out but in the felted layer around the spore mass two nuclei are usually found associated together.   In the long hyphae, also, that run across the intercellular spaces an arrangement of the nuclei in pairs seems to be the rule in the cells.

Many of these cells are so long that it is not possible to follow them to their ends but in the shorter ones (Fig. 43) that can be traced there are two nuclei. Whether the cells are all regularily binucleated cannot be decided with certainty but there is certainly a great tendency toward the binucleated condition.

This fungus has small haustoria by which it holds itself in place in the intercellular spaces and gets material from the host-cells. Sometimes two or three of these can be seen in a single section making their way into a single cell (Fig. 44). They are very small in proportion to the size of the host cell. Their form is unlike that of the haustoria of the rusts and mildews as they branch repeatedly as soon as inside the wall (Fig. 45); the branches are very short and give the haustorium the appearance of a bunch of grapes hanging down in the cell. On account of their small size it is impossible to differentiate their nuclei.

### *Urocystis Anemones* (Pers.) Wint.

This species occurs every spring in great abundance on all the aerial parts of Hepatica acutiloba. In the young stages of spore-formation the pustules are observable as white spots on the leaves or petioles. The smut from the petioles was found better for the study of the mycelium as the hyphal cells are compelled to run parallel to elongated host cells and can be traced more readily. Parts of the stem and leaves showing the white blotches of the smut were fixed in Flemming's weaker solution, and stained in the triple stain.

At the time when the white pustules appear, the smut is already far advanced, and many of the hyphae are beginning to form spores. At first it seems impossible to make anything out of the tangle but by carefully selecting a place where they are not so dense and where the filaments are forced to run parallel to the host cells it can be seen that the cells are binucleated (Fig. 21). Whether this is true of all is impossible to say but certainly the majority of the hyphal cells show two nuclei closely associated and frequently the end walls of the cells can be made out. While there is great variation in the size and length of

cells in this region those that can be followed are certainly bi-nucleated.

The spores begin as short side branches (Figs. 22–23) con-taining two nuclei in a very dense cytoplasm. The two nuclei may lie in the long axis of the cell or transversely. The branch increases in length and size, cuts up into new cells so that a chain of binucleated cells is produced (Figs. 24–26). In the meantime it becomes wound around on itself and contorted so as to make it impossible to follow its turns. Sometimes it seems to branch and the branches to grow in so as to become a part of the knot that is forming the spore-ball.

The cells of the ball grow very much in size and their two nu-clei can now be easily seen lying side by side in each spore (Fig. 27). In well stained preparations the nucleoles can be followed through all the stages in the progress of the fusion of the two nuclei up to that of the mature spore with its single large nucleus and nucleole. All the cells are binucleated at this time; both those that are to form the sterile cells and the young spores. The difference between the two lies in the cytoplasm; those that will become sterile contain little stainable material while the spores are quite dense, but both are binucleated. On account of the small size of the nuclei and cells it is impossible division. It seems probable that the nuclei of this chain of spores are formed in this fashion as the nuclei frequently lie in a position to suggest the occurence of such divisions and the cells are broad enough for the two spindles to lie side by side.

In this smut also, haustoria are present. The hyphae are found almost entirely in the intercellular spaces and apparently depend on the haustoria to get their food from the host cells. The haustoria originate as short side branches which penetrate the cells. Sometimes they are quite large, extending far down into the cell (Fig. 29), while at other times they are merely little branched hyphae (Fig. 28) that only penetrate the cell wall. Their characteristic feature is that whether large or small they branch extensively as soon as they are inside the cell wall; if the haustorium is a large one these branches may run all through the host cell. In the smaller types the branches are short and stubby. In the cases I have observed, there seem to

be two nuclei present in the larger haustoria; the two lying in the neck of the haustorium just at the point where it begins to branch.

### *Entyloma Nympheae* (Cunn.) Setch.

This smut is very common on our species of white water lily (Nymphaea reniformis) in the lakes around Madison, forming whitish patches on the under sides of the leaves. As the smut occurs in the intercellular air spaces, it was impossible to fix it in any of the osmic or chromic fixing fluids as they would not penetrate to the mycelium. Carnoy's fluid was found, however, to give most excellent results on the fungus although it caused some shrinkage in the host cells. The sections were stained in the triple stain or in the iron haematoxylin.

Raciborski (34) has described the spore formation and also the remarkable haustoria of this species in their histological features. His paper was not available to me in the original but he was kind enough to copy and send me his figures and a summary of his work.

The spores and mycelium lie in the intercellular spaces of the lily leaf (Fig. 56) and the spores are formed especially in the large air-spaces just above the lower epidermis and in the air chambers below the stomata, although they may be formed in any free space between cells in the leaf. The spores of this species are always borne as the ends of short side branches.

A short lateral branch, very dense with cytoplasm, is put out from one of the larger hyphae (Fig. 55). Inside of this are two nuclei usually arranged parallel to the long axis of the cell as it is narrow at this time. This branch grows both in length and thickness and the two nuclei come to lie side by side in it (Figs. 57–62). The stalk of the spore becomes vacuolate and finally a large vacuole seems to cut the spore off from the hypha bearing it and a wall is put in behind it. The wall of the spore thickens and the cytoplasm becomes filled with vacuoles. In the mature spore the wall has become very thick and is covered with minute spines (Fig. 61); the end is apiculate by the thickening of the end wall; and a large vacuole often fills a large part

of the spore, the nucleus being pushed to one side by it. It is rather difficult to distinguish the fusion nucleus inside the spore as the process of fusion is not complete until the spore has attained a very thick wall. This takes up the stain and makes it difficult to see any structures inside the spore.

The hyphae are the largest found in any of our common smuts. They lie in the intercellular spaces among the palisade parenchyma and pulp parenchyma cells of the leaf and form long strands across the air spaces above the lower epidermis. The branching is characteristic of this smut, and of Urocystis at least, if not of other species; a side branch always originating in front of a partition across the main hypha (Fig. 49–55). The cells are typically binucleated; the two nuclei usually lying in the part of the cell formed by the main hypha, although one of them may lie out in the branch.

The nuclei are large, occupying a large part of the transverse diameter of the cell lumen and always lying in its main axis. They show a well differentiated nucleole and chromatin reticulum (Fig. 51). While conjugate divisions were not observed, it seems entirely probable that they occur here as in the rusts where the nuclei are frequently similarily placed. In spore formation the tubes which lead to the small branch that is to form a spore are entirely too small to admit more than one nucleus at a time, and the two may be seen following one after the other into the young spore (Fig. 57). It is impossible, of course, at this time to distinguish whether the two going in, are sister nuclei, or nuclei that have been produced by conjugate division from a pair in the main hypha. I have been unable to find division figures in the nuclei.

The large haustoria are especially striking in this species and serve probably both as holdfasts and as organs by which the host plant is drained of its food. Raciborski (34) has given a good account of their external morphology with the exception of the appressorium but did not discuss their nuclei.

These haustoria originate like ordinary side branches from hyphae in the intercellular spaces. They are binucleated and usually short (Fig. 49). They are pushed out against the cell wall and there begin to branch, not to form a sack as Raciborski

(34) stated it, sending out short projections which become closely appressed to the cell wall to form a sort of appressorium (Figs. 63, 70, 71) such as occurs in the mildews except that in this case it is much larger and better developed. The host cell wall under this appressorium ultimately becomes dissolved away and a hypha is pushed down from its middle into the cell. In the meantime, however, the host cell is not entirely passive; as a response to the irritation of the appressorium it has thickened its wall under the spot where the stimulus occurs (Fig. 70).

There is apparently a struggle but the parasite ultimately penetrates the host cell. The hypha is very much constricted at this point where it enters the cell but once in, it swells out into a tube as large as the normal hyphae of the intercellular spaces. It now begins to branch profusely and almost fills the entire host cell with short hyphae. The host cell nucleus is frequently enclosed in a tangled knot formed of these haustorial hyphae and as Raciborski (34) noted is hypertrophied. One haustorium is found in a single cell and the absorbtion of material by it is continued until the cell is dead.

There are two nuclei in these branches that form the young appressorium and this number is maintained in the mature haustorium. They lie in the swollen ends of the appressorium (Fig. 70) and in the stalk of the haustorium just after it has entered the cell but has not started to branch (Figs. 64 & 65). While they may be seen taking other positions this location in the neck of the haustorium is probably the most favorable for their functions in relation to the entire haustorium. As noted before they take this same position in Urocystis Anemones.

### THEORETICAL DISCUSSION.

The similarity of the promycelium which comes from the chlamydospores of the smuts to that which comes from the teleutospores of the rusts has been the principal reason for assuming as Brefeld (6) has that the two groups are closely related. While this promycelial tube is always divided into four cells by three transverse walls in the rusts and the number of

6—S. & A.

cells may vary in the smuts or there may be a non-septate promycelium as in the Tilletias, still as Brefeld (6) has shown, there are variations in the basidia, especially those of the Tremellineae, which are perhaps parallel in some degree to those in the rusts and smuts. An exhaustive study of the basidium in all its forms has been made by Brefeld and the system of classification which he worked out is the quite commonly accepted one today for the groups of the Ustilagineae, Uredineae, and Basidiomycetes.

Our belief in the relationship of these three groups was still further strengthened by the discovery by Dangeard (12) and Sappin-Trouffy (36) that the formation of both teleutospore and chlamydospore was preceded by the fusion of two nuclei in the young spore which made a striking parallel to the fusion of the two nuclei in the young basidium.

It has now been well established that this fusion of two nuclei in the teleutospore of the rust and a similar fusion in the young basidium is universal for the two groups having these organs. And my results on the smuts above described confirm the views of Dangeard, Maire, and Raciborski that a similar fusion occurs regularly in the chlamydospore of the smuts. Microtome sections bring out very clearly as described, the great difference in the spore formation of the two divisions of the smuts: the Tilletiaceae and the Ustilaginaceae. In the latter group an entire group of hyphae break up into spores, while in the first the spores are borne as side branches or at the tip of the main hyphal branches.

The question whether the nuclear phenomena are the same in the two cases is a difficult one. Dangeard (12 & 13) has found a nuclear fusion in the formation of the spores of Doassansia Alismatis, Entyloma Glaucii, and Urocystis Violae of the Tilletiaceae; Maire (30) has found it in Tilletia Tritici, and Raciborski (34) in Entyloma Nymphaeae. In my own work I have been able to confirm the existence of the fusion in Doassansia Alismatis and Entyloma Nymphaeae and to show that it also occurs in Urocystis Anemones and Doassansia deformans. Practically all of the most decisive work has been done on this division of the smuts, while little has been done on the other.

Dangeard definitely asserts that a fusion occurs in Ustilago Tragopogi; in U. Violacea there are two nuclei present and sometimes only one but it is impossible to see the fusion. The reason for this uncertainty is, of course, the small size of the cells and nuclei. My own observations indicate that a fusion occurs in the young spores of Ustilago levis and U. Zeae. But further evidence is needed to make it perfectly clear that the same nuclear conditions are present in both groups. It may be that there are species with larger cell and nuclei that are more favorable for study than those of the species studied so far.

Soon after the discovery that the two nuclei fuse in the basidium and teleutospore, appeared the additional fact from the work of Sappin-Trouffy, Ruhland, Maire, and Harper that the mycelium from which the teleutospores and basidium arise is binucleated and especially that the sub-hymenial cells are binucleated. It is fairly well established that the two-nucleated stage in these fungi represents the sporophyte generation in their life history and hence it is of the first importance to discover whether in the smuts this condition of the cells is limited to the spore alone or whether it extends throughout a large part of the mycelium. Previous authors have done little on the nuclear conditions in the mycelium. All that we have are the statements of Dangeard (12), Fisch (16), and Schmitz (37) that the mycelium is composed of multinucleated cells.

My observations show, however, that while this statement that the mycelium is made up of multinucleated cells is probably true of the genus Ustilago it is probably not true of the Tilletiaceae. Entyloma Nymphaeae has binucleated cells. The same is probably true of Urocystis Anemones, Doassansia Alismatis, and Doassansia deformans; at least many binucleated cells are present in the later stages. Maire (30) also has made the statement that the spores of Tilletia Tritici arise from binucleated cells in the mycelium. Dangeard's figures of spore formation in Urocystis Violae, Entyloma Glaucii, and Doassansia Alismatis (12) show a mycelium in which the cells nearly all have two nuclei associated together.

It is true that all these observations relate for the most part to the time of spore formation and that this binucleated condition

may not in all cases extend far back of this, or even may be characteristic of it; still in Entyloma Nymphaeae the entire mycelium is binucleated. In general it may be said at present as to the nuclear conditions in the smut mycelium that in the genus Ustilago the cells are multinucleated up to the time of spore formation; in some of the Tilletia group the cells are certainly binucleated while in others they are probably so, at least to the extent that many binucleated cells are present.

The question arises for the smuts as for the rusts and Basidiomycetes as to the point of origin of this binucleated condition. For the rusts it is now generally agreed according to the work of Blackman (2) and Christman (7) that it arises in some way in the aecidium, or in the primary uredo, if the aecidium is lacking. In the Basidiomycetes Miss Nichols (32) found the binucleated condition arising irregularily at almost any point in the mycelium which formed the carpophore.

As described above we have a fairly complete life history of one of the types with multinucleated cells in Ustilago levis and there are a number of points of interest here to which it is necessary to call attention. The divisions which the nucleus undergoes in forming the nuclei for the promycelium should perhaps be assumed to be the reduction divisions on analogy with the rusts. Usually there are two of these divisions in the genus Ustilago while in Tilletia there are at least three, one of which must be on this hypothesis only a vegetative division. The promycelial cells on this hypothesis have the reduced number of chromosomes and the conidia that come from them also have the reduced number and hence are gametophytic. When the nucleus of this conidium divides without the conidial cell dividing, has the cell now become necessarily sporophytic? Certainly not, since the condition is evidently unstable and the nuclear divisions may continue till the cell contains twenty or thirty sister nuclei. The sporophytic generation cannot be assumed to begin until the binucleated condition is definitely established and it is very difficult to say at just what point this occurs. There seems to be no possibility that the binucleated condition in Ustilago is established by any thing in the nature of cell fusion or nuclear migration.

Tilletia and its related genera are presumably more specialized than is Ustilago and it is interesting to note that the binucleated condition is present in them at an earlier stage preceding spore formation. This may well be due to the working back of the binucleated condition in the life cycle from the spore in which only it is present in Ustilago.

That the sporophytic binucleated condition of the cells can work back in the mycelium has been recently shown by the work of Maire (31) on Galactinia succosa in which he found that the binucleated condition which was supposed to appear only in the ascus cell may appear in several cell generations back of it. Such a tendency might lead to a form with binucleated cells throughout its ascogenous hyphae and this condition might lead to the entire suppression of the original type of fertilization such as occurs in Pyronema, Sphaerotheca, etc. according to the work of Harper (19, 20, 23). The old, normal fertilization at the beginning of the sporophyte generation might thus become lost or suppressed.

In the rusts this may have happened. The binucleated condition of the teleutospore with conjugate divisions may have worked its way back until, as we find, the entire mycelium of the sporophytic generation is composed of binucleated cells. In the Basidiomycetes the process apparently has not gone so far. In them, as Miss Nichols (32) has shown, the binucleated condition does not arise at any one region in the mycelium but may start at almost any point in it.

In this connection the fusion of the cells in the promycelium of Ustilago is interesting. These fusions are entirely similar to those in the yeasts but in them the fusion leads to different results. In the yeasts the fusion is evidently a sexual process according to the accounts of Barker (1) and Guilliermond (17 & 18). It may lead to the production of ascospores or as in Saccharomyces Ludwigii it may occur at their germination. In the smuts also the process has all the appearance of being sexual; the fusion typically occurs in pairs; there is an apparent attraction of the nuclei for each other and a fusion of the nuclei in some cases.

In the yeasts since in some cases ascospores are produced after the fusion it has been assumed by Guilliermond that it corresponds to the nuclear fusion that occurs in the ascus, forming what he considers a zygospore. In the ascus, however, the nuclear fusion is not the direct sequence of a cell fusion, there being only one cell fusion occurring in the Ascomycetes, and that is between an oogonium and antheridium at the origin of the ascocarp. Therefore it would seem more natural to assume that we have in the yeasts of this type where fusions occur before spore formation a shortened life cycle with the formation of a single ascus bearing ascospores inside it.

In the smuts, however, the case is different. It is probable that at the present time the parasitic mycelium rarely or never starts from the conjugated conidia or promycelial cells even though they represent the old sexual form of conjugation. It is quite possible that this normal sexual fertilization has ceased to play any essential role in the life cycle of the smut even though it recurs regularly when the promycelial cells or conidia are brought under certain conditions which favor it. Functionally, though not morphologically, it may have been replaced by the intracellular nuclear fusions of the chlamydospore, preceded by a longer or shorter series of conjugate divisions. As Harper (23) has noted, the same process may be going on in certain Ascomycetes. A true fertilization between the oogonium and antheridium has been found by Harper (20) in Pyronema and the Mildews. In others of this same group these structures seem to be wanting or are rudimentary. In the smuts the morphological equivalents of the oogone and antherid are the fused conidial or promycelial cells. Functionally the fusion of the cells is no longer of much importance in the life cycle of the smut but they still represent the primitive gametes. In the Basidiomycetes cell fusion has disappeared entirely but in the smuts it is retained in a rudimentary state and is only functional in a limited fashion under certain conditions.

It seems probable also that there may be different degrees in which this fusion occurs in the different species of smuts. In Ustilago Tragopogi according to Federly (15), the nuclei of the fused conidia travel toward each other and apparently fuse as a

rule. In the promycelial cells of the species of Ustilago of the cereal grains, the nuclei travel toward each other, come to lie in the same cell, and occasionally fuse. In U. antherarum, as described by Harper (22), the nucleus of each conidium remains in its place and all that occurs between the conidia is a cytoplasmic fusion. Dangeard (12) has probably described a similar case in Tilletia where the cytoplasmic fusion of the conidia is never followed by a nuclear one. In addition to these cases where fusions of various sorts occur, there are probably numerous others, where no conjugation of any kind, cytoplasmic or nuclear, takes place between either conidial or promycelial cells.

The haustoria constitute another feature in which the two groups of the smuts are unlike. The Ustilagos apparently get sufficient nourishment from their host plants by occupying intercellular spaces and perhaps by occasionally passing through a host cell. It is true that they usually live in positions that are favorable for the collection of food materials such as the growing points of young seedlings, in the ovaries or stamens, etc. The smuts of the Tilletia group, on the other hand, have well developed haustoria in three species at least;—Urocystis Anemones, Entyloma Nymphaeae, and Doassansia deformans. So far as I have found in the literature the only species hitherto known to have haustoria was E. Nymphaeae in which they had been described by Raciborski. In all the species in which haustoria have been found the smut occurs in tissues less favorable for furnishing a concentrated food supply, i. e. in the leaves and stems. The intercellular spaces here are large and the smut has to develop organs that will actually penetrate the cells and thus come in direct contact with the protoplasts.

The development of the haustoria of Entyloma Nymphaeae is of special interest as bearing on the nature of the haustorium not only in this group but in the mildews. As has been described, the young haustorium originates as a side branch which fastens itself to the cell wall and there for a time appears as an appressorium, serving apparently as an organ of attachment. Soon, however, it sends a tube down into the cell which functions actively as a sucker. Originally perhaps, the appressoria

alone were produced, the penetrating hypha being a further development or a better adaptation to the life of the fungus. The mildews may have developed their haustoria in a similar manner.

The term, spore ball, is quite loosely used to include a variety of aggregations of spores. In general all that seems to be meant by the term is that a number of spores hang together for a longer or a shorter period, the question of their common origin not coming into consideration further than that they all originated in the same region. In Sphacelotheca all that the word, spore-ball, implies is that the spores which come from the breaking up of the hyphae in a certain region, and are formed in a similar way to those of Ustilago, remain clinging together after formation. In Doassansia Alismatis it is only the side buds from the tangle of hyphae in an intercellular space which form the spores of the ball, the fertile ones being on the inside and the sterile layer on the outside. In D. deformans the origin of the spores is probably the same but the relation of sterile and fertile cells is exactly reversed, the sterile cells in this species being on the inside and a single layer of fertile ones on the outside. In Urocystis the spore ball is a very definite structure composed of closely related cells all originating from one of the hyphal branches.

It is easy to see, of course, that the spore-ball originated as a group of spores, of more or less common origin, clinging together. It is a more difficult physiological problem, however, to discover how it should be that in one species, the sterile cells all lie at the interior of the ball, while in another species undoubtedly of the same genus they only form a single layer on the outside of it. I shall not attempt at present an explanation of the origin of these different types of spore-balls nor propose a new system of naming for the different varieties. The necessity for work in this line is, however, conspicuous and it is evident that we are at the present time including under the name of spore-balls a variety of structures that are not at all homologous in their origin.

In my opinion the results of my studies seem to indicate that the two divisions of the smut group may be more distantly re-

lated than has been commonly supposed. The simple spores produced by the breaking up of the mycelium; the multinucleated mycelium during the entire life cycle; the intercellular mycelium without haustoria; the typically four celled promycelium, which are all characteristics of the Ustilago group are in striking contrast with the elaborate and varied spore-balls; the spores produced on lateral branches; the haustoria; the presence of binucleated cells and the non-septate promycelium which are found in the Tilletia, Entyloma, Urocystis division.

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PLATE LXXXVII.

EXPLANATION OF PLATE LXXXVII.

*Ustilago levis.*

Fig. 1. Conidia budding to form tree-like structures.
Fig. 2. Conidia, one and two nucleated, putting out germ-tubes.
Fig. 3. Conidia from old cultures showing remains of old germ-tubes.
Figs. 4-6. Germ-tube penetrating the epidermis of young seedling.
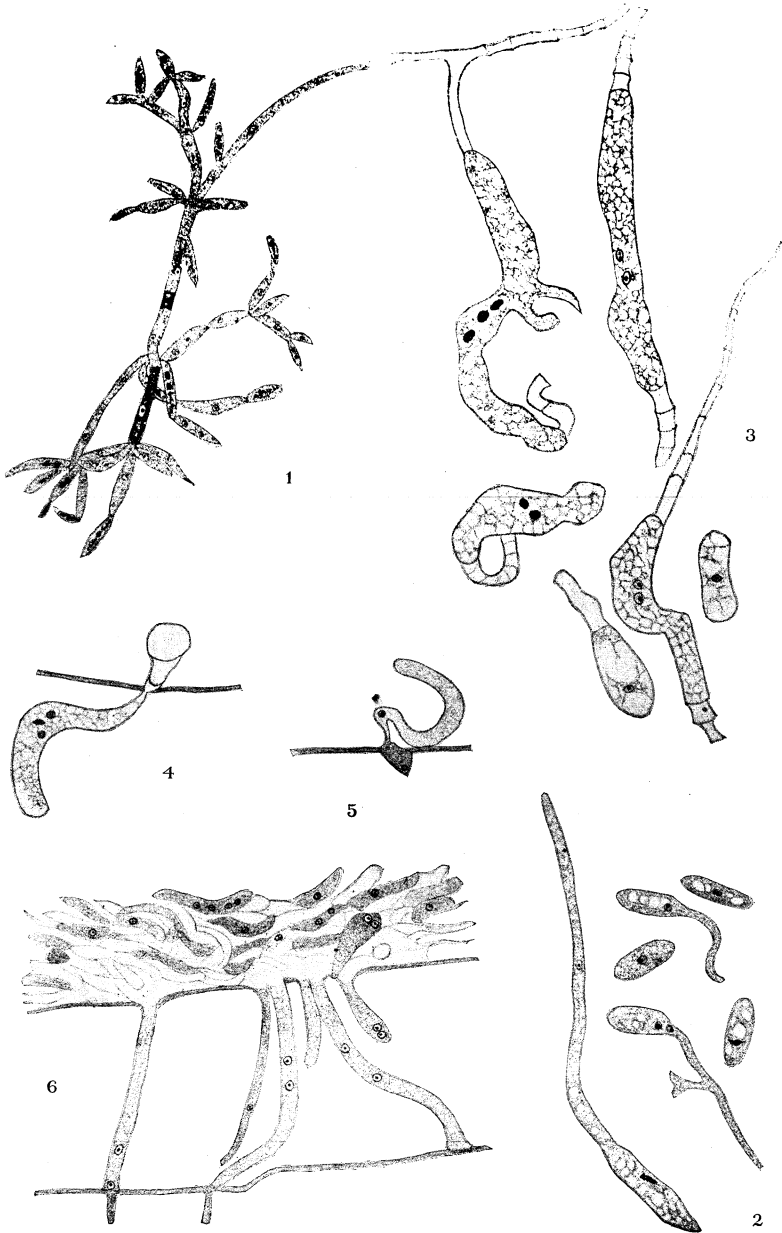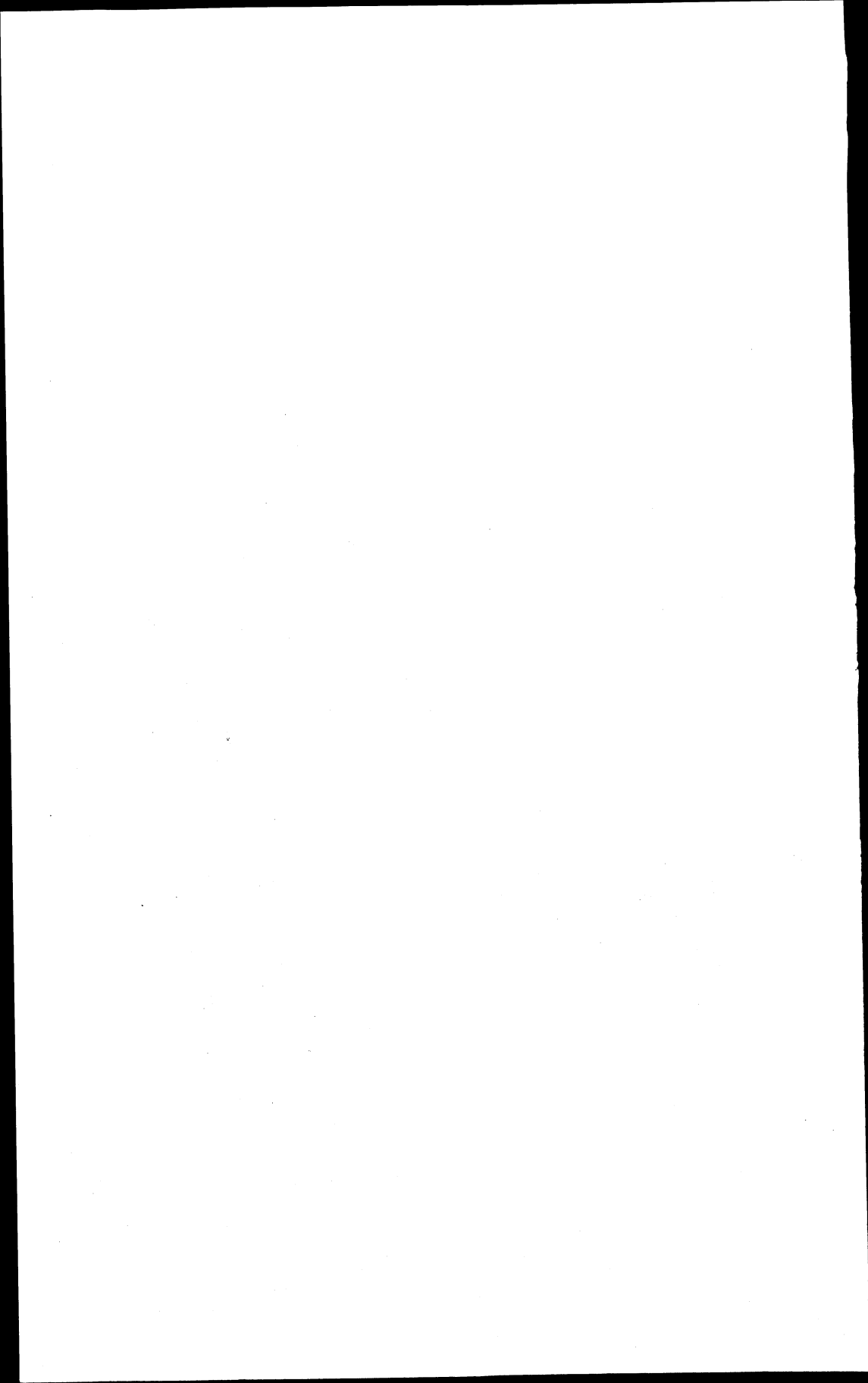
PLATE LXXXVIII.

EXPLANATION OF PLATE LXXXVIII.

Fig. 7. Longitudinal section of young seedling showing distribution of smut in young leaves and growing tip.

Fig. 8. Young leaf from preceding showing distribution of mycelium in it.

Fig. 9. Smut mycelium among growing cells, showing cell dividing.

Fig. 10. Young panicle with rudimentary flowers and floral bracts.
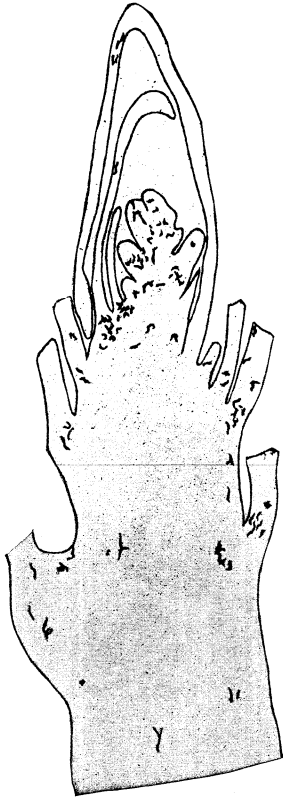
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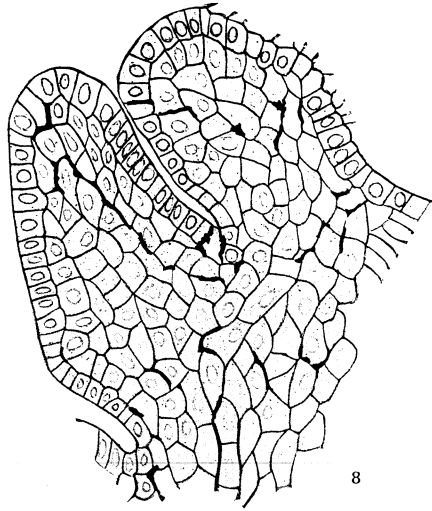
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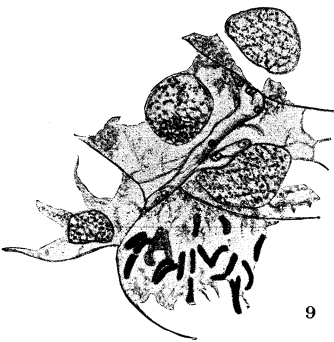
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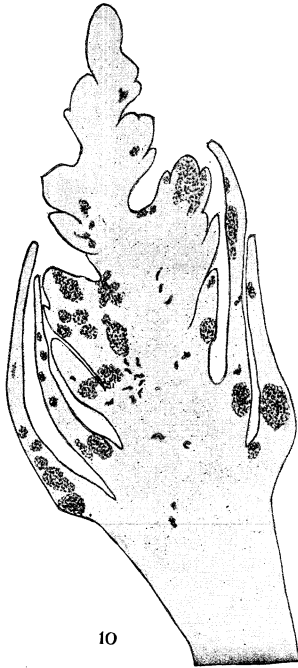
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LUTMAN : —

SMUTS
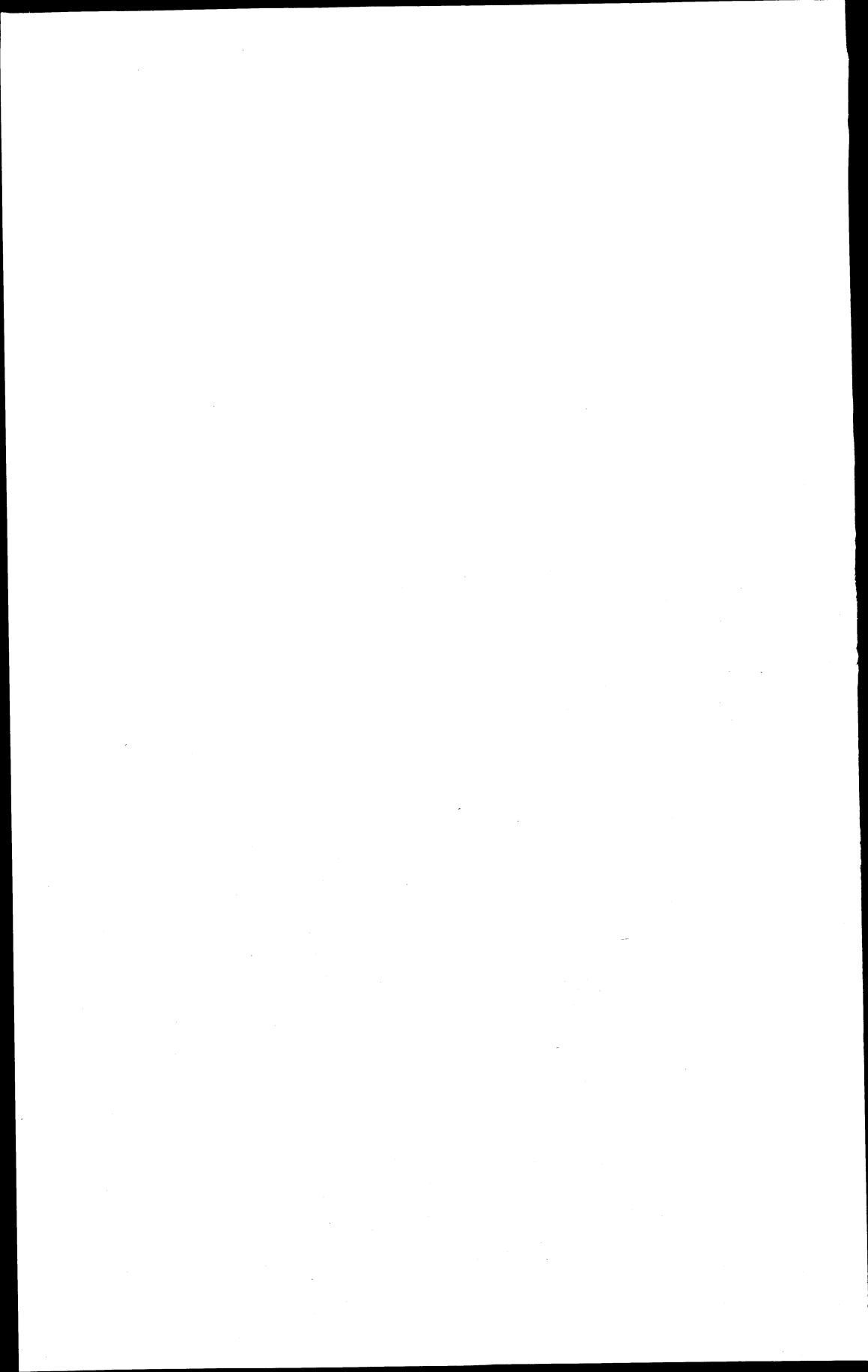
PLATE LXXXIX.

## EXPLANATION OF PLATE LXXXIX.

Fig. 11. Intercellular mycelium.

Fig. 12. Intracellular mycelium from leaf.

Fig. 13. Branching hypha tips that will soon begin spore formation.

Fig. 14. Section of branching end showing the beginning of the gelatination of the walls.

Fig. 15. The mycelium with walls gelatinized, broken up into segments.

Fig. 16. Some of the segments from a similar stage apparently showing nuclei.

Fig. 17. Segments, still later, showing nuclei; also young spores with nuclei.

### *Ustilago Zeae.*

Fig. 18. Mycelium.

Fig. 19. Segments of mycelium in spore-forming stage; two of them with gelatinized walls.

Fig. 20. Young spores showing nuclei.  ×1800.

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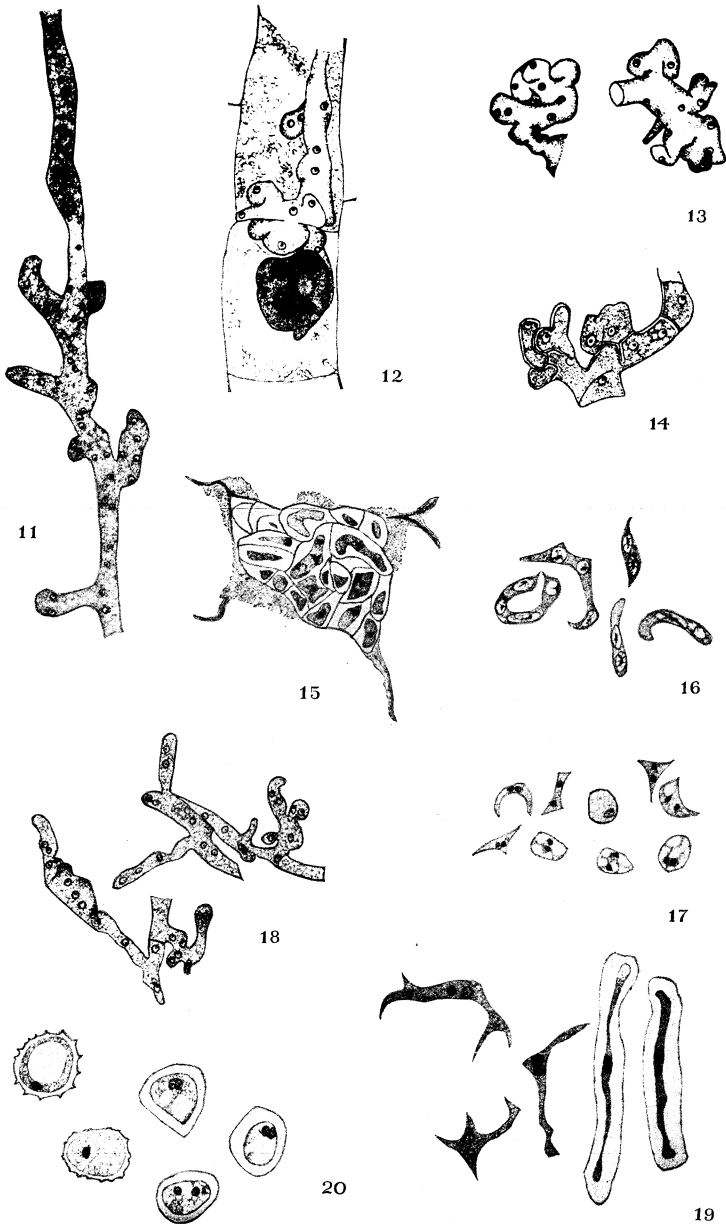
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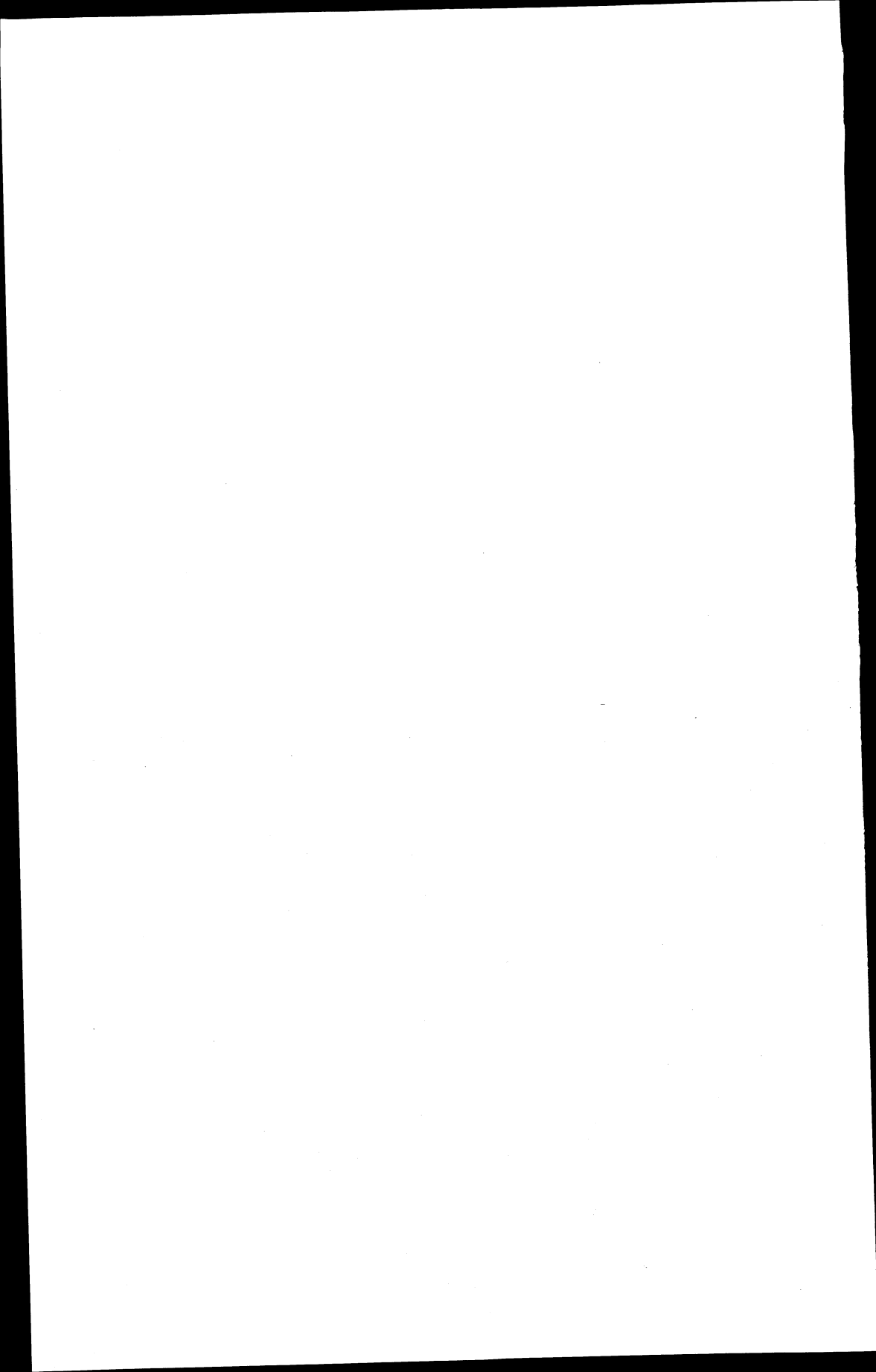
PLATE XC.

EXPLANATION OF PLATE XC.

*Urocystis Anemones.*

Fig. 21. Binucleated cells from the mycelium.
Figs. 22–23. Beginning of spore-ball formation; young branches.
Fig. 24. (a) Surface view of young spore-ball; dotted lines indicate contours of hyphal tips behind the part in view. (b) same showing nuclei.
Figs. 25–26. Young spore-balls.
Fig. 27. Nearly mature spore-ball showing all cells with two nuclei.
Figs. 28–29. Haustoria.

*Ustilago Hordei.*

Figs. 30–34. Fusions in promycelial cells with nuclei in various stages of migration or fusion.
Fig. 35. Three promycelial cells fused and nuclei in middle one.

*Ustilago Avenae.*

Figs. 36–37. Nuclei and most of the cytoplasm in the large conjugation tube.

PLATE XCI



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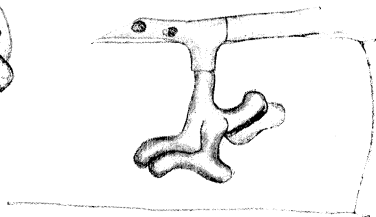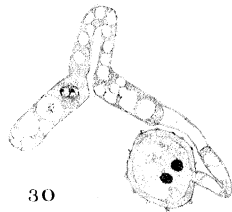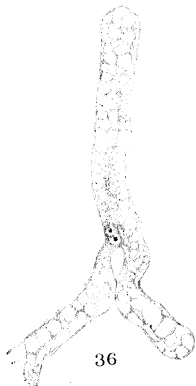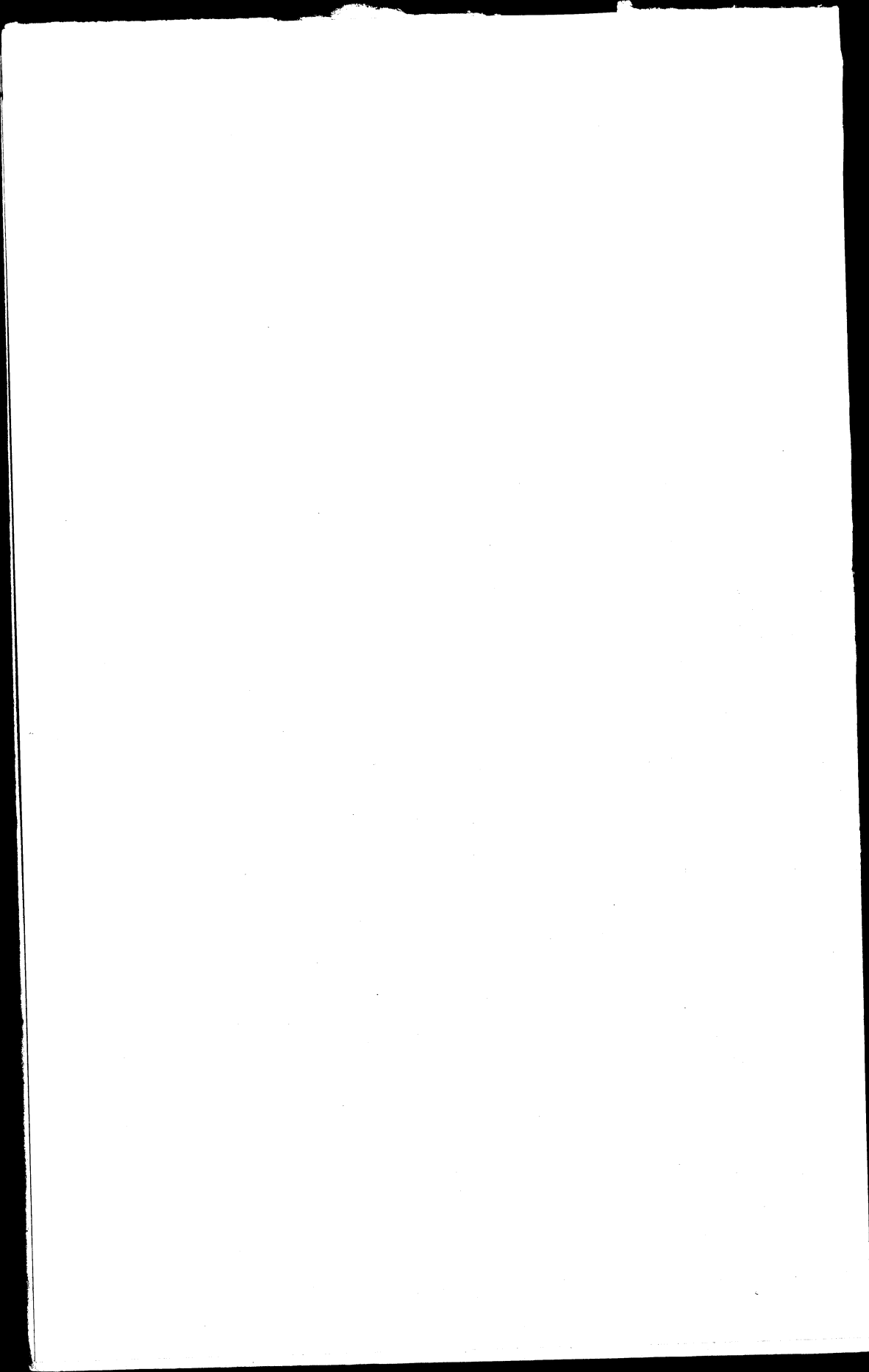
PLATE XCI.

EXPLANATION OF PLATE XCI.

Figs. 38–39. Cell and nuclear fusions in the promycelial cells. **Doassansia** deformans.

Fig. 40. Young spore-ball.

Fig. 41. Older spore-ball with sterile paerenchyma forming in center.

Fig. 42. Section of edge of spore-ball. (a) layer of hyphae around it. (b) fertile spores. (c) sterile cells.

Fig. 43. (a) and (b) Binucleated cells from the mycelium between the spore-balls.

Fig. 44. Haustoria in a host cell. ×500.

Fig. 45. Detailed view of a haustorium.

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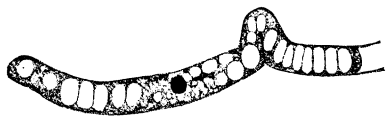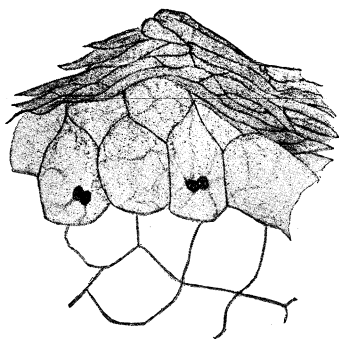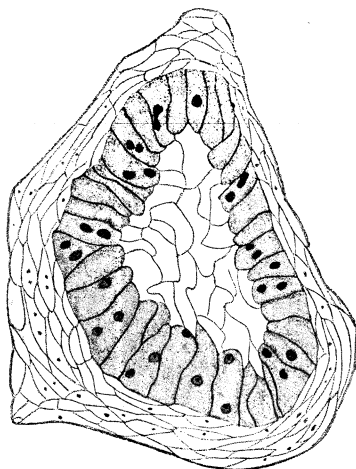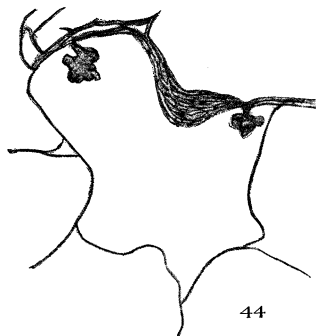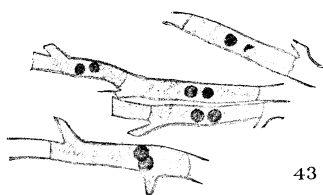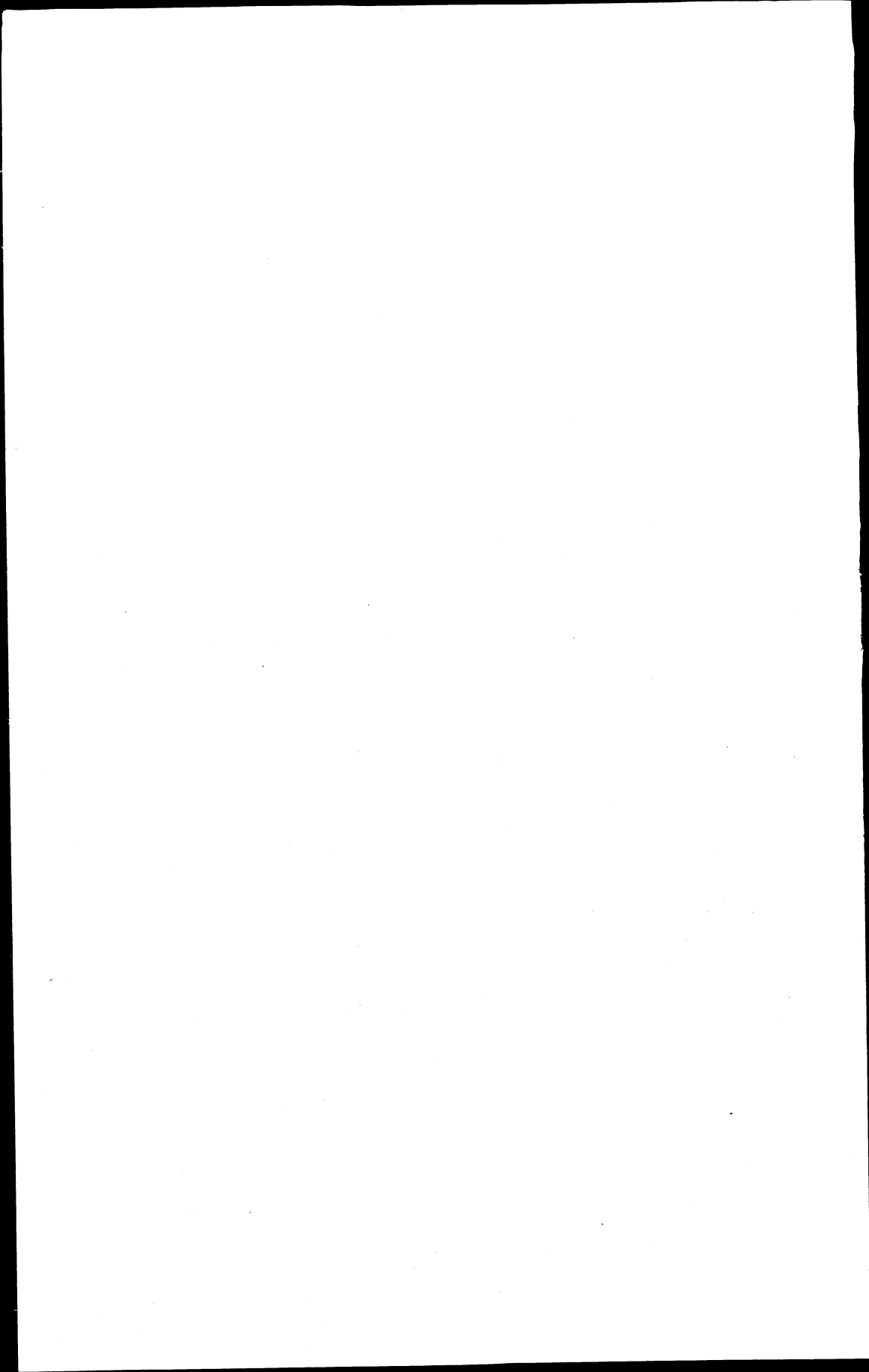
PLATE XCII.

EXPLANATION OF PLATE XCII.

*Doassansia Alismatis.*

Fig. 46. Binucleated cells from the mycelium between the spore-balls.
Fig. 47. Young spore-ball.
Fig. 48. Spore-ball germinating in position in the leaf.   ×300.

*Entyloma Nymphaeae.*

Figs. 49–52. Details of mycelium showing the binucleated cells, the method of branching, and the origin of the appressoria.
Figs. 53–55. Formation of spores.

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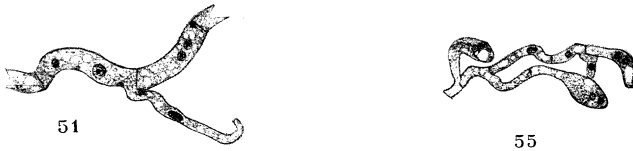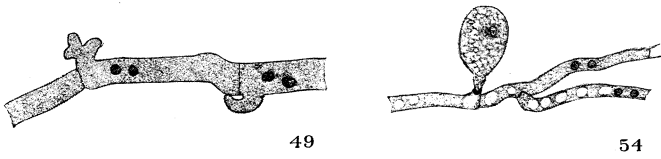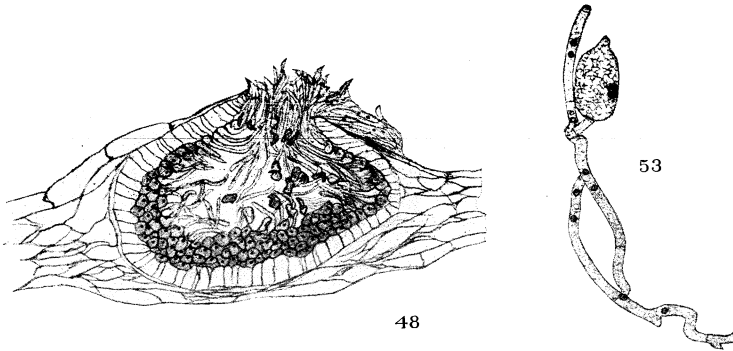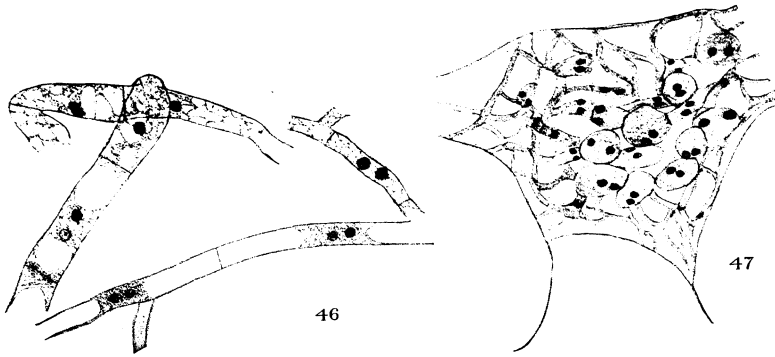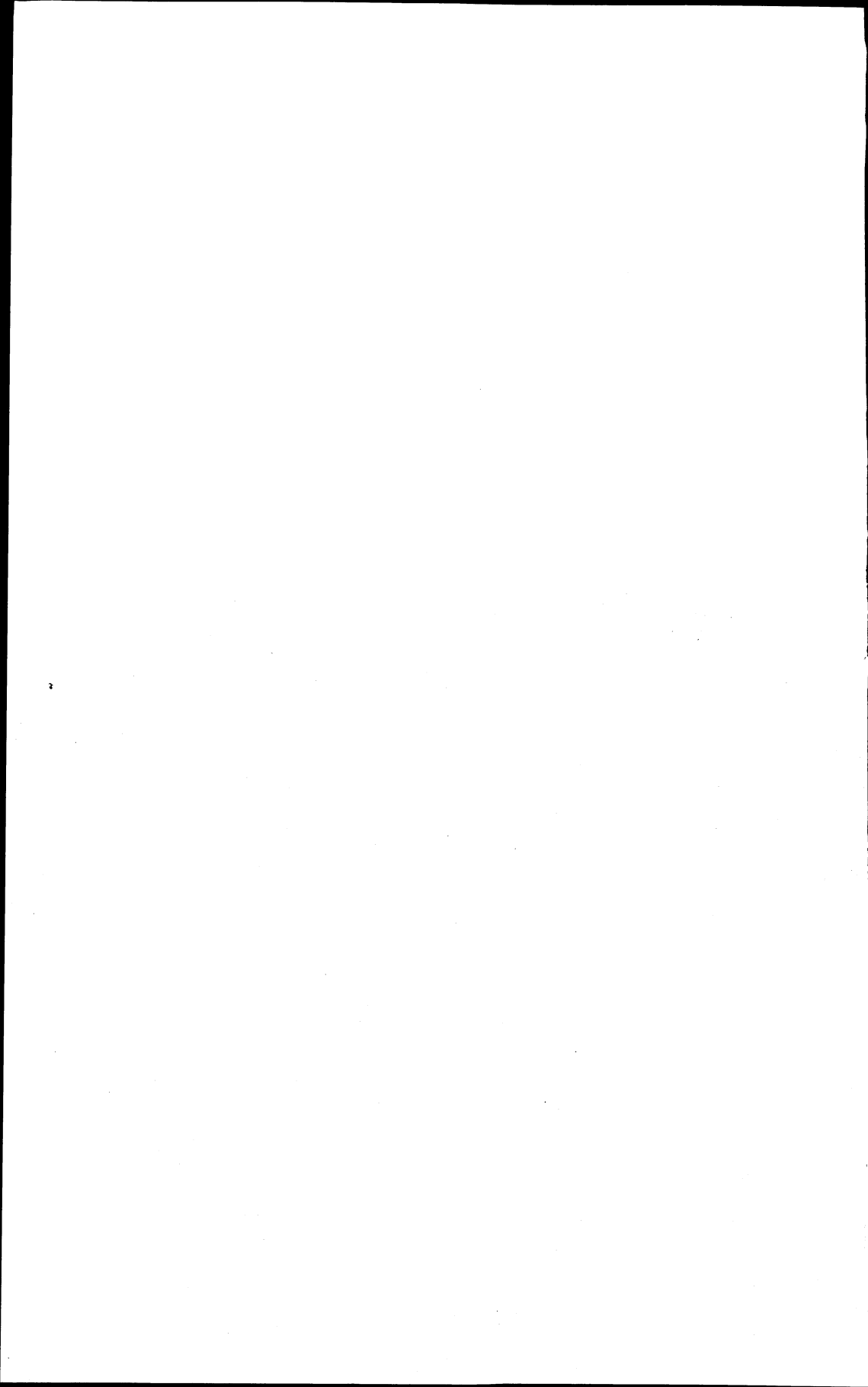
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PLATE XCIII.

EXPLANATION OF PLATE XCIII.

Fig. 56. Cross-section of leaf showing distribution of spores and myce-
    lium.   ×460.
Figs. 57–62. Stages in spore-formation.   ×2500.
Fig. 63. Surface view of a young appressorium.   ×2500.
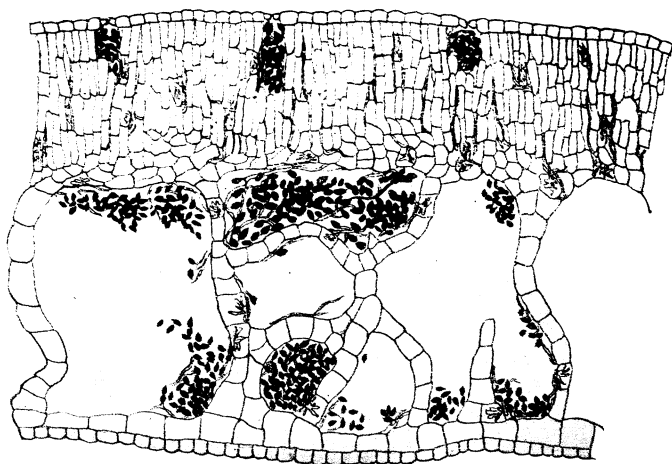
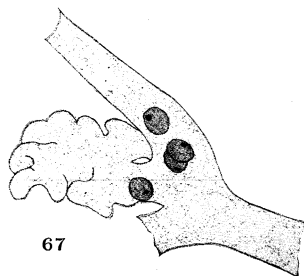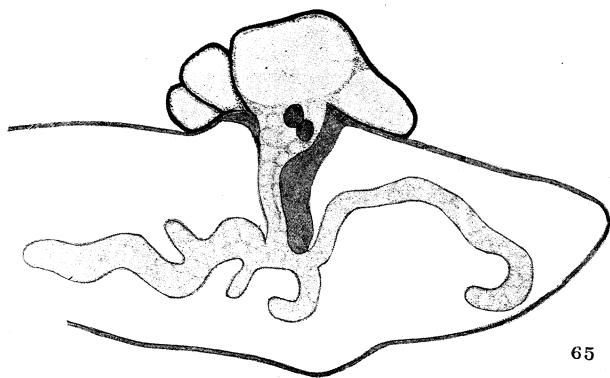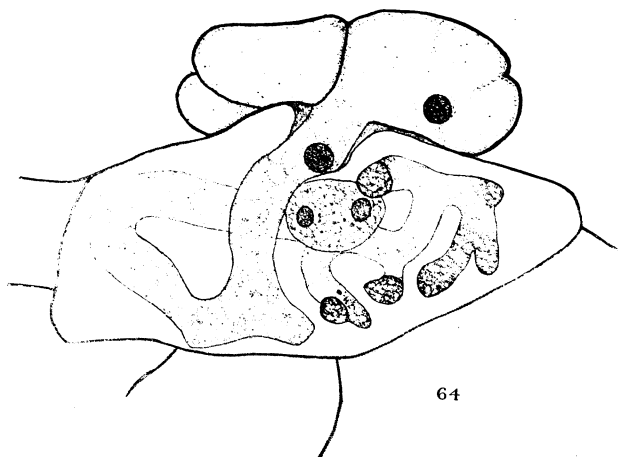PLATE XCIV

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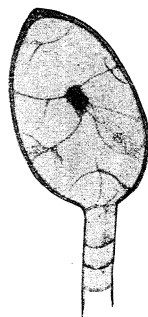
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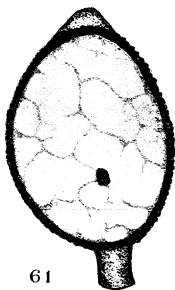
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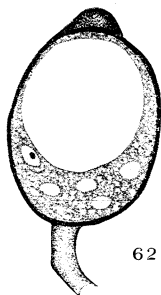
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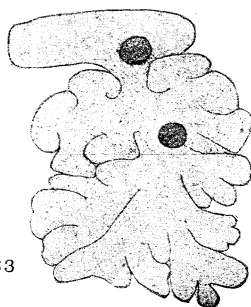
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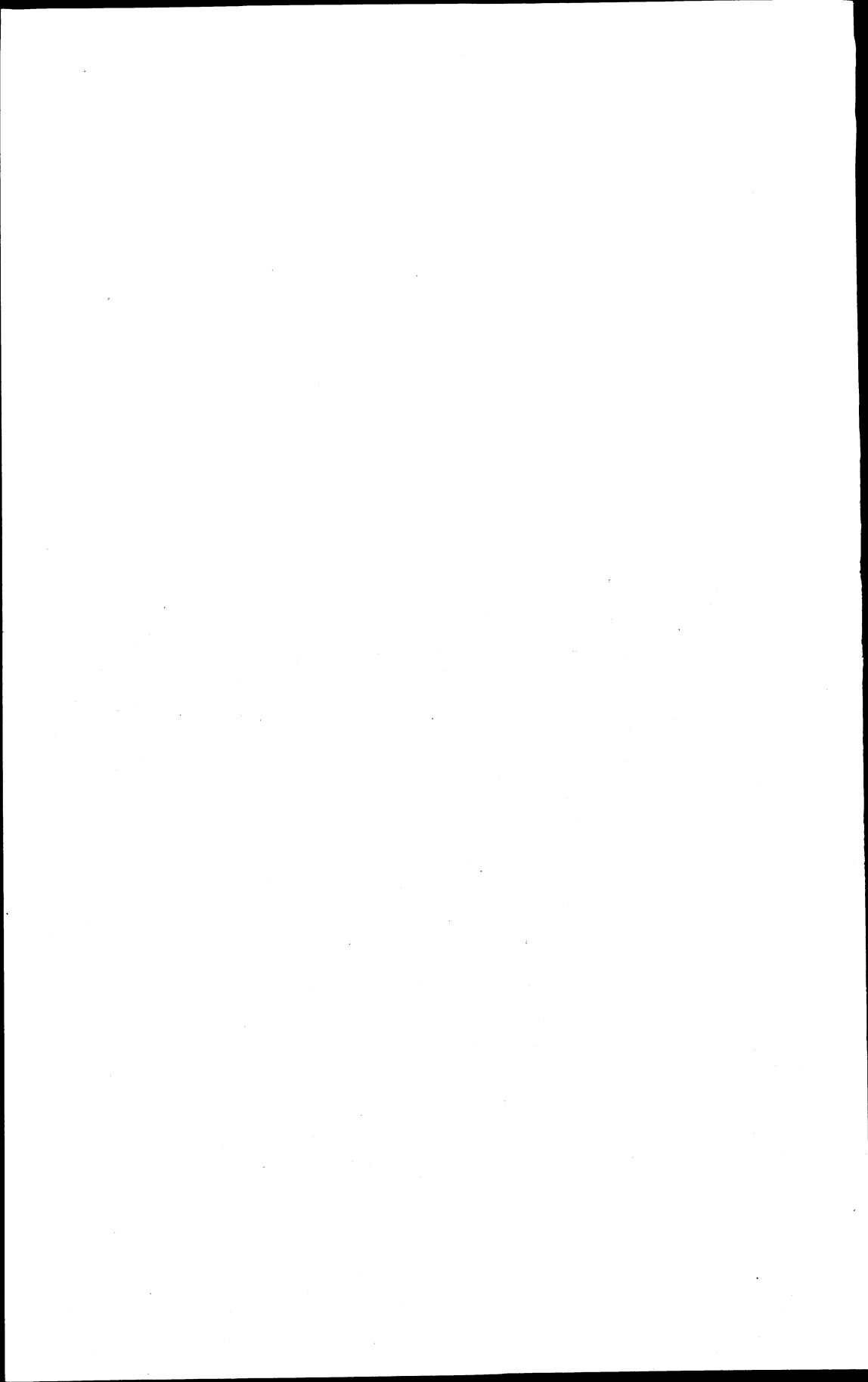
PLATE XCIV.

EXPLANATION OF PLATE XCIV.

Figs. 64–65. Section, showing haustorium; the walls of the appressorium are thicker than in the preceding figure.   2500.

Fig. 66. Section of young appressorium showing thickened host cell wall under it.   ×2500.

Fig. 67. Partial surface view of young appressorium showing nuclei migrating out into it.

MAGNIFICATION:—All figures are magnified about 1300× unless as noted differently after the figure.

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