A Study in Cereal Smuts of Minnesota.

A thesis submitted to the faculty of the Graduate School of the University of Minnesota by E. C. Stakman in partial fulfillment of the requirements for the degree of Master of Arts, May 23, 1910.

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There are in Minnesota various cereal smuts causing an aggregate annual loss of at least several millions of dollars. Of these there are six which do most of the damage, viz: the loose and covered smuts of wheat, loose and covered smuts of barley, oat smut, and corn smut.

The identity and essential character of these smuts were very imperfectly understood until Kellerman and Swingle, in about 1888, began making a critical study of the germination of spores obtained from the various hosts. They had been studied before this time, but the knowledge concerning them was in a chaotic condition. In fact, the studies of these forms began with the ancients. By the Romans the name Uredo, from urere - to burn - was applied, very evidently on account of the resemblance of the spore mass to charred remains. Pliny the Elder, in his Naturalis Historia, speaks of the smuts, and discusses the effect of weather and location upon their prevalence. The commonly accepted idea was that the smut mass was an abnormality of the plant itself. This view prevailed until the time of Linnaeus. Persoon was the first to give smuts a definite place among the fungi. In 1801, in his Synopsis Fungorum, he classes them with the fungi. Subsequent to this time, however, some botanists maintained that the smut mass was an abnormally developed group of the plant cells.

Prevost, in 1807, showed that the spores, by some considered as abnormal cells of the plant, were capable of germination. Tulasne, in 1854, substantiated this claim, thus removing all doubt as to the true nature of smuts. All of the so called loose smuts of wheat, barley, and oats were still included in the same species under either of the names, Ustilago segetum (Bull,) Ditt., or Ustilago carbo (DC.) Tul. It was shown, however, by Jensen in 1888, that each of these various smuts was able to parasitize only the host upon which it is found. By conducting cross inoculations he proved to his own satisfaction that there were distinct forms upon each of the hosts with which he experimented. Kellerman and Swingle, and, about the same time Brefeld, working out the details of germination, substantiated Jensen's claim that there were different species, very much alike in macroscopic characteristics, but yet essentially different.

As finally worked out, then, the six smuts which will be considered are loose smut of wheat, Ustilago tritici (Pers.) Rostr.; loose smut of barley, Ustilago nuda (Jens.)

Kellerm. & Swingle; covered, stinking smut or bunt of wheat, Tilletia foetens (B & C) Trel.; covered smut of barley, Ustilago hordei (Pers.) Kellerm. & Swingle; smut of oats, Ustilago avenae (Pers.) Jens., and maize smut, Ustilago zeae (Beckm) Unger.

Ustilago tritici (Pers.) Rostr.

History.-

As mentioned before, all the loose smuts were originally considered the same, so Ustilago tritici was first Ustilago segetum and had a variety of names before the present one was finally applied. The smut was known as early as 1552 when Tragus called it Ustilago. He used this name on account of the resemblance of the smutted head to a charred mass; the derivation is from ustus (pp. of uror, to burn). Tragus did not recognize it as a fungus; hence the name. C. Bauhin, in 1595, in his Phytopinax, named it Ustilago secalina, but he also failed to recognize it as a fungus.

It remained for Linnaeus and Bulliard to discover that the so called brand was in reality a parasitic plant. Recognizing this fact, various men applied names. Persoon called it Uredo tritici, a variety of Uredo segetum, while De Candolle considered it as a variety of Ustilago carbo, as did Tulasne who named it Ustilago carbo a vulgaris a tritici. Wallroth applied still another name, calling it Erysibe vera tritici. None of these men observed any difference between the form on wheat and those forms found on oats and barley.

Jensen, in 1888, showed the individuality of these various forms. He conducted cross inoculation experiments with wheat, oats and barley smuts. By dusting spores from wheat, oats and barley smut on wheat, he found that only those plants which had been dusted with spores from smut found on wheat produced smutted heads. His percentage of smutted heads in wheat was low, but the reason for this is evident in the light of the experiments carried on and the discoveries later made as to the methods of infection of loose smut of wheat. Jensen also asserted that spores of Ustilago tritici retained their vitality a much shorter time than those of most other forms. Consequently, he said that wheat was much less liable to be smutted than other cereals.

Plowright noted that Ustilago segetum, when found on wheat, had a decided golden lustre, while on Avena elatior he observed that it was sooty black. He predicted that careful physiological research would, in the future, show two distinct species. However, even such a man as Brefeld was still skeptical, and he declared that some loose smut of wheat which he had obtained from Halle was identical with naked smut of barley. Von Waldheim, in studying the germination of various smut spores, noted the greater length of promycelia of wheat smut spores, and also maintained that the promycelium disorganized earlier in this form than in those from either oats or barley. Kellerman and Swingle's studies of germination, in 1888, showed clearly that the loose smut of wheat was a distinct species. It was named Ustilago tritici (Persoon) Rostr.

The following is a synonomy of the species.

1552 - Ustilago tragus - Lobelius, De Stirpium Nomenclature pr. lib. III,p. 666; Lobelius, obs. plant, p. 22; Stirp. adv. nov.p.11

1596 - Ustilago secalina - Bauhin, Phytopinax, p 52.

1797 - Uredo segetum - Persoon, Disp. meth. fung., p 56.

1801 - Uredo (Ustilago)segetum b. Uredo tritici - Persoon, Syn. meth. fung., p 224.

1809 - Caeoma segetum - Link., obs. I, 4.

1815 - Uredo carbo b. tritici - De Candolle, Flor. Trans. VI, p 76.

1833 - Erysibe vera b. tritici - Wallroth, Flor. Crypt. Germ.,pars post., p 217.

1837 - Uredo carbo tritici - Phillipar, Traite, p 92.

1847 - Ustilago carbo a vulgaris a triticea R et Ch. Tulasne, Memoir sur les Ustilag. - Ann.Sci.Nat., Ser.3, t 7, p 80.

1888 - Ustilago hordei - Brefeld, Neue Unters.,II in Nachr. aus d. Klub der Landw.zu Berlin Nr. 221, 28 Juni, 1888, S 1581.

1888 - Ustilago segetum var. tritici - Jensen, Om Kornsortornes Brand (Anden Meddelelse) S 61.

1890 - Ustilago tritici Jensen - Prop. & Prev.of Smut - J. R. A. S. XXIV S.S. Pt. II, p 11.

1890 - Ustilago tritici Jens. - Kellerman & Swingle, Ann. Rep. Kan. Agr. Exp. Sta. 2, p 262.

1894 - Ustilago f folicola - P. Henning, Zeitschr. Pflanzenkr., IV, p 139.

1895 - Ustilagidium tritici - Herzb. in Zopf, Beitr. Phys. Morph. Org. V, p 7.

Methods.

For all germinations, both of this and the other forms, various media were tried. Tap water, distilled water and nutrient solutions were used for purposes of comparison. For all careful work the Marshall Ward Cell was used, while for comparison, germination tests were also made in watch crystals. Tests were made at intervals of a few weeks, beginning in July 1909, and continuing until April 1910.

Description of Spores.

The spores of Ustilago tritici are oval, subglobose or globose - variable both in shape and size. The shape is probably more often subglobose, or even globose, but not infrequently more elongate. The color is olive brown to golden, with often a bronze tint. One side of the spore is conspicuously lighter in color than the other, the lighter side being not infrequently almost hyaline. This side is also usually thinner than the darker. The spore wall consists of two distinct coats, the outer, or epispore, and the inner, the endospore. The dividing line between epi and endo spore is sometimes difficult to see on account of the fact that the endospore is usually hyaline. When both are visible in optical section, it is usually on the darker side of the spore where the contrast between the dark coloration and the hyaline endospore is more marked than on the lighter side. By the use of potassium hydroxide the two parts of the wall may be quite readily distinguished; sometimes it is possible to trace the line of demarcation around the entire spore. The epispore is practically always echinulate, sometimes minutely and sometimes quite distinctly so. In all cases the spines show only on the light colored side, sometimes only along the edge and, in other cases, covering about one half of the spore (Plate I, fig. 1). In size the spores vary very considerably; they measure from 4.75μ to 9.5μ in diameter, averaging about 5.5μ- 7.5μ x 5μ- 6μ .

Germination in Water.

The spores do not germinate as readily as those of some other species, especially Ustilago avenae, Ustilago hordei, and even Ustilago nuda. It requires from 14 to 17 hours for them to germinate. Some, of course, germinate much more readily than others, but the very late germinating spores are probably abnormal.

Germination proceeds always, as far as could be observed, from the light area of the spore. In a few cases the promycelium comes out from the very edge of this region, but in no case was it seen to come from the dark side. This seems quite natural in view of the fact that the spore wall is thinner on the light than on the dark side. Very rarely is the spore wall split by the young promycelium; in fact there seems to be a definite pore through which the germination tube comes. Normally only one promycelium is produced, but, in some cases, germination proceeded from two places. When this occurred, there were usually two light areas and the promycelia grew out from these.

The promycelium is usually constricted at the base, just where it emerges (Plate I, figs. 1-11), and sometimes has a swollen tip (I, 5, 8, 9; VII, 1-4). It may be either curved or straight, at first about 35 long and 4 in diameter. Up to 20 hours, the septations are usually quite indistinct, sometimes none being visible. Some of the promycelia are one or two septata, while others are continuous. At this time there are practically no vacuoles in the protoplasm which is still very finely granular.

By 22 hours the promycelia have ordinarily reached a length of from 30 to 40μ, and are very clearly 1 - 5 septate, usually 2 - 3. As yet there are normally no branches or few just beginning to form, and vacuoles in the protoplasm are just beginning to appear. By 24 hours branching has begun and, in some cases, vacuoles are already quite conspicuous (Plate I, fig. 9). A few fusions have sometimes taken place by this time; usually these are between branches of the same promycelium, or between the promycelium itself and one of its branches. In some cases tubes from different spores fuse and a single promycelium is formed above the fusion, but this is infrequently seen (Plate II, fig. 8). So called knee joint fusions, appearing often merely as a slight protuberance, were fairly common. These knee joint fusions came about as a result of the outgrowth of two short, stubby branches, one on each side of a septum. These branches grow together and produce a knob like protuberance which later on, as the cells swell up, may open and appear as though a tube had grown out from one cell and fused with that next to it (Plate II, fig. 1).

At about 25 to 27 hours, or, quite often, before this time, the promycelia have attained practically their full size. Fully grown, they are of various forms and sizes. Some are straight, unbranched, and 2 - 3 septate, while others are quite profusely branched, irregular in shape and many septate. The branches arise most often just below a septum and are usually curved. They may in turn branch, but, until after the expiration of four or five days, this does not usually occur to a very great extent.

After the promycelium itself has ceased growth, it sends out germ tubes or infection threads. These arise first either as branches or as continuations of the promycelium, differing in no essential particular from ordinary branches. However, as they grow out, the basal portion becomes first vacuolated and then vacant. As the protoplasm goes forward, leaving nothing but the walls behind, septations appear, so that there is a basal, segmented, vacant portion, and a swollen tip filled with protoplasm. Sometimes more than one germ tube is sent out from the same promycelium, usually when the promycelial tube itself grows out and also sends out a branch. However, the branching may not occur until the germ tube is already well formed and grown out to a considerable length. In the earlier stages the promycelial cells still retain their protoplasm, even after the bases of the germ tubes have become entirely vacant. This is rather peculiar; the promycelium may be well supplied with protoplasm, and, growing out from it, an infection thread with a vacant basal portion much longer, very often, than the promycelium itself. This condition does not last long, however. The promycelial cells soon lose their protoplasm also. Very often the first cells to become vacant are the basal ones, but this is not necessarily the case. By 96 hours nearly all of the original promycelia were entirely vacant and the germ tube seemed to come directly from the spore. There were some exceptions to this, one of which is shown in fig. 3, Plate III.

These germ tubes may branch; in fact they quite often do. Usually branching takes place near the tip. When branches are sent out they are protoplasmic throughout at first. Sometimes, however, in a comparatively short time, they become very much like the original germ thread. Fusions occur either with branches from the same filament or with those from others, so that sometimes a net work is formed. This is rarer in water, however, than in nutrient solutions. A number of these cases of branching and fusions are shown on Plates II & III. More often the germ threads appear as do those in Plate III. They are long and narrow, usually 1μ- 2μ wide in the basal portion, and about 2μ- 3½ μ wide in the swollen tip. After 4½ days there was but little change. The tubes were fully grown and reached a length of 300 - 400μ, the tip, not always continuous, measuring about 30 - 90μ x 3 - 4 , and the vacant, segmented, basal portion about 1 - 2½ .

Summary of Germination in Water.

Germination in water takes place in about 14 or 15 hours although sometimes it requires a longer time. A 2 - 5, sometimes more, septate promycelium, about 30 - 40μ long and 3 - 4μ wide is sent out from the lighter colored portion of the spore. This branches in some cases, but produces neither sporidia nor free segments, remains attached to the spore and sends out very long, narrow germ tubes or infection threads which consist of a long, segmented basal portion and a swollen, protoplasmic tip which may or may not branch. Fusions sometimes take place, especially "knee joints", from which germ tubes often grow.

Germination in Nutrient Solution.

Of the nutrient media used, a 5% solution of sugar seemed most favorable, and the following description is of spores germinating in that medium.

The early stages are very nearly the same as just described for germinations in water. The growth of promycelia proceeds more vigorously, perhaps, but in essential characters, such as branching, fusions, etc., there is no important difference. The promycelium is 2 - 5, or, in rare cases, even more, septate; it is branched or unbranched, often constricted and sometimes vacant at the base.

However, distinct differences appear after about 20 hours. The septations are much more distinct, and the cells of the promycelium seem much less firmly united than water; in fact there was a distinct tendency for the promycelium to break up into component cells. This tendency seemed especially strong when the spores were still fresh. Spores collected in July 1909, were immediately tried in watch crystals and Ward Cells. Germination took place within 14 hours, and the promycelia formed very quickly. The tendency to break up, however, was quite pronounced. In some cases the entire promycelium became detached from the spore first and then broke up into segments; or, in other instances, the end cells were freed while the others remained attached. These free cells were very closely watched, but none of them divided again; nor did any germinate. Spores collected at the same time as those whose germination was studied in July were tried in March. They did not form free segments, although in other respects, they resembled the others quite closely.

The later stages were somewhat different from those in water. Germ tubes, such as those previously described, are sent out in great abundance. They are of the same general character as those produced in water, except for the fact that they are generally thicker, and the swollen tips are more coarsely granular (Plates IV & V). There seems, too, to be a greater tendency toward branching in the nutrient solution. At ten days the tips which, by this time, were swollen to a thickness of 4 - 5 and were very coarsely granular, had sent out many septate or unseptate branches, a majority of which were quite short and blunt. The typical appearance of the tips at this stage is shown on Plate V. As will be noticed from the figures, the tips are very often irregularly septate, long and short, thick and narrow cells alternating in no definite manner. Quite frequently the tip sent out long, irregular tubes, often non septate and resembling primary branches in most respects except for their irregular shape.

Beginning about the twelfth day, or even before that, in some cases, the tips, now about 75 - 100μ long, of the germ tubes, begin to form very definite septations which are often at quite regular intervals. The swelling continues, the diameter of the segments being not uncommonly 6 or even 7μ. This process continues until at 25 days nearly all of the tips were of this nature (Plate VI, figs. 1-8). At the same time the vacant segments of the germ tube, and even the basal cells of the filled tip in some cases disintegrated and left chains of cells such as those shown on Plate VI, fig. 7. Forms such as that shown on the same plate, fig. 8, were very common; in fact by this time nearly all of the tips showed a structure very closely resembling in all essentials the figure shown on the plate. By 27 days the nourishment was practically exhausted and the cell had begun to dry up. A large percentage of the chains resembling the one shown in fig. 8, Plate VI had now divided at the septa, leaving the individual segments entirely free from each other, as in fig. 11. These segments had now rounded up to a diameter of about

6 - 9μ, being about the size of the original spore. The wall thickened until it could be plainly distinguished even while the segments were still grayish in color. This wall became thicker, while the segments, at first grayish in color, now assumed an olive hue or a somewhat golden lustre. In few cases were they as dark as the original spores; neither did they appear as distinctly echinulate; but in size, shape and color they resembled them very closely.

The cell was now allowed to dry out completely. Then more sugar solution was added and the cell placed under the same conditions as it had been originally under. Within 16 hours the rounded up segments sent out tubes resembling very closely the promycelial tubes of the original spore. It is probable then that owing to the exhaustion of nourishment, the protoplasmic contents rounded up and formed resting cells which, from their method of origin, would be chlamydospores such as Brefeld described for some of the smuts. The culture was kept for 42 days and by that time a large percentage of the segments had germinated.

Summary of Germination in Sugar Solution.

The germination in sugar solution proceeds very much as in water in the earlier stages. However, as contrasted with water germinations, in some cultures free segments were formed within 48 hours by the breaking up of the promycelium. The growth of promycelium and branches was more vigorous than in water and fusions were probably more frequent. The germ threads were coarser and branched more. The tips of these threads broke up into free segments which surrounded themselves by a wall, assumed an olive brown color and germinated under favorable conditions.

Vitality of Spores.

Attempt was made to determine as far as possible in the short space of 9 months the relative vitality of spores of the different species. Spores of U.tritici collected July 8, 1909 were kept in a dry place until Dec.10, 1909. Then some smutted heads were buried under about an inch of soil in pots and placed outside where they were fully exposed to the weather. On April 8, 1910, some of the spores were taken from the pots and placed in hanging drops in Ward Cells beside other cells containing spores which had been in a dry place all winter. It was found that those spores which had been kept outside germinated as readily and the promycelia grew as vigorously as did those of the spores which had been kept in a dry place during the winter. In fact, the vigor of germination was very surprising, for the supposition was that the spores of this species retained their vitality for a comparatively short time-a few months at most-, and could very uncertainly survive winter conditions. The figures on Plate VII are of frozen spores and give some idea of the character and vigor of germination.

Ustilago nuda (Jens.) Kellerm.& Swingle

History.-

The earlier history of this smut is identical with that of Ustilago hordei, since the two forms were long considered the same. There seemed to be some idea that the two forms might be separate, but it was not at all clear. In fact no clear distinction, as has already been mentioned in the discussion of U. tritici, was for a long time made between the smuts. The smut on barley was first separated from that on oats by Lobelius in 1591. He applied two names viz.,Ustilago polystichi and Ustilago hordei distychi. However, it is uncertain whether or not he separated loose barley smut from covered smut and put them under different names. Bauhin also separated oats and barley smuts, applying the name Ustilago hordeacea to that on barley. It is not known whether or not he recognized the two forms. Persoon in 1801 gave the first really accurate description of barley smut, but he probably had in mind the covered and not the naked smut, since in his description he speaks of the spore mass as "pulvere latente".

It was not until 1888 that the loose and covered smuts were definitely separated. Jensen noticed the difference in the character of the spore mass and the difference in color and size of the individual spores. He first considered what is now Ustilago nuda as a variety, giving it the name Ustilago segetum var. nuda; and later he called it Ustilago segetum var. hordei nuda. In addition to recognizing the distinction between the two smuts on barley, Jensen also proved by his inoculation experiments that the naked barley smut attacked only barley. Brefeld also carried on infection experiments and arrived at the same conclusion. He also studied the germination and noted the long narrow promycelia producing no sporidia. He also asserted that the vitality of the spores was very low when compared with spores of oat smut. Kellerman & Swingle worked out the germination carefully and cleared up any doubt which may have remained concerning the individuality of the species.

The following is a synonomy of the species.

1552-Ustilago - Tragus, De Stirp. nomen. pr., lib.III,p 666.

1591-Ustilago polystichi - Lobelius, Icones, p 36.

1591-Ustilago hordei distychi - Lobelius, Icones, p 36.

1596-Ustilago hordeacea - Bauhin, Phytopinax lib. I,sec.IV, p. 52.

1767-Chaos ustilago - Linnaeus, Syst. Nat. Ed. XII, II, p. 1356.

1791-Reticularia ustilago-Linnaeus, Syst. Nat. Ed. XIII, II, p 1472.

1791-Reticularia segetum- Bulliard, Hist. des Champ., I, P 90, tab. 472, lit. E.

1809-Caeoma segetum - Link, Obs. I, p 4; Sp. pl. Willd., VI, II, p 1, No. 1.

1813-Ustilago segetum (Bulliard) Dittmar, in Sturm, Deutchl. Fl., Bd.III., Heft 3,S 67, T. 33.

1815-Uredo carbo a hordei - De Candolle, Flor. Fran., VI, p 76.

1833-Erysibe vera a hordei - Wallroth, Flor. Crypt. Germ. pars. post., p 217.

1837-Uredo carbo a hordei - Phillipar, Traite, p 92, pl 3.

1847-Ustilago carbo a vulgaris c hordeacea, R & Ch. Tulasne, Memoir sur les Ust.- Ann.Sci.Nat.,Ser.3,t 7, p 80.

1856-Ustilago segetum b hordei - Rabenhorst, Klotzchii, Herb. viv. myc. ed. nova, Cent. III, No. 397.

1888-Ustilago segetum var hordei f nuda - Jensen, Om Korns. Brand., S 61.

1888-Ustilago hordei - Brefeld, Neue Unters. uber d. Brandp. u. Brandkr. II, in Nachr. aus der Kl. d. Landw., zu Berl., Nr. 221, 28 Juni, 1888.

1888-Ustilago segetum var. nuda - Jensen, Prop. & Prev. of Sm. in J.R.A.S. XXIV s.s., P II, p 10; Plowright, Brit. Ured. & Ustilag., p 274.

1888-Ustilago segetum var. hordei nuda - Jensen, Prop. & Prev. Sm., l.e., p 11.

1888-Ustilago hordei (Rabenhorst) Lagerheim, Rev. d. in Exsic. "Krypt. Bad. v. Jack, Leiner u. Stitzen" S. 2, Nv. 41.

1889-Ustilago hordei var nuda - Jensen, Le Charbon des Cereales, p 4.

1890-Ustilago nuda hordei - Jensen, in letter dated Jan. 24, 1890.

1890-Ustilago hordei - Rostrup, Overs. K. Danske Vid. Selsk. Forh. 1890, p 10.

1895-Ustilagidium hordei - Herzb. in Zopf, Beitrage Phys. Morph. Org. 5, p 7.

Description of Spores.

The spores of Ustilago nuda resemble quite closely those of U. tritici. In shape, size, color and character of wall the two are very similar, and it is sometimes difficult to distinguish between the two. The spores are globose, subglobose, or oval, sometimes irregular in shape, but not always. In color they are olive brown or golden, but quite frequently they are a darker, duller brown. They are very much lighter on one side than on the other. On the lighter colored side are rather irregularly disposed, warty excrescences, except for a spot from which the promycelium later emerges. The spore wall seems thicker on the dark than on the light side. In comparison with U. tritici it seems thicker all around, and in most cases darker. Often the spores appear to be hollowed out toward the center where they are also lighter in color than around the edge, thus giving the spore a concave appearance. The spore wall, as in U. tritici, consists of two parts, the epispore and endospore. Sometimes it is hard to distinguish clearly the two coats, but by the addition of potassium hydroxide, they can be quite clearly seen. In size the spores vary quite considerably, averaging probably about 5-7 x 6-6-6½ µ, although some are larger, and the more elongate ones not infrequently are 10 or even 11 µ long. They may be distinguished sometimes from spores of Ustilago tritici by the greater thickness of the spore wall and the somewhat concave appearance. However, this is not a universal characteristic, so it is sometimes almost impossible to distinguish between the two.

Germination in Water.

The spores germinate quite readily in water-in shorter time than those of U. tritici; but they require a longer time than those of U. hordei. The time required of course varies quite considerably with conditions, but under normal conditions at a temperature of 32°C, about 25% of fresh spores had sent out promycelia within 12 hours.

The promycelium appears at first as a delicate, hyaline protuberance. It always so far as could be seen arises from some point in or very near the lighter colored area of the spore. The promycelium is usually not so much constricted at its base as is that of U. tritici. In fact, very often it is constricted practically not at all (Plate VIII, fig. 10). Usually the spore wall is not ruptured by the promycelial tube, but in some cases it is slightly split (Plate VIII, figs. 8 & 9). This is especially liable to occur when the promycelia are short and blunt, in which case they seem to make up in thickness what they lack in length. The germ pore then must be large; it cannot always be seen, but in the earlier stages of germination it is sometimes possible to see it quite distinctly.

At first, of course, there are no septa, but within the course of a few hours they appear. Eventually in a full grown promycelium, there are two or three septa, but some never become septate at all, while others have more than three septa. Three, however, is very commonly the number in a normal promycelium. Usually there are not many branches, but sometimes they grow out from near the septa (Plate VIII, figs. 13 & 14). Typically, then, the promycelium is rather long and slender, measuring about 25-50 x $2\frac{1}{2}$-3μ. Fig. 15, Plate VIII is a very characteristic form. Some fusions, mostly of the "knee joint" type were seen, but aside from these they were quite rare. So far as was observed the promycelium remained attached to the spore throughout. Quite frequently segments became vacant; and quite frequently the entire protoplasmic contents were lost, but even then the filaments did not separate. After about 25-30 hours there were few changes in the original promycelium except that some of the segments became vacant.

When the promycelium has reached its maximum size, very often a long, very slender germ tube is sent out from one of the "knee joint" fusions or from near a septum, or even from the tip of the promycelium. Atfirst these germ threads are filled throughout their entire length with protoplasm and may or may not be septate. In the earlier stages there are usually no septations, but by about 50 hours they begin to appear, especially as the thread grows, leaving the basal portion vacant. These germ threads keep on growing for five or six days, sometimes reaching a great length, 200-450μ. After a few days the basal portion is always vacant and septate, while the tip contains protoplasm and is usually non septate. The basal portion is finally much longer than the tip: in a thread whose total length is 350μ, the tip is ordinarily from 60 to 100μ long. In diameter the tips are about 2μ wide, while the vacant, basal portion is usually narrower. The tips are very often wavy in outline, appearing much as did the swollen tips of Ustilago tritici before breaking up into resting cells. However, in U. nuda none of these tips were seen to break up, although the cultures were kept fully as long as those of U. tritici.

The germination in water is characterized by the development of a slender promycelium from a large opening in the spore; by the comparatively rare branching; by the development of long, very slender germ threads and by the absence of free segments or sporidia.

Germination in Sugar Solution.

The early stages of germination in sugar solution are quite similar to those in water. The promycelia, however, are often much thicker and, in consequence, the spore wall more often splits when germination begins. Branching is much more common and begins earlier. Often there are fair sized branches by 20 hours, and by 27 hours they are sometimes quite extensive. The branches usually arise from the upper end of a cell, just below a septum. Fusions are commoner than in water; they may be either "knee joints", or they may be formed by a branch growing out and fusing with the promycelium. Very often the intervening cells become vacant.

By 25 hours germ tubes had begun to form. These grow out from the promycelium as lateral branches, as direct continuations of it, or from fusions. By 27 hours many of the promycelia had become wholly or partly vacant, and a simple or complex germ tube was growing out. Germ tubes also arise from branches, so that there may be a number of them from a single original promycelium. When these germ tubes grow out from fused portions, the fused parts themselves usually become vacant. The threads as in other cases become septate at the base, and the tip may branch, the branches in turn fusing (Plate IX, figs. 1 & 2). The basal portion of the germ tube does not necessarily become vacant first, although usually it does. An intermediate or even an end cell may become vacant while the others are still filled with protoplasm. Rarely two promycelia come from the same spore and each sends out a long infection thread; or sometimes the promycelium forks very near the base and two germ threads nearly equal in length arise in this way.

After seven or eight days a rather curious net work, sometimes difficult to follow, is formed as a result of the growth and branching of a single promycelium. Such a one is shown on Plate X, fig. 2. In this particular case the promycelium sent out two branches very near the base. One of the branches sent out a long germ tube, while the other one, after growing for a time twisted back and fused with the original promycelium. From this fusion two germ tubes were sent out. The original branch also sent out a secondary branch which in turn sent out a long, typical germ tube. Later a second promycelial filament (a) was sent out from the spore. This filament eventually fused at b with a protuberance of the branch which fused with the original promycelium. Later, a germ thread was sent out from this fusion also so that from the various branches and fusions of one original promycelium, five distinct, very long germ threads were sent out. Another specimen showing the same general method of branching, fusing and producing germ tubes is shown on Plate XI, fig. 2. This is rather peculiar in the fact that at 16 days some of the promycelial segments still retained their protoplasm; usually they had lost it long before that time.

The two cases just described are not oddities by any means; in fact such combinations as are shown in these figures occur quite frequently. However, they could scarcely be considered as typical. More often the development is simpler; a single tube is produced from the promycelium , or two may be sent out. These reach great length. After about 16 days the basal portion which is almost hyaline is very hard to trace, so that some of the longest of the germ threads were probably not measured. A fairly representative one, picked out at random, measured 484 . Some of them were longer, so that a length of 500μ or more is not at all exceptional. The length seems all the more remarkable in view of the fact that the threads are very narrow, averaging about 1½-2μ, while the tips are a little thicker, usually about 2½ to 3½μ wide. The tips are 50 to 110μ long and usually wavy in outline; this wavy outline seems characteristic of the threads of U. nuda, since it did not appear so uniformly in any other form.

The tips keep on increasing in thickness up to about 26 or 27 days when some of them are as much as 7 or 8μ wide. Some are continuous and non septate, while others at this stage seem to exhibit a tendency to round up into segments as did those of U. tritici (Plate XII, fig. 1). From these rounded portions about the 26th day, branches began to be sent out. Usually they were short, measuring 25 to 35μ, but in some instances they reached a considerable length (Plate XII, fig. 1). Sometimes these branches appeared very similar to the original germ tubes, the basal portion becoming vacant and segmented, and the tip still remaining filled with protoplasm (Plate XII, fig. 2).

Fusions between different filaments are quite common. Two of the protoplasmic tips, lying side by side, often sent out short branches which fused. Some filaments were observed in which two fusions took place (Plate XII, fig.7) while cases in which branches of the same tube fused with each other, or a branch fused with the tip were common. The culture in which the forms described above grew was kept for 46 days, but it failed to show in that time further important changes. It was kept in the expectation that resting cells such as were observed in U. tritici would be formed, but so far none have appeared; in fact there were no free segments up to the 46th day unless the basal portion of the germ threads separated from the spores. This was not seen to occur, but it might easily have escaped notice, since after 35 days the filaments were hard to trace.

Summary of Germination.

The characteristic features in the germination of this form then are the development of a rather narrow, commonly three septate promycelium which sends out very narrow infection or germ threads to great length; the absence of sporidia, and non liberation of free segments.

Vitality of Spores.

Spores which were collected July 8, 1909 germinated very readily. Tests were made at intervals of about three weeks during the interim between July and April. Not much difficulty was experienced at any time in obtaining a fairly large percentage of germinations. Some smutted heads were buried in pots Dec. 12, 1909 and placed out of doors where they were left until March 8, 1910 when they were tested for germination. These spores germinated normally, showing great vigor in their growth, especially in nutrient solutions. The figures on Plate XIII are of spores which germinated after having been outside all winter.

Ustilago hordei (Pers.) Kellerm & Swingle.

History.

The history of this smut is rather uncertain. It was until so recently considered as identical with Ustilago nuda that there is much question as to its exact history. The history of U. nuda has already been given, and the history of U. hordei, during the earlier period, is practically the same. Originally of course this form was included with the other "loose smuts" under the name Uredo or Ustilago segetum. In 1591 Lobelius distinguished barley smut from that on oats, but it is not clear whether or not he recognized the difference between the two forms on barley. He gave two names, Ustilago polystichi and Ustilago hordei distychi, but he may have used both names to include Ustilago nuda and Ustilago hordei. Bauhin in 1596 in his Phytopinax, calls barley smut Ustilago hordeacea, but this name may have been used to designate both forms. In 1801 Persoon, in his Synopsis Methodica Fungorum, names a barley smut Uredo (Ustilago) segetum a uredo hordei, and refers to the spores as covered or hidden ("pulvere latente"). This shows that he had in mind Ustilago hordei.

Brefeld in 1883 described in great detail the Ustilago carbo spores from barley, and evidently he had Ustilago hordei. In 1888 Jensen definitely established the identity of the two smuts on barley. In 1888 he named the covered smut Ustilago segetum var tecta and in 1889 he called it Ustilago hordei var tecta. Subsequently he considered this smut as an independent species, giving it the name Ustilago tecta hordei Jensen. Kellerman and Swingle, working out the spore characters and germination peculiarities of the various smuts, contributed still more to the knowledge concerning the species, and it was finally named Ustilago hordei (Persoon) Kellerman & Swingle. Following is the probable synonomy of the species.

1552-Ustilago-Tragus-De Stirp. Nomen. pr., lib.III, p 666.

1591-Ustilago polystichi-Lobelius, Icones Stirpium p 36.

1591-Ustilago hordei distychi,Lobelius,Icones Stirpium p 36.

1596-Ustilago hordeacea-Bauhin, Phytopinax,lib.I,Sec.IV,p 52.

1767-Chaos Ustilago-Linne,Syst.Nat.Plant.Ed.XIII,II,p 1472.

1791-Reticularia Ustilago-Linne,Syst.Nat.Plant.Ed.XIII, II, p 1472.

1791-Reticularia segetum-Bulliard,Hist.des Champ.I, p 90.

1801-Uredo (Ustilago) segetum a Uredo hordei-Persoon, Syn. Meth. Fungorum, p 224.

1809-Caeoma segetum-Link, Obs., I, p 4.

1813-Ustilago segetum (Bulliard)-Dittmar, in Sturm - Deutschland's Flora,Bd.III, Heft 3, S 67.

1815-Uredo Carbo a hordei-De Candolle,Flora Francaise, VI, p 76.

1833-Erysibe vera a hordei-Wallroth, Flora Crypt. Germ. pars. post., p 217.

1837-Uredo Carbo-hordei-Phillipar, Traite sur la Carie, p 92.

1847-Ustilago Carbo a vulgaris c hordeacea- R & Ch.
Tulasne Mem. sur les Ustilag. comp. aux les Ured.
p 80.

1856-Ustilago segetum b hordei- Rabenhorst, Klotzchii,
herb. viv. myc., Ed. nova, cent. 3, No. 397.

1888-Ustilago segetum var. hordii f tecta - Jensen, Om
Kornsorternes Brand, S 56, et seq.

1888-Ustilago segetum var. tecta - Jensen, Prop. & Prev.
of Sm. in J.R.A.S. XXIV s.s. p 10; Plowright,
British Uredineae and Ustilagineae, p 274.

1888-Ustilago (Rabenhorst) Lagerheim, Revision der im Ex-
siccat "Kryptagamen Badens von Jack, Leiner und
Stizenberger" enthaltenen Chytridiaceen, Peron-
osporeen, Ustilagineen and Uredineen S.2, Nr. 41.

1888-Ustilago segetum var. hordei tecta-Jensen, Prop. &
Prev. of Sm. l.c. p 11.

1889-Ustilago hordei v tecta-Jensen, Le Charbon des
Cereales, p 4.

1890-Ustilago tecta hordei-Jensen, in letter, Jan. 24, 1890.

1890-Ustilago Jensenii-Rostrup, Overs. K. Danske Vid.
Selsk. Forh. 1890:12.

1890-Ustilago hordei (Pers.) Kellerman & Swingle, Ann.
Rep. Kans. Agr. Exp. Sta. 2:268.

Description of Spores.

The spore mass of Ustilago hordei is different from that of either U. tritici or U. nuda. Whereas the spore mass of the latter two forms is dry and powdery, that of U. hordei is usually more or less closely compacted, and seems sooty rather than powdery. In color too the spore mass of U. nuda and U. tritici is of a brownish black, while that of U. hordei is purplish or jet black.

In microscopic characters the spores are also different in important respects, those of U. hordei being, on the average, larger and rounder. More conspicuous is the fact that the spores in this form are absolutely smooth, there being no spines on the epispore. The spores vary some in shape, but not as much as those of the two forms described; in a great majority of cases they are subglobose or globose. They may be somewhat angled but scarcely ever do they present the oval forms which are quite common in the two preceding species. The size is also fairly uniform; the average would probably be 6-$9\frac{1}{2}\mu$, although some are larger, measuring up to $11\frac{1}{2}\mu$ in diameter. In color they are usually brown, and lack the bright lustre which is often characteristic of U. tritici or U. nuda, being of a duller brown. There is sometimes a somewhat peculiar mottled appearance due to the alternation of light and dark areas. The spores, as in the preceding species, are conspicuously lighter in color on one side than on the other, and on the dark side the spore wall often seems thicker.

Germination in Water.

The spores germinate very readily either in water or nutrient solution; in fact germination proceeds in much shorter time than in any of the other forms studied. A large percentage of fresh spores had germinated by 6½ hours, and nearly all had germinated by 12 hours. As in the other forms the promycelium grows out from some part of the lighter colored area of the spore. The promycelial tube is often slightly curved, but may be straight. Septa appear in the course of a few hours and when fully developed the promycelium is usually 3 septate, although it may be more or less.

At about 15 to 18 hours or less, the promycelium has begun to branch and bud. A few vacuoles also appear at about this time. At 18½ hours there were already some vacant cells but this was rare (Plate XIV, figs. 3-5). There seems to be no very definite order in which the cells lose their protoplasm; any one of them may first become vacant, and the others follow in irregular order. At this time, sporidia have already been formed; they may be abjointed either from the tip of the end cell or from septa, in which case they are usually pinched off just below a septum. In the formation of sporidia, a small protuberance is first sent out; this elongates, rounds up toward the tip, leaving a slender, very short basal portion by which it is attached to the promycelium, and then abstricts. Often it is possible to see the scar on the cell from which the sporidium was abjointed (Plate XIV,fig. 8). Usually the sporidia fall off almost immediately after formation, but more rarely they remain attached while they in turn bud and produce secondary sporidia. After one sporidium has fallen off from the promycelium, others may be produced in the same place, so that a continuous process goes on. Those already separated from the parent cell may grow and keep on budding off secondary sporidia, but this is not so common in water as in nutrient solutions. In shape the sporidia are usually elliptical or broadly oval; in size they average about 4-5 x 2 μ. Sometimes they germinate very soon after formation, but more often a considerable time elapses before they send out germ tubes.

At 48 hours a few germ threads had been sent out from promycelial cells. They arose in much the same manner as did those already described for the two preceding forms; by 48 hours they were already fairly well grown, the vacant, septate, basal portion averaging about 70 x $1\frac{1}{2}$-2μ , with swollen tips about 20-30μ long and a little wider than the basal portion. Vacant cells in the promycelium are now quite common, especially where branches or germ tubes are being sent out. At first in such cells the protoplasm disappears from the inner portion, leaving a film of protoplasm around the edge, but this may eventually disappear also.

After a few days practically all growth ceased leaving such structures as those which have been described. A few fusions took place, but they were infrequent in occurrence. The tips of germ tubes swelled slightly, and a few of the sporidia germinated, but nearly always the tube sent out by the sporidium was short and not at all thrifty.

Summary of Germination in Water.

The spores germinate quickly, sending out from the lighter colored portion of the spore a promycelium averaging about 45μ in length. Sporidia are rather sparingly produced from tip and septa; these may send out infection threads, but they are usually comparatively short. Branching takes place, but to no such extent as in nutrient solutions.

Germination in Nutrient Solutions.

There are very early marked differences between germination in water and in nutrient solutions. There is a much greater vigor in the germination and growth, some of the spores sending out two or in rare cases even three promycelia. When this occurs, there is uaually more than one light colored area in the spore, and it is from these that the tubes are sent out (Plate XV, fig. 8). The promycelia are uniformly thicker, although some times shorter, than those developed in water; often they are straighter, but they may be curved. The cells are shorter, thicker and more constricted at the septa, giving them a plump appearance. Oil globules are more abundant than in water, both in the promycelia themselves and also in the sporidia. The promycelium grows to a length of 30 - 43 , although some are longer, and is ordinarly completely formed by 30 hours.

One of the most striking features appears in the vigor of growth, especially in the production of sporidia. Sporidia are abjointed in great profusion both from the tips of end cells and at the septa; often they fall off very shortly after formation, but they may remain attached and go on budding very vigorously, producing secondary, tertiary and even quaternary sporidia (Plate XV, fig. 9). After detachment the sporidia also may grow and keep on budding energetically, often from both ends, until after about 30 or 40 hours, the culture is completely filled with them and, even with the naked eye, one can see on the cover glass a white mass of luxuriantly growing sporidia and filaments.

The sporidia vary in size and shape. They may be oval, ellipsoidal, subglobose and rarely, somewhat elongate and slightly angular. In size there is also considerable diversity: when first abjointed they are 7μ x 4-6μ, but they later grow larger. They are thus seen to be larger than those produced in water. There is more of a tendency to assume a subglobose or globose form, the thickness being usually greater. The secondary sporidia may be nearly the same size as those from which they are formed; usually they are a little smaller. For a few days the sporidia do not germinate: all their energies, as it were, seem to be devoted to the formation of as many more sporidia as possible. After three days, however, some had sent out tubes to a length of 20-25μ. The tips of these germ tubes, even when short, are frequently swollen. Sporidia are sometimes formed from the tubes before they grow out into a long, typical germ thread.

As time passes on, the sporidia increase in size as do the promycelia. Fusions between branches of the same promycelia or different ones are quite common, and from these or from cells of the promycelium, germ tubes are sent out (Plate XVI). They resemble in general features of development and appearance those found in U. tritici and U. nuda, but were usually quite a little shorter and thicker. At about this stage manyof the promycelia became detached and kept right on budding or branching. A few of the spores which germinated tardily sent out short, very thick, blunt 3 or 4 septate promycelia from which germ threads were sent out. Often from these belated spores there were two promycelia, one usually larger than the other (Plate XVI, figs. 9 & 11).

Upon the approaching exhaustion of the nutrient medium, the tips of the germ tubes become swollen to about 6-7μ and round up, as do the short promycelial cells referred to above, into thick, very coarsely granular segments. Fusions between various segments, either of the same or of different filaments, are quite common. These segments may break apart when the nutrient material is exhausted; or they may remain attached. When they break up they may break up into single segments or a number may remain attached, forming a chain as is shown on Plate XVII, fig. 16. The sporidia have by this time grown to a considerable size, many of them being as large as the original spore. The culture was in this condition at 9 days when the cell was allowed to dry out. More sugar solution was then introduced and within 10 hours, the segments which had rounded up sent out tubes (Plate XVII, figs, 4,7,13,16, 17, 18, 19). These tubes often resembled original promycelia, while in other cases they were very much like germ tubes (Plate XVII, fig. 4). The sporidia also send out germ tubes or keep on budding. Sometimes the sporidia germinate from one end and keep on budding from the other. The growth in the culture by 12 days became very rank; in fact there was such a conglomeration of budding and germinating sporidia, free segments, germ tubes and promycelial remains that it made accurate observation very difficult. In the dense masses, however, sporidia did not germinate; neither did free segments, so only those around the edge sent out tubes.

Summary of Germination in Sugar Solution.

The essential features of the germination in sugar are the abundant production of sporidia which may keep on budding off other sporidia, or germinate sending out eventually long germ threads; the frequent occurrence of free segments, and their long continued activity; and the typical rounding up of resting segments upon approach of unfavorable conditions. The resting segments formed earlier in this form than in U. tritici and germinated more readily. This seems very natural in view of the fact that there is a greater tendency toward formation of free segments and a more ready germination in U. hordei than in U. tritici.

Vitality of Spores.

The ability of spores to undergo successfully winter conditions was tested in the same way as in the two other forms so far described. There was no apparent diminution of vitality or vigor due to the exposure during the time the spores were outside.

Ustilago avenae (Persoon) Jensen.

History.

The ancients probably knew smut of oats, but did not distinguish it from other smuts. For a long time it was not recognized as a fungus parasite, but considered as an abnormality of the plant itself. Linnaeus at first failed to recognize it as a fungus, but later he did so. Tragus in 1552 gave it the name Ustilago[1], while Lobelius[2] in 1591 and C. Bauhin in 1596 called it Ustilago avenae[3] and Ustilago avenaria respectively. None of these men recognized the true nature of the malady; in fact some even considered that it was due to the presence of small animal organisms.

Bulliard[4] in 1791 recognized it as a fungus, but did not distinguish between the different smuts. He applied

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1) Tragus-De Stirp. Nomence. prop. lib. III, p 666.
2) Lobelius-Icones Stirpium plantorum tam exoticum quam indigenarum Antoerpiae, 1591, p 36.
3) C.Bauhin-Phytopinax, p 52.
4) Bulliard-Histoire des Champignons de la France I,p 90.

the name Reticularia segetum to all of the cereal smuts. This was the first fungus name applied to the parasite. A number of writers between 1750 and 1800 mentioned the loose smuts, but except for Tessier in 1783, none of them take any particular notice of the smut on oats.

Persoon[1] adopted Bulliard's name, Ustilago segetum, for the loose smuts in general but he separates them into varieties on the different hosts, calling that on oats Uredo (Ustilago) segetum g Uredo avenae. The smut was placed in the genus Ustilago in 1813 by Dittmar.[2] He applied the name Ustilago segetum, thus retaining Bulliard's specific name. De Candolle[3] called all the loose smuts Uredo carbo, retaining for the forms on various hosts the varietal names of Persoon. The oat smut thus became in his system of nomenclature, Uredo carbo g avenae. Other names were applied by Wallroth, Phillipar and Tulasne, but not a great deal of real knowledge was added until Jensen made his inoculation tests and showed the various forms distinct. His ideas were substantiated by the extensive germination tests made

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1) Persoon-Synopsis Methodica fungorum, pars. prima - Gottingae 1901, p 224
2) Dittmar-in Sturm, Deutschland's Flora, Band III, Heft 3, S 36
3) DeCandolle- Flora Francaise, V, p 76

by Kellerman & Swingle. Oat smut, together with other "loose" smuts is often referred to as Ustilago segetum (Bull.) Dittm. and as Ustilago carbo (D.C.) Tul.. Jensen[1], however, removed the necessity for confusion so far as oat smut is concerned by naming it first Ustilago segetum var. avenae[1] in 1888 and Ustilago avenae[2] in 1889.

The following is a synonomy of the species.

1591-Ustilago avenae-Lobelius, Icon. stirp., p 36.

1596-Ustilago avenacea-Bauhin, Phytopinax, p 52.

1767-Chaos ustilago-Linne, Sys. Nat.,Ed. XII,II, p 1356.

1791-Reticularia ustilago,Linne, Syst.Nat.,Ed.XIII,II,p 1472.

1791-Reticularia segetum-Bulliard,Histoire des Champignons, I,p 90. Poiret in Encyc. Method. de Botanique, VI, p 181. Withering, Bot. Arr. IV, p 356. Johnston, Flora of Berwick-on-Tweed, II, p 203. Greville, Flora Edin., p 442.

1797-Uredo segetum-Persoon, Disp. Method. fung., p 56; Syn. Meth. fung., p 224.

1801-Uredo (Ustilago) segetum g Uredo avenae, Syn.Meth. fung., p 224.

1809-Caeoma segetum- Link, Obs. I, p 4.

1815-Ustilago segetum (Bulliard) Dittmar, in Sturm, Deutschland's Flora, Band III, Heft 3, S 67. Fries, S.M. III, p 518. Berkeley, in Smith's English Flora, V,

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1) Jensen-Prop. & Prev. of Smut in Oats & Barley, J.R.A.S. of England, XXIV, s.s., part II, p 11

2) Jensen-Le Charbon des Cereales, Copenhague, Jul.1889,p 4

pt.II, 1847, p 374; Outl. Brit. Fungi, p 335. Cooke, Micro. F. 4th ed., p 229. Loudon, Ency. of Plants, Loudon 1872 pp. 1044, 1045. P.A.Karsten, Mycologia Fennica, pars IV, in Bidrag till kannedom af Finlands natur och folk, Trettion-deforsta Haflet, p 6, No. 1. Winter, Die Pilze, I p 90, Nr. 103. Oertel, Beitrage zur Flora der Rost und Brandpilze Thuringens, in Deutsche Botanische Monatschreft, IV, Nr. 3, Marz 1886, Seite 267, Nr. 418. Plowright, British Uredineae and Ustilagineae, p 274. De Toni, in Sacchardo Syllage Fungorum VII, II, p 461.

1815-Uredo Carbo g avenae-De Candolle, Flora Francaise, VI, p 76.

1833-Erysibe vera g avenae-Wallroth, Flora Cryptogamica Germaniae, p 217, No. 1672.

1837-Uredo Carbo avenae, Phillipar, Traite sur la carie et la Charbon, p 92, pl. 2.

1847-Ustilago Carbo a vulgaris b avenacea, Tulasne, Memoir sur les Ustillginees comparees aux Uredinees, in Ann. Scie. Nat. z e serie t VII, 1847, p 80.

1871-Ustilago Carbo-Tulasne, Cooke, Handbook of British Fungi, II, p 512, No. 1520. Hazslinsky, Magyarhon uszokgombai es ragyai, in Magy. tud. akad. math. es termesz. Kozelmenyek, XIV, Iot. 1876-7, 1-110 Fischer von Waldheim, Aper. System. des Ustilag., p 12, No. 6. Gholovneviuya, Monoghraficheskei ocherk, Chast II, str. 13.

1888-Ustilago segetum var avenae-Jensen, Om Kornsorternes Brand (Anden Meddelese), S 61.

1889-Ustilago avenae (Pers.) Jensen, Le Charbon des Cereales, p 4.

1903-Ustilago avenae f. foliicola, Olmeida, Revista, Agron. I, p 20.

Description of Spores.

The spores resemble somewhat those of U. nuda and U. tritici. In size they are very nearly the same, ranging from 5 to 11 μ. They lack, however, the lustre of the spores of the two forms just mentioned. Whereas those of U. tritici and U. nuda often have a golden tinge, those of U. avenae are usually of a dull brownish color, except in the lighter colored areas. In shape they approach more often subglobose or globose than those of U. tritici. Sometimes they are oval or elliptical, but not typically. They may sometimes be angular and somewhat irregular in outline. As in the cases of the other spores mentioned, they are conspicuously lighter in color on one side than they are on the other. On the light side the echinulate character of the spore wall is very often clearly perceptible. On the darker side it is possible more often than in other forms to see the endospore without the use of reagents. When visible, it usually appears as a faintly outlined hyaline portion just inside of the epispore.

Germination in Water.

The spores germinate quite readily in from 10 to 13 hours. As in the other forms, the promycelium extrudes from the lighter colored side of the spore. Two germ tubes are not uncommon; when there are two, both usually grow out from the lighter colored side of the spore (Plate XVIII, fig. 8). The promycelia are at first rather slender and non septate. Very shortly, however, septa begin to appear. By 24 hours the promycelia are often fully grown, measuring 20-40x4-5μ. They are either straight or slightly curved and usually 2 or sometimes 3 septate. They abjoint sporidia from the septa or from the tip, more often from the tip, although it is not at all uncommon for them to be formed at the septa. They are pointed at the end by which they were attached, are oval or elliptical, sometimes elongate in form, and measure 4x2-7x7μ. These sporidia may again bud, either in situ or after detachment, and form secondary spores. This, however, was not very prevalent in water, occurring by no means as often as in nutrient solutions.

When fully grown the promycelia are 1-3 septate, although sometimes more. The usual number of septations is two. There are quite frequently branches arising from some point near the base of the promycelium which in some cases grow up and fuse with one of the promycelial cells. A very prevalent tendency is the production of knob-like protuberances on opposite sides of a septum. These stubby branches fuse and there is formed as a result a knob at the septum. As the promycelia grow, the parts of the knob are forced apart and such a condition as is shown on Plate XVIII fig. 7 is seen. Very often the promycelium is quite sharply bent at these fusions (XVIII, 13).

After a few days the sporidia have usually germinated; in water few are formed after about 2 days. They may eventually send out a long infection thread showing the typical characteristics of those already discussed for the other forms. The promycelia in the meantime often become detached and swell up; the swelling is especially apparent at the ends of the cells which seem rather loosely held together. The protoplasm becomes vacuolated, many of the cells becoming entirely vacant, while those which retain their protoplasm keep on swelling slightly until they are often as thick as the original spore. Long germ threads are now usually sent out, and with their formation the culture usually becomes dormant.

Summary of Germination in Water.

The germination and growth in water, then, is characterized by the meager production of sporidia and by the fact that they seem to be formed from the tip of the promycelium more often than from the septa. The promycelia are somewhat shorter than those of other forms, are usually 2 septate, although not necessarily so, very frequently become detached, very often form "knee joint" fusions, and eventually either become vacant or swell up at the ends and send out germ tubes before becoming vacant. Infection threads are sent out either by sporidia or from one of the cells or a fusion of the promycelium.

Germination in Nutrient Solution.

In nutrient solutions growth is always much more vigorous. Quite frequently two promycelia were sent out from the same spore. Often after the promycelium was fairly well grown, another one was sent out, or in some cases two were sent out simultaneously. Apparently in one or two cases the second germination took place on the darker side of the spore. This, however, may have been apparent only since the tube may have come from under the spore.

Sporidia are formed in much greater abundance than is the case in water. They are abjointed both from septa and from the tips of the end cells. Ordinarily they fall off soon after formation but they may sometimes remain attached and produce secondary sporidia. Usually they are larger than those formed in water. As was the case with U. hordei they are also typically of a different shape. Those formed in water were elliptical or oval, while those formed in sugar solution are very often subglobose. In size they averaged shortly after detachment about 5x9μ.

Promycelia often become detached and kept on budding just as though they were still attached. The sporidia also kept on budding very vigorously after detachment. When the nourishment began to be exhausted, however, this activity seemed to cease and germ tubes were sent out. The drying up of the culture did not seem to impair the vital properties of either the thickened promycelial cells or the sporidia, for upon the addition of more nutrient solution solution they again became active.

The chief difference between germination in water and in nutrient solution is the greater vigor of germination in sugar solution and the more abundant and prolonged production of sporidia. In other respects, such as branching, fusions, production of germ tubes and freeing of segments there is no vital difference. The vitality of the spores was not lessened by exposure to winter conditions; germination was nearly as prompt and vigorous as when the spores were fresh.

Ustilago zeae (Beckm.) Unger.

Maize smut has been known for about one hundred and fifty years. It was noticed first by Aymen in 1760 on corn tassels. He seems, however, not to have named it and not to have given an idea of its true nature. He says for instance that the source of the disease is in the tassels[1]. De Candolle[2] in 1806 included it under the name Uredo segetum, designating it as a variety, Mays zeae. Burger[3] in 1810 gives some information about it, but his account is brief. He noted, however, that the smut may attack any part of the plant as contrasted with the smuts of the small grains. He also noted that the seed corn might be treated with the "smut dust" without the smut necessarily appearing on the plants grown from this seed. Bonafous[4] in 1836, in his Histoire Naturelle, describes the smut in some detail, giving also an interesting account of the controversy as to whether the trouble was due merely to a vegetative abnormality or a fungus parasite. He cites for instance as evidence on the part of those who hold that it is not due to a fungus parasite, the fact that seed corn treated with smut does not necessarily produce smutted plants, while if the stalks be twisted and thus slightly bruised, smut results. Many at that time regarded smut on corn as a lesion caused by the rupture of cellular tissue with the ensuing diffusion of an altered cell sap.

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1) Aymen-Rech. sur les pragres et les causes de la Nielle, 7 77 (1760)
2) De Candolle, Flora Francaise (1805) II, 596
3) Burger, Akhandlung uber Mais (1809) p 242
4) Bonafous-Historie Naturelle, Agricole et Economique du Mais (1836) p 94, pl. 18

Tulasne[1] in 1847 gave a full description of the fungus and gives it a specific name, calling it Ustilago Schweinitzii. De Bary[2] in 1853 threw much light on the method of infection and development of the fungus. Others, such as Kuehn, Fischer von Waldheim, Wolff and Kuhn, contributed papers and observations on the smut. Kuehn[3] in 1859 tried to germinate the smut spores but found that fresh ones could not be induced to germinate with any degree of readiness in water. He found, however, that in the following January they germinated fairly well. Brefeld[4] gives a complete and interesting account of the germination of the spores. His experience with the germination of fresh spores in water is similar to Kuehn's. He received a smut "boil" from a friend and at once attempted to germinate the spores in water, but was surprised to find his efforts not at all successful. Subsequent to this time he accidentally dropped the spore mass and a cloud of spores arose, some of them falling into cultures of spores growing in nutrient

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1) Tulasne-Memoire sur les Ustilag.comp. aux Uridin, (Ann.Sci.Nat.S. III,T.VII,1847,p 83)
2) De Bary-Untersuchungen uber die Brandpilze (1853) p 4
3) Kuehn-Die Krankheiten der Kultur gewachse (1859) p 70
4) Brefeld-Botanische Untersuchungen uber Hefenpilze-V Heft, p 68 et seq.

media. He found that these spores germinated very readily. However, he was not successful in obtaining germinations in water until the following spring. He describes in detail the various phases of germination, and later gives the results of extensive infection experiments which he carried on.

A synonomy, probably incomplete, is given-

1768-Lycoperdon zeae, Beckman, Hannov. Mag.6, p 1330.

1805-Uredo segetum var. Mays zeae. DeCandolle, Flora Francaise, II, p. 596.

1815-Uredo maydis, De Candolle, Flora Francaise, VI, p 77.

1822-Uredo zeae-Schweinitz, Schr.Nat.Gesell.Leipzig, I, 71.

1822-Caeoma Ustilago zeae-Schweinitz, Synopsis Fung. Carolinae Superioris, No. 485.

1824-Caeoma zeae-Link-Species Plantarum II, p 2.

1833-Erysibe maydis-Wallroth, Flora Cryptogamica Germaniae IV, 215.

1836-Ustilago zeae (Beckm.) Unger, Einfl. Bodens, p 211.

1840-Ustilago zeae-Unger in Corda, Icones Fungorum, IV, 9.

1842-Ustilago maydis Corda, Icones Fungorum V, p 3.

1847-Ustilago Schweinitzii-Tulasne, Ann.Sci.Nat.Ser.III, Vol 7, p 86.

1881-Ustilago zea Mays, Winter, Pilze Deutschlands I, p 97.

1882-Ustilago Euchlaenae-Arcang.Erb.Critt.Ital.II, 1152.

1884-Ustilago zeae mays, Winter, Rab.Krypt.Fl.I, p 97.

1895-Ustilago mays zeae, Magnus, Deutsche Botanische Monats schrift XIII, 50.

Description of Spores.

In shape the spores are mostly globose or subglobose, sometimes ellipsoidal, and infrequently irregular; by far the greater percentage are either globose or subglobose. They are very prominently echinulate, the spines showing distinctly on all sides. Unlike all the other spores so far described, those of Ustilago zeae are not lighter on one side than on the other. In size they are larger than any of the four already described, varying from 8½ to 15μ. They are mostly about 10μ in diameter and seem to vary less from the normal than do those especially of Ustilago tritici and Ustilago nuda. Very few were seen which measured 15μ, although a few abnormally long ones did. When that long, however, they were considerably thinner than average, being of a narrowly oval shape, which is rather uncommon.

They are usually reddish brown in color, although some are darker, especially around the edges where they are often brownish black. Often they are considerably lighter in color toward the center than around the edge. The wall consists as in other forms of two parts, the outer epispore and the inner endospore. The endospore is sometimes difficult to see without the aid of reagents. However, when treated with either potassium hydroxide or acetic acid, the two coats may be very clearly seen.

Germination in Water.

The spores, especially when fresh, germinate either very tardily or not at all in water. Fresh spores just taken from the corn plant were tried but they failed to germinate. Many attempts were made during the fall and early winter to germinate them, but with no success. It was not until the earlier part of January that they could be induced to germinate. These negative results, together with the fact that the result with nutrient solutions was very nearly the same, was puzzling at first: it was thought that the spores were not yet wholly matured when first tried. To establish this point, spores were gathered every few days up to the time the corn was cut, and germination tests made, but always with negative results until the early part of January. The experience of Brefeld and Kuehn has already been cited. As opposed to their experience and the results obtained here, Hitchcock and Norton found no difficulty in germinating fresh spores in water; in fact it was their experience that fresh spores germinated more readily although not quite so luxuriantly in water than in nutrient solutions. The difference may be one of acclimatization and adaptation to local conditions.

The spores after a resting period germinate fairly well in water, requiring less than 14 hours. The promycelium more often than in preceding forms ruptures the spore wall. Ordinarily the rent in the wall is only short, but very often conspicuous. The promycelium is at first very delicate and non septate. The base is often constricted where the promycelium emerges from the spore (Plate XX, figs. 1-9). Within 20 hours septations usually appear, and at about the same time there are outgrowths from near the septa. These delicate protuberances develop into small fusiform conidia measuring from 8 - 18μ x $\frac{1}{2}$ -2μ. Usually the conidia break off very soon after formation, and only rarely were they observed to produce secondary conidia in situ. However, after being abjointed, they very often form secondary conidia, usually smaller than themselves. Occasionally the promycelia lost their protoplasmic contents in the formation of sporidia (Plate XX, fig.8), but this was not so often the case with attached as with detached promycelia.

When full grown, usually within 25 hours or less, the promycelia range in length from 20-60μ and are comparatively narrow. They are mostly 2 or 3, usually 3 septate, but not universally so and are either straight or curved; usually when they are slender they are also curved. They may or may not be branched; when branches appear, they often arise from the base (XX,5). The promycelia become detached very readily. At first either the entire promycelium or two of the cells break off, leaving the others attached to the spore. Later the rest of the cells separate from the spore so that by four days there were practically no attached promycelia to be found. These detached promycelia do not seem to suffer any diminution of vegetative activity in consequence of their detachment from the spore. They may even keep on growing, and frequently do, or they may produce conidia just as vigorously as they did while still attached to the spore. Often they become vacuolated, and later lose their protoplasm entirely as a result of the continuous formation of sporidia (XX,14, 16,17,22). Sometimes short chains of conidia are formed in the water, but this chain formation is much more apt to occur when the promycelia of spores growing on the surface of the water in watch crystals are partially or wholly exposed to the air. In such cases, chains of considerable length are often formed. (XX,21) This figure is of air - sporidia in a manure decoction, but is very similar to those formed in watch crystals filled with water.

The promycelia, sometimes while still attached, and often after detachment, send out a germ thread, protoplasmic at first but soon becoming vacuolated or entirely vacant but segmented in the basal portion (XX, 7,24,25,27). The sporidia too germinate quite readily. Some had already germinated at the expiration of 24 hours, while others required a much longer time. At first the filament is narrow and often wavy in outline, but as growth progresses these infection threads assume practically the same character as those sent from the promycelial segments. The sporidia themselves become vacant after the germ tube is well established. One peculiarity is the total absence of fusions; none of any nature were seen. After five days very little if any change took place; the germ tubes may have grown a little but it was almost imperceptible.

Summary of Germination in Water.

Fresh spores germinated with difficulty or not at all in water, but after a resting period they germinate quite readily. The promycelia are rather slender, usually 2-3 septate and often slightly curved. Fusiform, rather small sporidia are sparingly produced, mainly from the septa. These may rarely form other sporidia; more often they send out infection threads. The promycelium usually separates from the spore, continuing its activities as though still attached. There were as, contrasted with other forms, no fusions observed. The germ tubes from either promycelia or sporidia were in no case as long as those of other forms.

Germination in Nutrient Solution.

The promycelia produced in nutrient solution are usually much shorter and blunter than those produced in water. The base is not so often constricted; in fact very often the base is thicker than the portions out toward the tip, thus giving a pyramidal appearance. The promycelium as in water becomes 2 or 3 or even four septate and abjoints sporidia from septa, or in some cases from the tip. The sporidia are much more numerous than in water; in fact within a couple of days the culture teems with them. They may either bud again, forming secondary sporidia, or they may send out germ tubes soon after formation. More often they keep on budding while the nutriment lasts, sometimes forming dense masses of sporidia-so dense in fact that only those around the edge ever germinate. They are usually considerably larger than those formed in water, in some cases measuring as much as 30x5μ. Their shape is also different; they have rounded edges and are thicker, being less often spindle shaped. Their large size, together with the fact that they are often of the same shape as individual segments of the dismembered promycelium makes it rather difficult at times to distinguish between them. Upon germination, sporidia give rise to a long, slender germ thread resembling quite closely those formed in water. The promycelium may branch and send out long filaments such as is shown in fig. 28, Plate XX, or it may break up, go on budding and finally send out germ tubes from the various segments. When growing on the surface, long much branched chains of air conidia are produced, differing from those in the liquid mainly in the fact that they do not break apart so readily as those in the solution. They are also sometimes thicker walled and resemble ungerminated sporidia in having a large supply of oil. See Plate XXII.

Summary of Germination in Nutrient Solution.

The promycelia formed in nutrient solution are thicker and sometimes shorter than those formed in water. Sporidia are much more abundantly produced, are larger, are more of an oval shape, and bud more vigorously than in water. Sporidia are produced as long as the nutrient material lasts; when it is exhausted, or approaches exhaustion, germ tubes are sent out either from sporidia or from promycelial segments.

Tilletia foetens (B & C) Trel.

Introductory.

This is the smut popularly known as bunt or stinking smut of wheat. There are two species of bunt which cannot very well be distinguished except by microscopic examination of the spores. Tilletia foetens, the form described here, has smooth spores while Tilletia tritici (Bjerk) Winter has very pronounced winged reticulations. In size they are nearly the same, although those of Tilletia tritici are a little smaller. Both of these forms are reported by Clinton[1] as occurring in Minnesota. Both may occur in the state, but Tilletia foetens is probably the more common form. All of the bunt collected at St. Anthony Park was of the latter variety, and specimens obtained from St. Peter, Belgrade, St. Hilaire, Detroit, Olivia, Watson and Mendota also proved to be Tilletia foetens, while spores from Pullman, Washington, were those of Tilletia tritici. The places in Minnesota from which spores were obtained are fairly representative of the different areas of the state, so it is probable that there is not a very distinct regional distribution of the two forms, but

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1) Clinton, G.P. - N.A. Flora, Vol. 7, Pt. 1, p 48.

rather a local one.

History.

The early history is probably confused with Tilletia tritici. The ancients knew the stinking smut, but there is of course no way of knowing whether it was Tilletia foetens or T. tritici. Tilletia foetens was first recognized by Wallroth[1] in about 1833, and was first definitely described by Berkeley and Curtiss[2]. It was given the name Tilletia laevis by Kuehn in 1874, and is so referred to by Frank[3] and other European writers. Frank[4] states that it occurs especially on summer wheat in the Alpine regions. However, he says, it is locally distributed, alternating with Tilletia tritici in various places.

Synonyms-

1815-Uredo Caries-De Candolle, Flora Francaise, VI, p 78.

1847-Tilletia Caries-Tulasne, Ann. Sci. Nat. III, 7, p 113;
Ustilago sitophila - Dittmar;
Caeoma sitophilum - Link.

1873-Tilletia laevis, Kuehn, Rab. Fungi Env. 1697.

1884-Tilletia foetens (B&C) Trelease, Parasitic Fung. Wisc. p 35.

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1) Wallroth-Flora Cryptogamica Germaniae, II p 213.
2) Ravenel-Fungi Car. V, 100-Berkeley, Notices of N. Am. Fungi, 573-Grevillea, III, p 59.
3) Frank, Krankheiten der Pflanzen p 117.
4) " " " " " p 118.

Description of Spores.

The spores are the largest, by far, of all the species studied. They measure 14-27μ , mostly about 20μ. In shape they are usually globose or sometimes subglobose, occasionally more elongate and oval..The wall is composed of epispore and endospore. Like that of Ustilago hordei, the epispore is entirely smooth. The two spore coats are more easily distinguishable than those of the other forms, probably due to the greater size of the spores. The addition of potassium hydroxide makes it possible to see them very clearly. The epispore is often dark brown, sometimes of a lighter color, while the endospore is almost hyaline. In color the spore is usually light or dark brown, sometimes almost golden. Some of the spores are of the same color throughout, presenting a bronze colored surface, while others have a large number of oil globules such as the spores shown on Plate XXI.

Germination in Water.

Spores collected in August 1909 were immediately tested for germination, but none germinated either in water or in nutrient solutions. Repeated attempts were made throughout the fall and winter, but always with negative results. Some bunted heads were put outside, Dec. 10, 1909, and allowed to remain there until April 8, when some were put in hanging drops in Ward Cells. These spores germinated in 72 hours. Brefeld states that the spores of Tilletia tritici which he tried germinated readily when fresh. Spores of T. tritici from Pullman, Washington, were tried time and again in the fall and winter, but did not germinate, while those which had been frozen germinated within three days.

In tests made during the latter part of April, 1910 spores of T. foetens germinated in 48 hours. Not more than 10% had germinated by this time, but by 70 hours practically every spore had sent out a germination tube. The epispore is nearly always ruptured when the promycelium is formed. Sometimes the rent is not very long, extending not more than 1/6 of the length of the spore, but in others it is long and very conspicuous, often reaching very nearly the length of the spore. In many cases there was a very decided crack from which others extended (XXIII, 3 & 4). Sometimes part of the endospore seems to be carried out of the spore with the promycelium and then torn especially when an unusually thick promycelium is sent out.

The promycelium appears first as a straight or sometimes wavy protuberance; it is sometimes coarsely granular, but usually not exceptionally so. The spores which germinate late often send out much thicker and blunter promycelia which frequently branch almost as soon as they extrude from the spore (XXIV, 7 & 8). The branches in these cases are, however, nearly always short and stubby, appearing at first like irregular knobs. They frequently have a knarled appearance and are almost as broad as the spore itself. The longer germination is delayed beyond that of the average, the more prevalent is this tendency.

Normally the promycelia are very much like those shown in figs. 6 & 16, Plate XXIV. Septations in filaments with no vacant portion are rare or absent. However, within a short time vacuoles appear, and as the promycelium grows, the protoplasm moving continually forward, the portion next to the spore becomes vacuolated, then entirely vacant and finally septate. Sometimes a promycelium may lose its protoplasm without becoming septate (XXIV,14). Simple or compound branching is very common at this stage. In most cases the branches are very nearly the size of the original promycelium. At 76 hours some of the promycelia had assumed the characteristic appearance of germ threads, and by 96 hours practically all of them had taken on this form, but they grew for some time longer. They varied in length from 90 to 250μ, being in comparison with U.tritici and U.nuda, especially considering the relative sizes of the spores, rather short. The tubes continued to produce vacant cells until by 7 days many of them were entirely vacant and hyaline. By 8 days the filaments had nearly all disintegrated, or at least were dormant and ready to break up. This is an account of a germination in a Ward Cell. Many more like it were observed; no sporidia were formed, nor was there any apparent tendency toward their production except in a few cases.

Spores were also put in water in watch crystals to determine whether or not there were important differences from those growing in Ward Cells. Many of the spores had sent out promycelia in 48 hours. By 75 hours small protuberances began to appear on the tips of some of the promycelia which still retained all of their protoplasm. These knobs varied in number; not all of them being visible, it was sometimes difficult to make absolutely certain of the exact number. They grew out into finger-like projections and finally into an even number of long, slender, somewhat cylindrical or narrowly sickle shaped sporidia. By 4 days these sporidia were very numerous.

Usually there were 4,6,8, or 10 sporidia, 6 and 8 being very common. However, odd numbers sometimes occur, but only as rare exceptions. At first they are arranged in a whorl on the end of the promycelial tube. Often they diverge toward the distal extremity, but in some cases they converge, forming a spindle shaped bundle. The individual sporidia were at first well filled with protoplasm containing but few vacuoles, and as far as could be seen, no septa. Often while still attached to the promycelium, little knob like branches were sent out toward each other by neighboring sporidia. These branches meet and fuse. Often all the sporidia of one whorl are united in pairs; The sporidia may become detached from the promycelial tube before fusion; in that event, they fuse with the nearest sporidium(XXIV,46), or if none be in close proximity they fail to fuse. However, the chances of one sporidium becoming isolated are not very great, since they are produced so close together.

One of the pair of fused sporidia now often sends out, commonly from near the tip, but not necessarily so, a slender branch (XXV, 18 a). This branch grows out into a very long, narrow, often much coiled germ tube, filled with protoplasm at first, but eventually becoming vacant and segmented from the base outward, and retaining protoplasm only in the somewhat swollen tip. As the germ tubes increase in length, the protoplasm of the sporidia becomes vacuolated at first and finally disappears (Plates 24 & 25) altogether. At the same time septa, varying in number from 5 to 7, appear in the fused sporidia which, now fully grown, are often 70-80μ long by 3-4μ wide. It is not absolutely essential that a sporidium fuse in order that it may send out a germ tube, but in the great majority of cases, the fusion takes place.

Instead of immediately sending out germ tubes, the sporidia often send out short, rather thick little branches which gradually swell up toward the tip. These branches keep on getting larger toward the tip until they appear to be attached by a slender filament resembling a sterigma. These are secondary sporidia; they are often bean shaped or crescent shaped and measure 20-45μ x4-6μ. They may send out long slender germ tubes very closely resembling those sent out by the primary sporidia, or in some cases they send out a filament which keeps on producing more secondary sporidia (XXV, 4). The germ threads may branch from the filled tip and may produce more secondary sporidia from these lateral branches (XXV, 7).

When mature, the germ threads are very long, some of them measuring at least 450μ with a tip 75-110μ long. Some of them may very probably be longer, but on account of the fact that they have such a strong tendency to become much coiled, absolutely accurate measurements were very hard to make.

Summary of Germination in Water.

The spores seem to require a resting period; at any rate the spores of the particular material used germinate only after such a period of rest. Germination occurred in 48 hours to 4 days, depending upon conditions. The spore wall in most cases is ruptured by the simple or branched promycelium which grew out to a length of about 200 μ and assumed the appearance of a germ thread. In case the promycelium reaches the air, long, cylindrical, often curved sporidia are produced in whorls on the tip. These unite in pairs before or after detachment from the promycelium and either send out long infection threads or produce secondary sporidia which send out the threads. On these threads produced by secondary sporidia short lateral branches may arise which in turn produce sporidia like the one from which thread grew. Although usually the secondary sporidia produced by germ are formed on lateral branches, sometimes they are formed in a string. In case there is an odd number of sporidia or if fusion fails for any other reason to take place, the single sporidium may send out a germ tube, but it is shorter than the average.

Germination in Nutrient Solution.

In practically all trials in nutrient solutions, except soil infusion, both with T. foetens and T. tritici, the presence of the nutriment seemed to have a deleterious effect. The spores under the same conditions of temperature etc. required a longer time to germinate than they did in water. The germinations, when they did occur, were usually abnormal. In a large number of cases the promycelia exhibited a very well defined and persistent tendency to round up into an irregular or sometimes even subglobose or globose mass of protoplasm, resembling somewhat an overgrown amoeba with few or no pseudopodia. Occasionally the entire contents of the spore seemed to be making an unsuccessful effort, as it were, to come out of the spore at the same time. In rare cases even the endospore became ruptured and the whole pushed partly out of the epispore like the contents of a grape partly squeezed out of the skin. In these cases no promycelium was sent out, although the distal portion sometimes became somewhat pointed as if to send out a tube. However, there was seldom much growth from such protoplasmic masses. The whole, after seven to nine days became vacant, leaving a bubble like remnant. When promycelia were sent out they were short lived, and in no case were they seen to produce sporidia. This, however, may have been due to other causes.

General Summary.

The smuts considered fall naturally, as far as germination is concerned, into two general classes, those which produce no sporidia and those which do. Those producing sporidia may again be subdivided. A brief key might be made as follows:

I Producing no sporidia.
  A. Germ tube often branched - Ustilago tritici
  B. Germ tube spraringly branched - Ustilago nuda
    Germ tubes very narrow

II Producing sporidia.
  A. Sporidia produced mainly at septa, singly or in chains.
    a. Spores smooth - Ustilago hordei.
    b. Spores echinulate.
      1. Minutely so; spores small, 5-9μ - Ustilago avenae.
      2. Prominently so; spores larger, 8-15μ - Ustilago zeae.
  B. Sporidia produced in whorls at tip of promycelium - Tilletia foetens.

The germinating period varies considerably in different forms. Ustilago hordei germinates most readily, only 6½ hours being in some cases required. Tilletia foetens required the longest time, the minimum for these spores being 48 hours. The intermediate forms show differences of a few hours in the length of time required.

The germination characteristics are quite closely connected with the parasite's life history. The two forms which live over within the seed produce no sporidia, while those which live over in the soil or on the grain kernels produce sporidia which help insure their chances of persistence.

As far as the behavior of promycelium itself is concerned, there are differences. Fusions occurred in all except Ustilago zeae and Tilletia foetens. The sporidia of Tilletia foetens conjugate in pairs and send out germ threads from these fused pairs. In all other forms germ threads were sent out either by the promycelium or by sporidia, when these occurred. The promycelia very commonly became detached and free segments formed in U. zeae, U. hordei and U. avenae. This occurred sometimes in the case of U. tritici, promycelia growing in nutrient solution, but was never observed in Ustilago nuda or Tilletia foetens. The different forms behave differently; the spores of the same form behave differently under different conditions, and individual spores behave very differently in many cases, indicating that much may yet be learned concerning the phenomena of their germination and growth.

Explanation of Plates.

Plate I. Ustilago tritici.

Fig. 1. Group of spores showing various shapes and sizes.

Figs. 2-11. Early stages of germination in H_2O, Fig. 9 showing premature vacuoles. Figs. 10 & 11, promycelium beginning to branch.

Figs. 12-18. Stages at 54 hours, showing branching and beginning of germ tubes in figs. 16 & 18. (X 1000)½

Plate II. Ustilago tritici at 74 hours in H_2O.

Fig. 1 Promycelium showing common form of fusion.

Figs. 2 & 3. Formation of germ threads as continuations of the promycelium.

Fig. 4. Germ thread forming from tip of promycelium; the basal cell vacant.

Fig. 5. Branched and fused promycelium with vacant tips on 3 of the branches.

Fig. 6. Promycelium which has sent out two branched germ tubes.

Fig. 7. Promycelium apparently continuing as a germ tube after one has been sent out as a branch.

Fig. 8. Two promycelia fused and continuing as a single tube.

Fig. 9. Two germ tubes from the same promycelium, one of which has sent out another germ tube which fused with the other original one.

Fig. 10. Apparently 3 germ tubes from the same promycelium, two of which fused. [X 1000]½

Plate III. Ustilago tritici. Germination in water at 96 hours. Various fully grown germ threads. [X 1000]½

Plate IV. Ustilago tritici. Germination in 5% sugar solution.

Figs. 1-10. At 22 hours. 1-6 showing various forms of promycelia.

Fig. 8. Promycelium with loosely connected segments.

Fig. 9. Promycelium broken from spore.

Fig. 10. Cells of promycelium separated from each other.

Figs. 11-15. Various forms of swollen tips of germ threads. [X 1000] ½

Plate V. Ustilago tritici in 5% sugar at 10 days. Tips of germ threads dividing contents into segments and sending out branches. [X 1200] ½

Plate VI. Ustilago tritici in 5% sugar.

Figs. 1-7. Tips of germ tubes showing tendency, especially in fig. 5, to round up into segments. 25 days.

Figs. 8 & 9. Some of the segments freed - 27 days.

Fig. 10. Free segments, some of which show formation of a wall - 28 days.

Fig. 11. Free, or resting segments which have surrounded themselves by a wall.

Fig. 12. Resting segments germinating at 30 days, (a) at 36 days. X 1500.

Plate VII. Ustilago tritici. Germination, in distilled water, of spores which had been frozen. All at 23 hours. [X 1000] ½

Plate VIII. Ustilago nuda.

Fig. 1. Group of various shaped spores.

Figs. 2 - 6. Promycelia in water at 24 hours; Fig. 12, a typical form.

Figs. 9 - 11. Growth in 2½% sugar at 5 days, showing fusions and long, unusual germ threads.

Plate IX. Ustilago nuda in 2½% sugar. [X 1200] ½

Fig. 1. Germ tube growing out from promycelium, the two end cells of which have become vacant. Tip branched and fused.

Fig. 2. A fairly common form at 7 days.

Fig. 3. A simple form of young germ thread at 7 days. [X 1200.]½

Plate X. Ustilago nuda in 2½% sugar.
Various stages and types of germ tubes at 9 days.

Fig. 2 showing a much branched form with a number of fusions; a and b later fused also and sent out a long germ thread from the fused portion. [X 1200.]½

Plate XI. Ustilago nuda in 2½% sugar solution at 16 days.

Figs. 1-3. Various forms of germ tubes.

Fig. 4. Typical, wavy tip of single germ tube. [X 1200.]½

Plate XII. Ustilago nuda in 2½% sugar solution at 26 days.

Various forms of tips of germ tubes as finally developed; fusions shown in figs. 5 & 7. [X 1400.]½

Plate XIII. Ustilago nuda in 5% sugar solution.

Various stages of germination of spores which had been outside from Dec. 10 to April 8. [X 1200.]½

Figs. 1-5, at 24 hours.

Figs. 6-9, at 27 hours.

Plate XIV. Ustilago hordei. Germination of spores in water.

Figs. 1-5, at 18½ hours, showing beginning of formation of sporidia in figs. 3 & 4.

Figs. 6-13, at 21 hours showing formation of sporidia in figs. 8, 12 & 13. Fig. 8 shows sterigma which sometimes appears in formation of sporidia. [X 1200.]½

Plate XV. Ustilago hordei. Germination in 1% sugar solution.

Figs. 1-4. Early stages of germination, showing formation of sporidia.

Figs. 5 & 6. Various forms of sporidia.

Fig. 8. A spore which has sent out 2 promycelia both of which are producing sporidia vigorously at 18 hours.

Fig. 9. More unusual form in which sporidia are remaining attached while forming other sporidia.

Fig. 7. Sporidia germinating.

Fig. 10. Detached sporidia budding.

Fig. 11. Sporidium sending out long, narrow tube.

Fig. 12. Sporidium of large size, budding from both ends at 92 hours.

Fig. 13. Sporidia germinating at 4 days.

Fig. 14. Two unusual sporidia one of which is germinating while the other is budding.

Figs. 15 & 16. Sporidia at 5 days; unusual shaped tube in 15; fig. 16 showing germinating from one end and formation of sporidium from the other.

Fig. 17. Typical promycelium at 34 hours.

Fig. 18. Promycelium abjointing sporidia from tip only at 92 hours; unusual.

Fig. 19. Germination of sporidium at 5 days.

Fig. 20. Germination of sporidium at 8 days.

Fig. 21. Formation of sporidia at 5 days; the one at the tip germinating in situ.

Fig. 22. A resting segment.

Fig. 23. Typical germ thread sent out by a sporidium 9 days. [X 1200.] ½

Plate XVI. Ustilago hordei in 1% sugar solution. All [X 1200.] ½

Illustrating development of germ tubes.

Figs. 1-5. Various stages in development of germ tubes at 48 hours.

Fig. 6. Two tubes from same spore, fused and branched. 8 days.

Fig. 7. Detached promycelium sending out germ thread at 12 days.

Fig. 8. Unusual form; apparently no septations in vacant portions. 94 hours.

Fig. 9. Two germ threads from same promycelium at 9 days.

Fig. 10. Two promycelia from same spore; one sending out a germ thread which is unusually short - 12 days.

Plate XVII. Ustilago hordei.

Figs. 1-5. Various forms in 1% sugar at 9 & 12 days.

Figs. 1 & 2. Tips of germ threads with characteristic fusions.

Fig. 3. Tip of germ thread showing tendency to round up into segments.

Fig. 4. Sporidium producing germ tube.

Fig. 5. Very thick promycelium which has sent out no germ threads.

Figs. 6-17. Forms at 12 days.

Figs. 7,9,15,16,17 & 18 show resumption of activity by resting segments and sporidia after culture had been dried out. Fig. 17 shows an almost globose sporidium, which rounded up and became rich in oil contents, beginning to germinate. Fig. 9 shows a resting sporidium like that shown in fig. 17 sending out a tube appearing much like the original promycelium. X 1400.

Plate XVIII. Ustilago avenae. Germination in water.

Figs. 1-6. Early stages of germination showing typical promycelia with formation of sporidia in 3,4,5,&6.

Fig. 7. A "knee joint" fusion which, due to the growth of the promycelium, has opened up.

Fig. 8. Development of two promycelia from the same spore.

Fig. 9. Production of sporidia from septa and tip; sterigma at septum.

Fig.10. Non septate promycelium.

Fig.11. Promycelium in which vacuoles are appearing.

Fig.12. Germ tube being sent out from basal segment.

Figs.13-18. Various forms at 32 hours.

Figs.19-22. Forms at 60 days; figs.20-22 showing free segments.

Fig.23. Tip of germ tube at 13 days.

Fig.24. Germ tube at 13 days budding sporidia from portions filled with protoplasm. [X 1200.] ½

Plate XIX. Germination of Ustilago avenae in sugar solution.

Figs.1-14. Various forms of promycelia at 24 hours.

Fig.15. Detached sporidia budding.

Fig.16. Branched promycelium producing sporidia.

Figs.17 & 18. Germinating sporidia.

Fig.19. Promycelia producing germ tubes which have fused at 11 days.

Fig.20. Germ tube at 7 days. [X 1200.] ½

Plate XX. Ustilago zeae. Germination.

Figs. 1-6. Germination in manure decoction at 19 hours.

Fig.7. Formation of germ tube in water at 2 days.

Figs. 8 & 9. Promycelium at 7 days producing sporidia.

Figs. 10 & 11. Typical sporidia; germinating in fig. 11, a, b, & c.

Fig. 12. Detached sporidium which has germinated from both ends and become vacant as a result.

Figs. 13 & 15. Free segments budding.

Fig. 14. Sporidia, some of which are budding.

Fig. 16. Sporidia sending out germ threads at 4 days in water; 2 sporidia have lost protoplasm after germination.

Fig. 17. Chain of sporidia in manure decoction at 44 hours.

Figs. 18 & 20. Detached segments sending out germ threads.

Fig. 19. Detached segments budding.

Fig. 21. Promycelium sending out germ tube. [X 1200.] ½

Plate XXI. Ustilago zeae in 1% sugar solution at 27 days.

Fig. 1. Germinating sporidia.

Fig. 2. Ungerminated sporidia which have become rich in oil.

Fig. 3. Individual promycelial cells sending out germ tubes from both ends.

Fig. 4. Promycelium sending out a number of germ threads.

Fig. 5. Attached promycelia which have sent out no germ tubes and have large oil globules.

Fig. 7. Detached promycelial cells sending out tubes; the one on the left has two air conidia.

Fig. 8. Promycelial segments sending out long threads. [X 1400.] ½.

Plate XXII. Ustilago zeae in 1% sugar at 27 days.

Showing formation of much branched chains of air conidia, usually fairly thick walled and rich in oil globules. X 1200.

Plate XXIII. Tilletia foetens. Germination in water at 5 days- showing typical spores with many oil globules and the split epispore out of which the promycelium comes. Typical forms of germ tubes at 5 days. X 430 ½

Plate XXIV. Tilletia foetens. Germination in water.

Figs. 1-19. Various forms of promycelia at 3½ days.

Figs. 20 & 21. Beginning of process of formation of sporidia at 4 days.

Fig. 22. Typical whorl of sporidia, two of which have fused. 4 days.

Figs. 24 & 25. Fused sporidia which have formed secondary sporidia and lost their protoplasm.

Fig. 26. Whorl of sporidia, two of which have fused and sent out a narrow germ thread.

Fig. 27. Sporidia fusing after detachment.

Fig. 28. Fused sporidia which have formed a secondary sporidium. Secondary sporidium sending out germ thread.

Fig. 29. Fused sporidia which have sent out a short germ tube from the tip. X 430. ½

Plate XXV. Tilletia tritici. Germination in water.

Fig. 1. Typical whorl of sporidia at 5 days.

Fig. 2. Fused pair of sporidia with no protoplasm.

Fig. 3. Abnormal promycelium at 5 days.

Fig. 4. Conjugated sporidia which have sent out two branched germ tubes from the tip. The germ tubes are producing secondary sporidia.

Figs. 5 & 6. Detached secondary sporidia sending out germ threads.

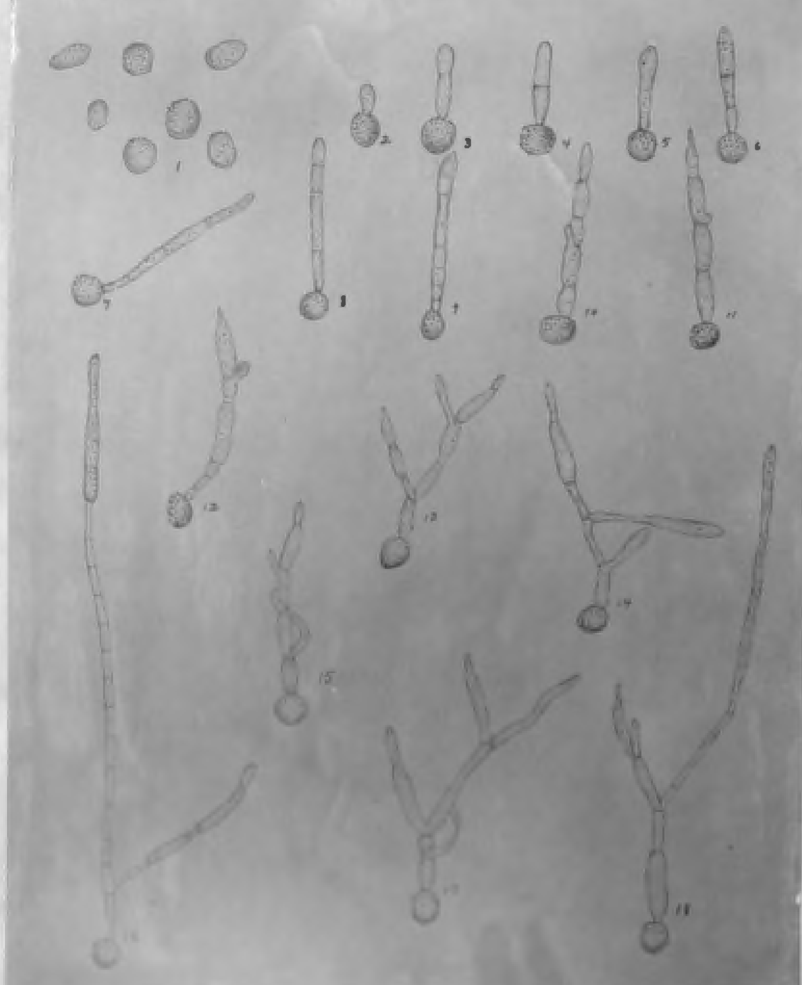
Fig. 7. Fused sporidia sending out a germ tube which is producing a secondary sporidium.

Fig. 8. Whorl of sporidia, mostly fused, sending out germ tubes directly and from secondary sporidia.

Fig. 9. Formation of secondary conidia in a linear series - unusual.

Figs. 10 & 11. Typical, long narrow germ tubes sent out by fused sporidia. (X 430). ½.

Plate I.



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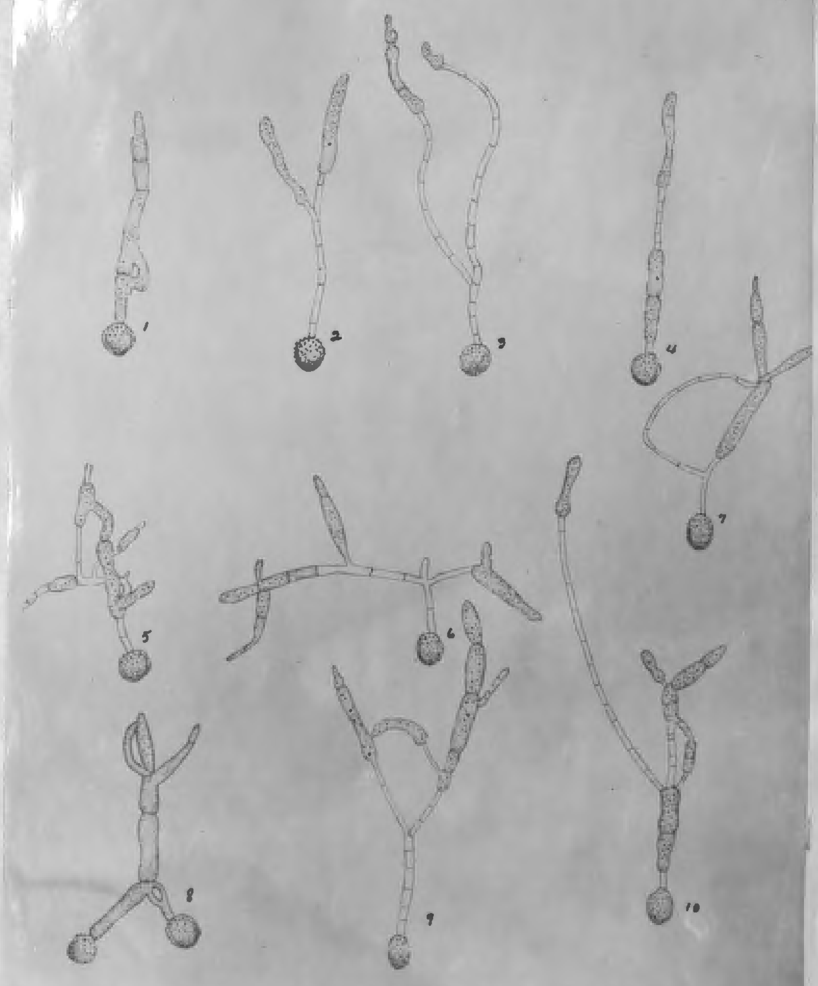

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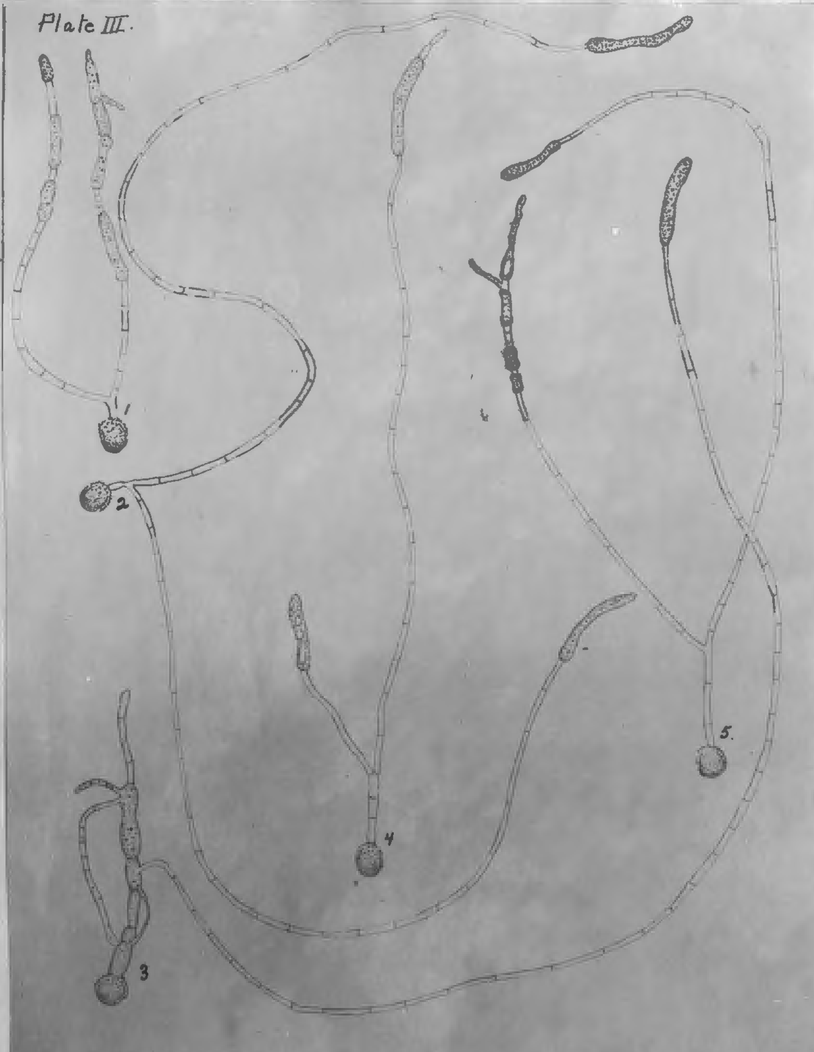

Plate IV.

Plate V.

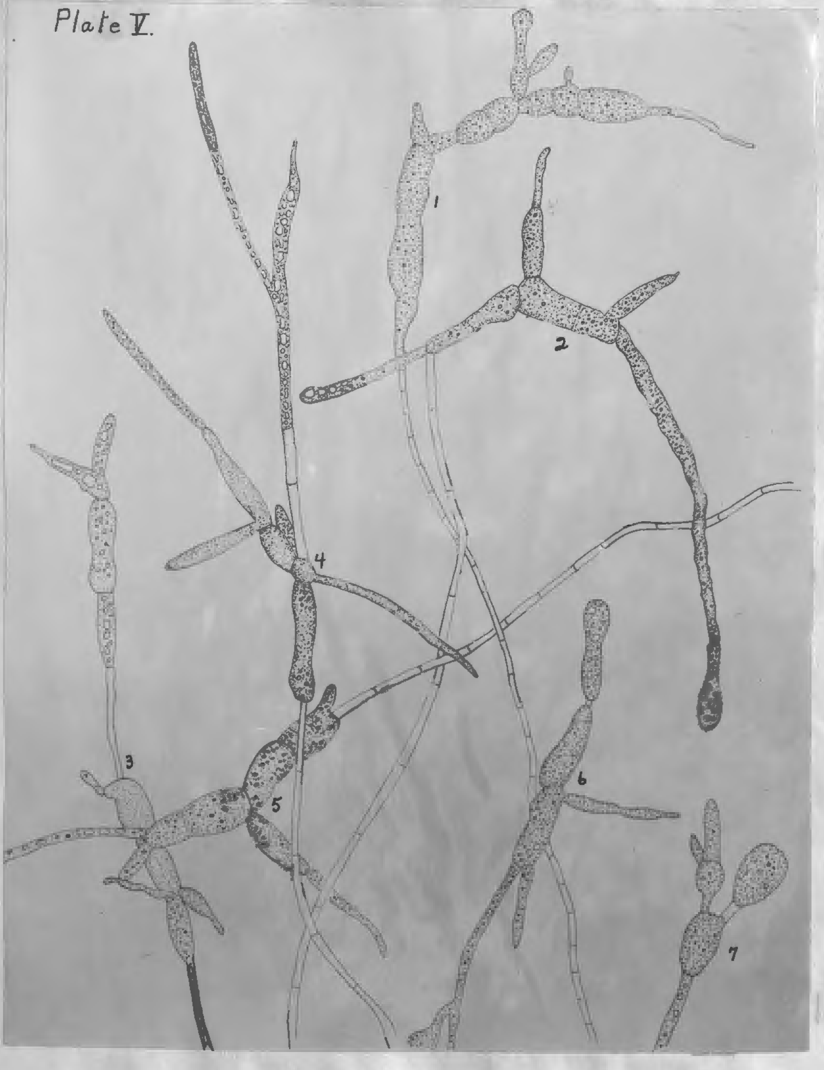

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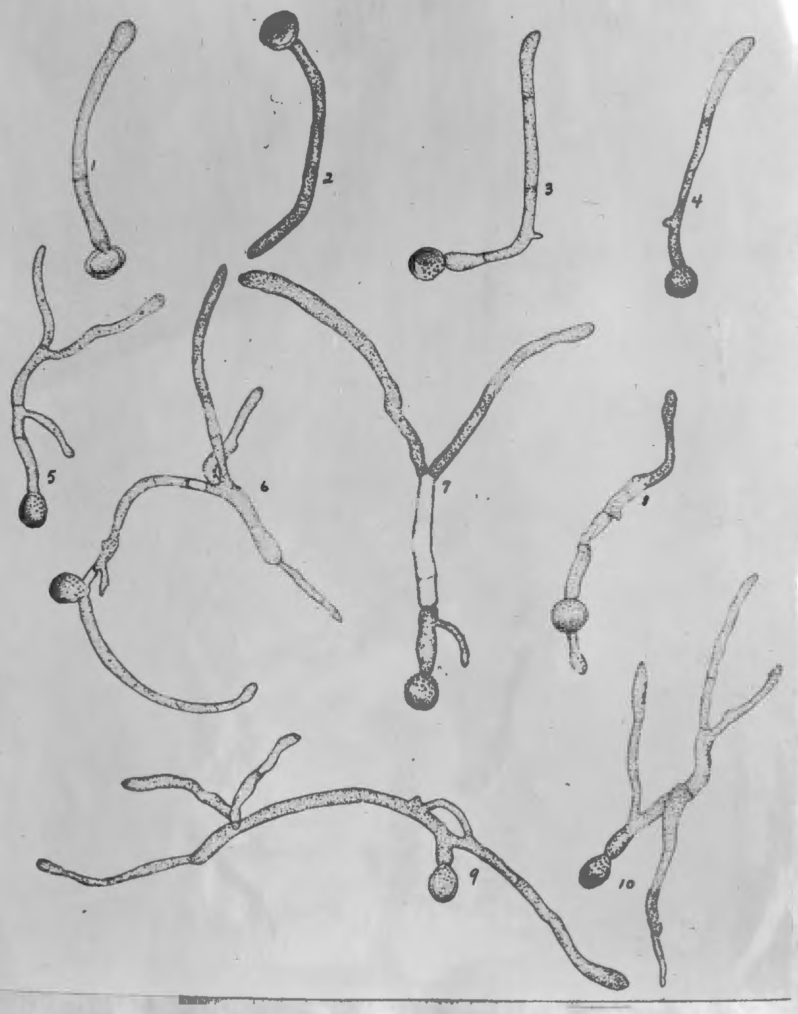
Plate VII.



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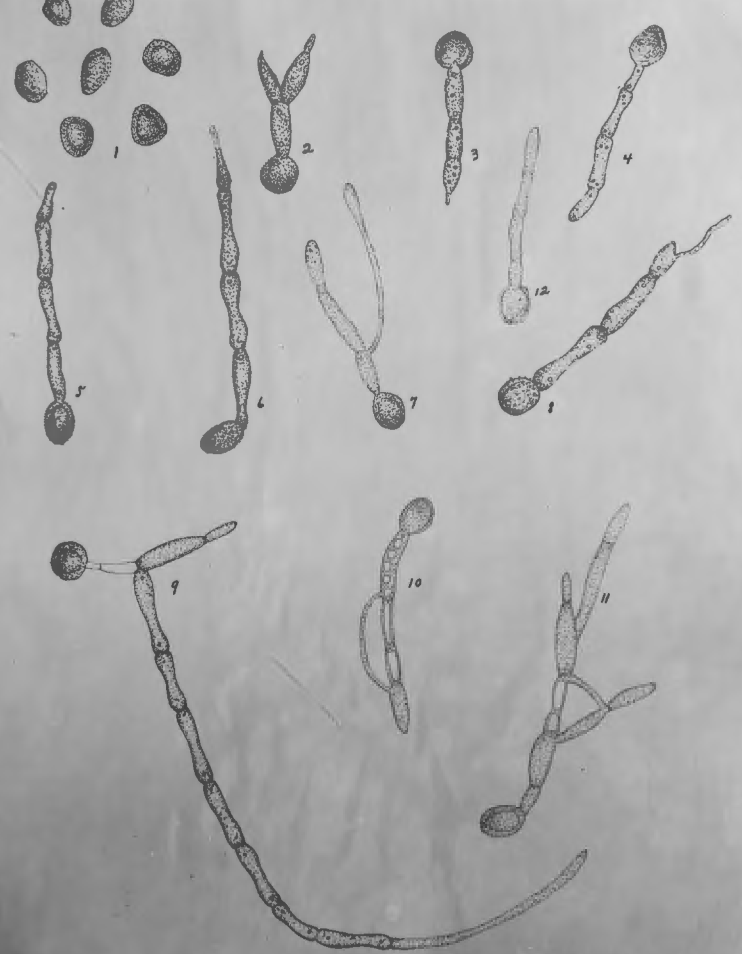

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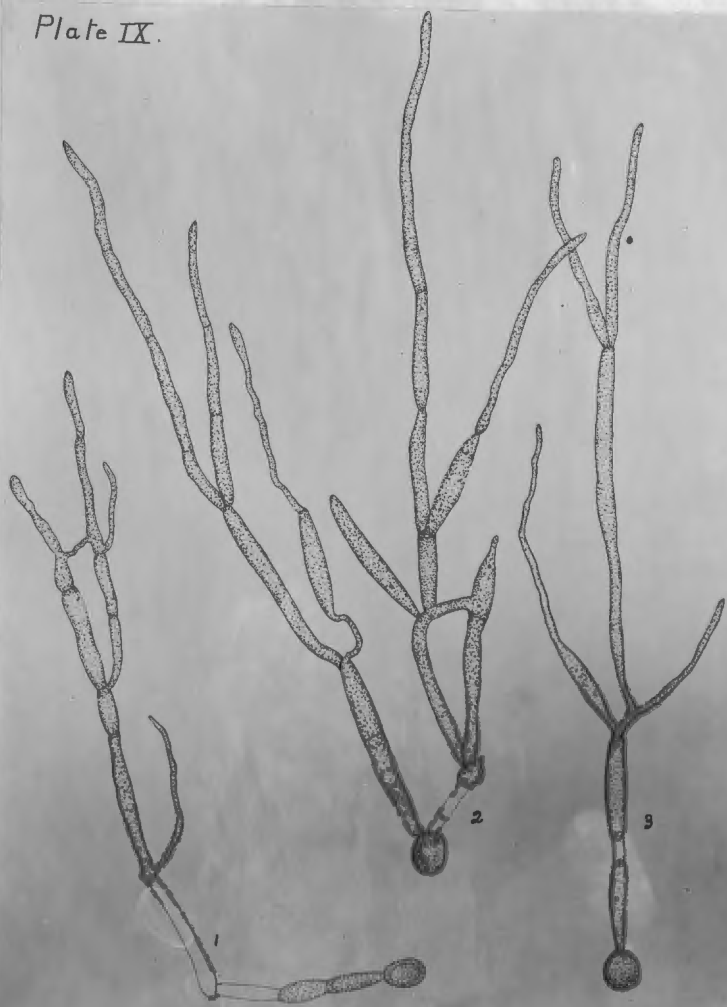

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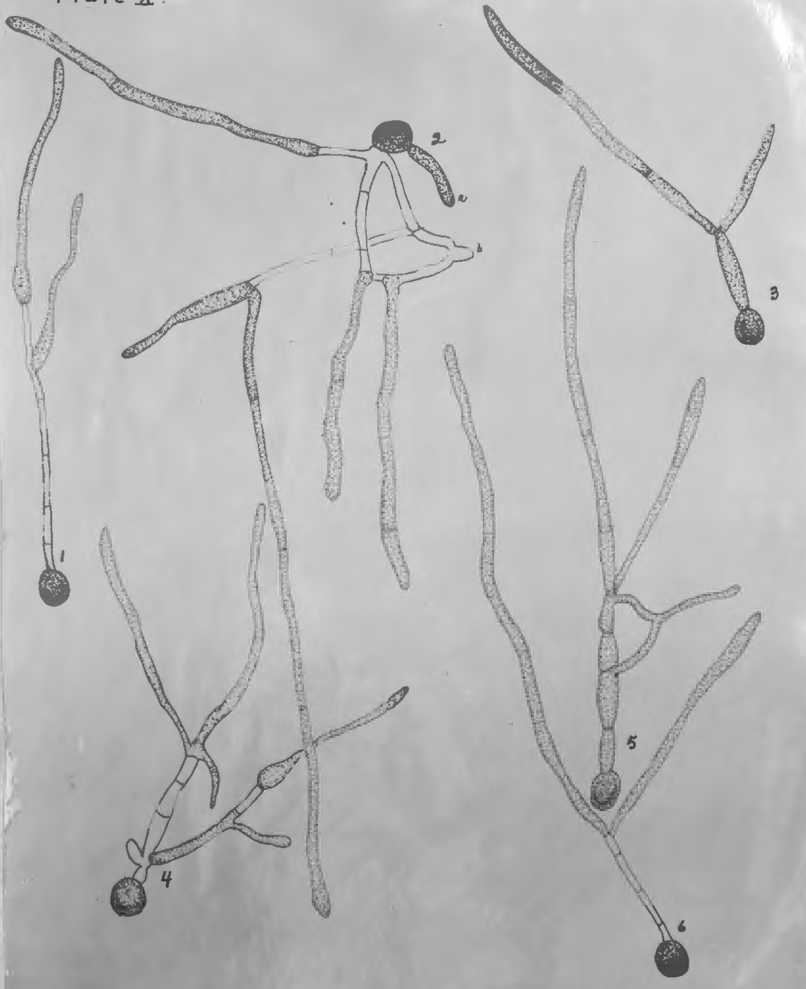

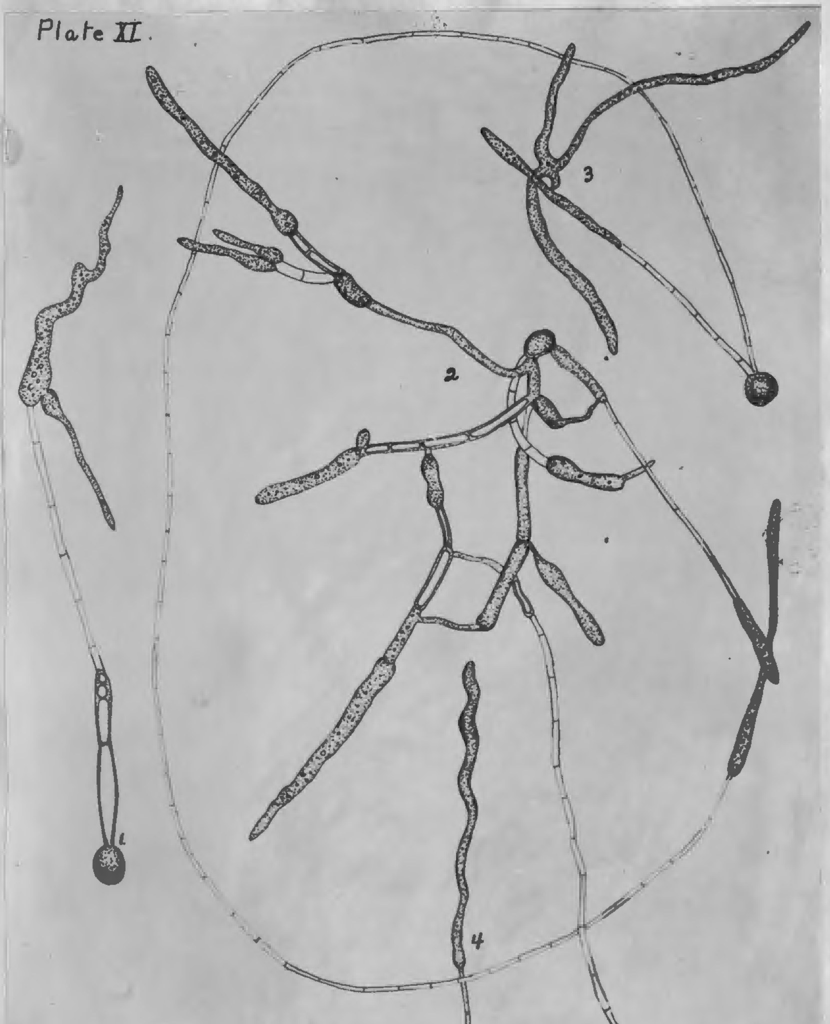
Plate II.
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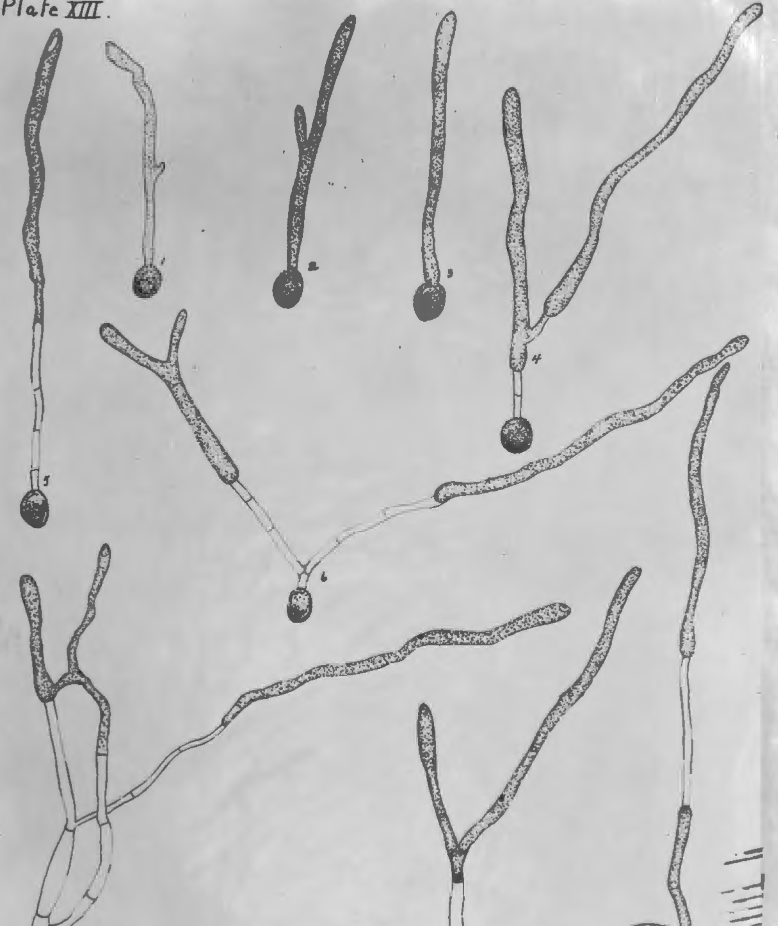
Plate XII

Plate XIII.



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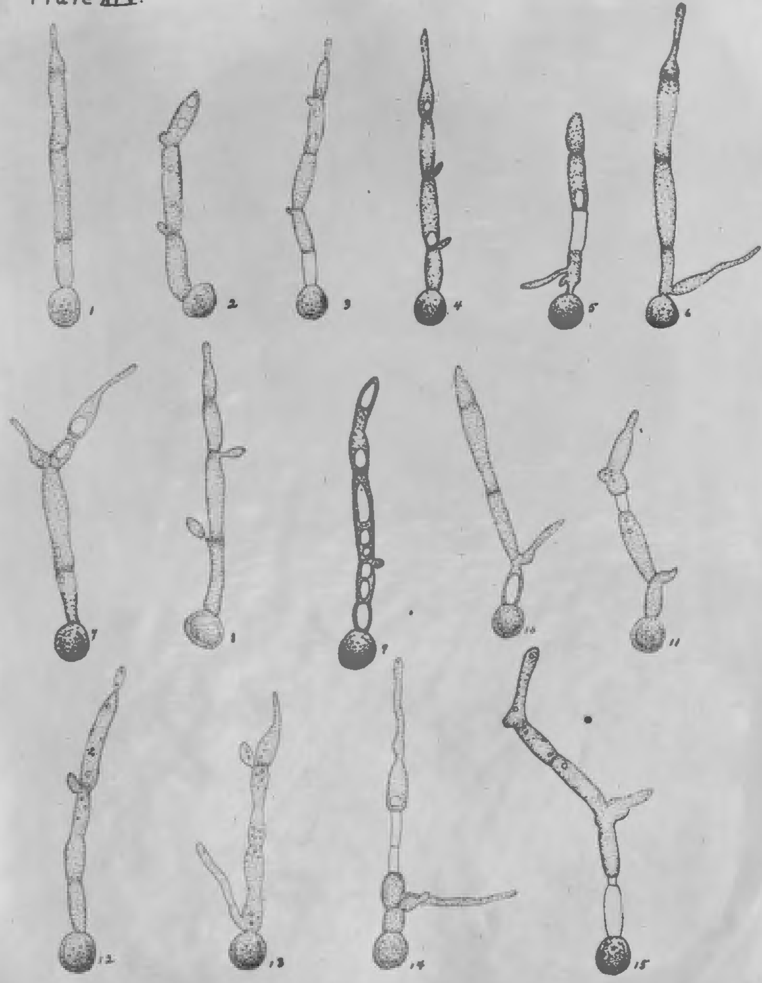

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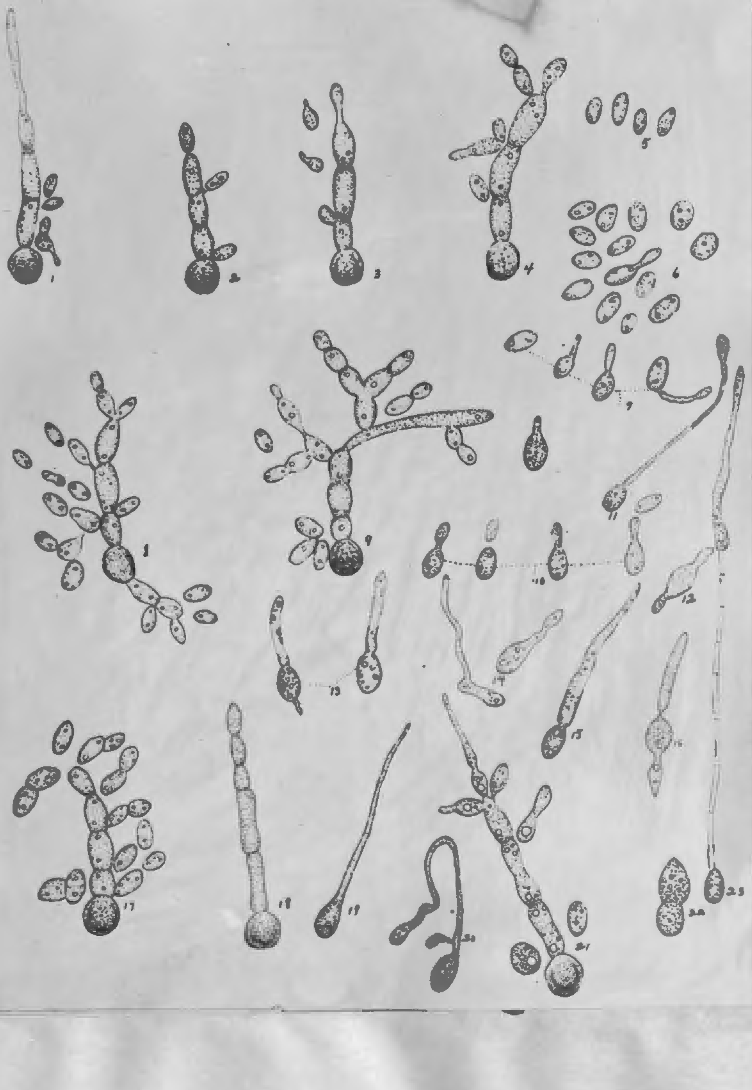

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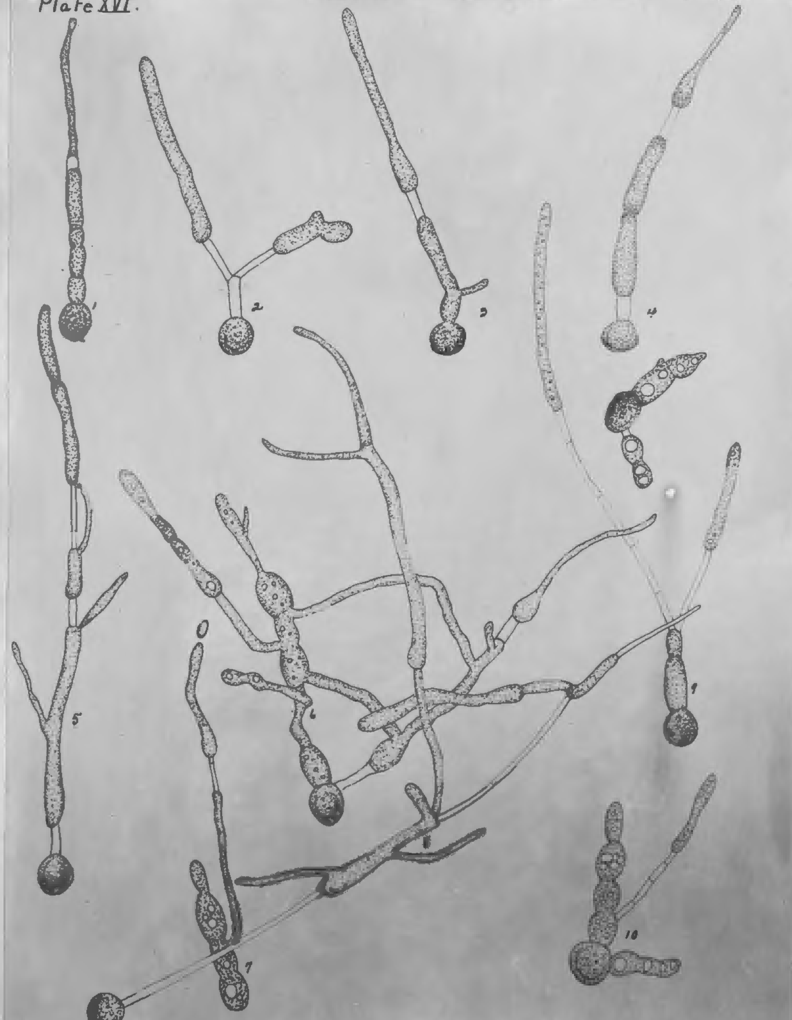

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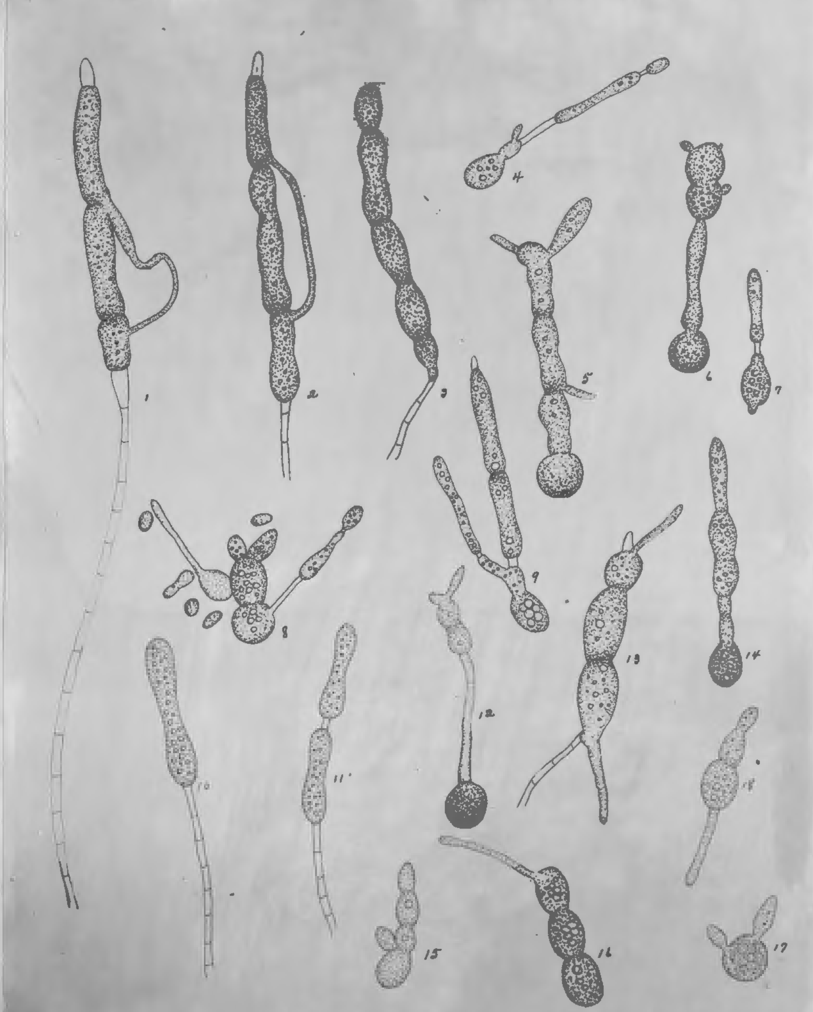

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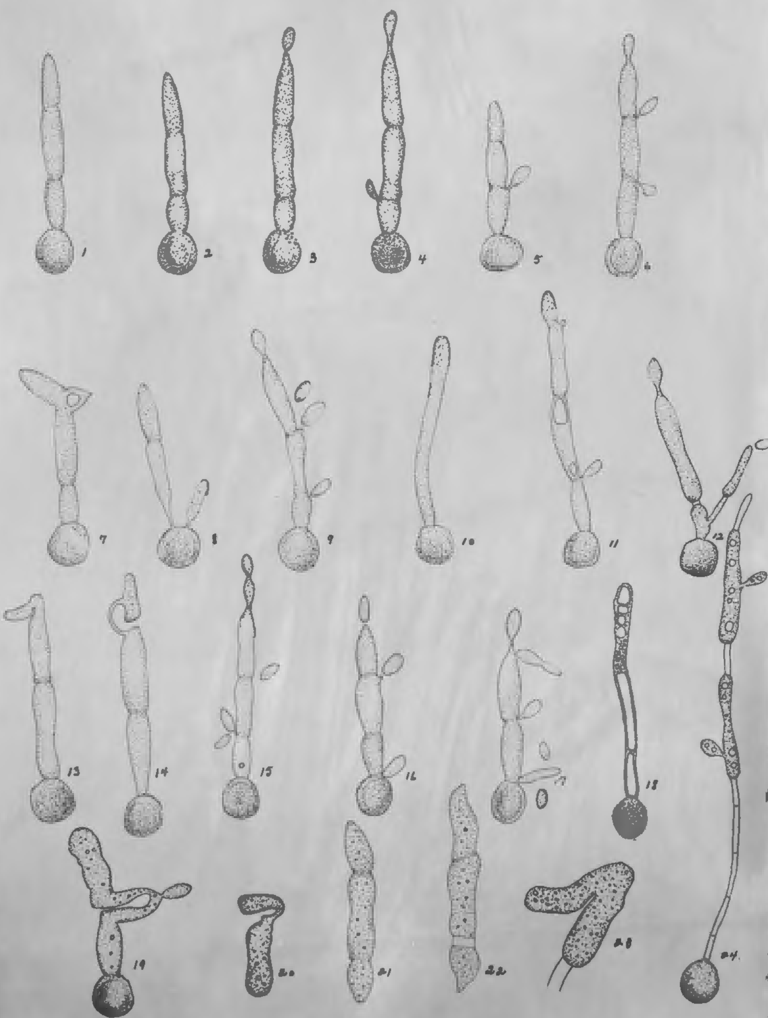

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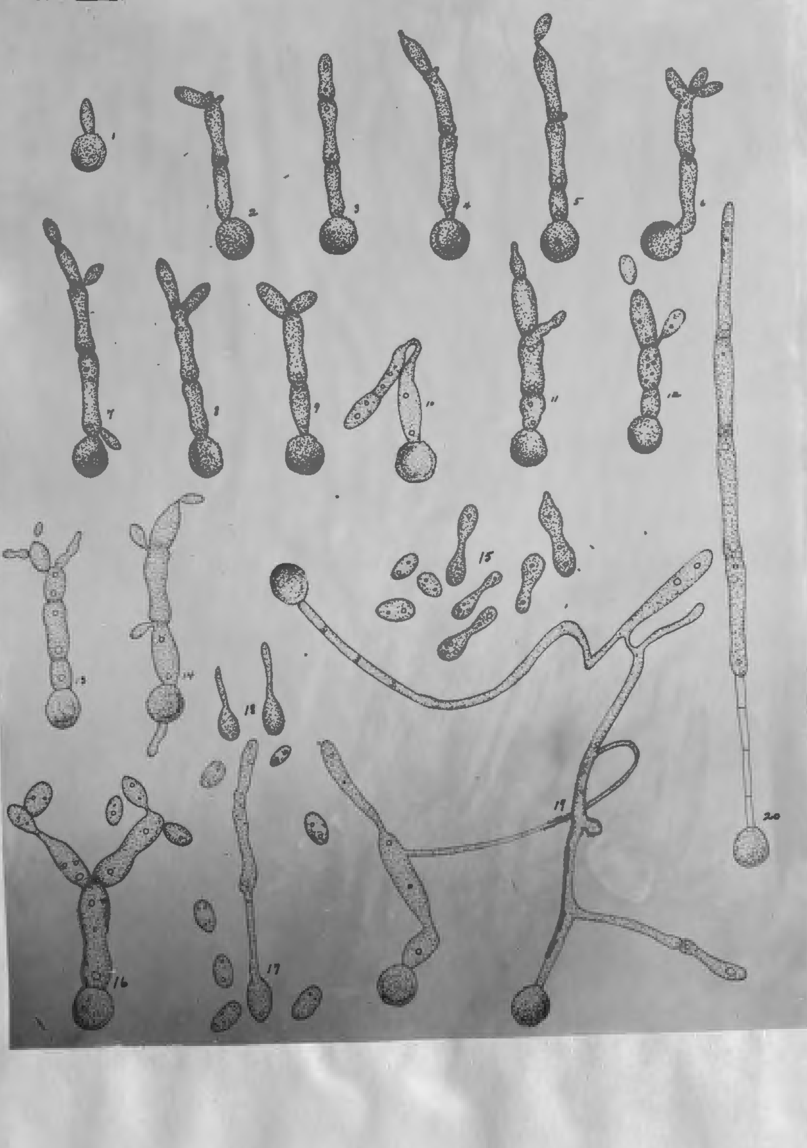

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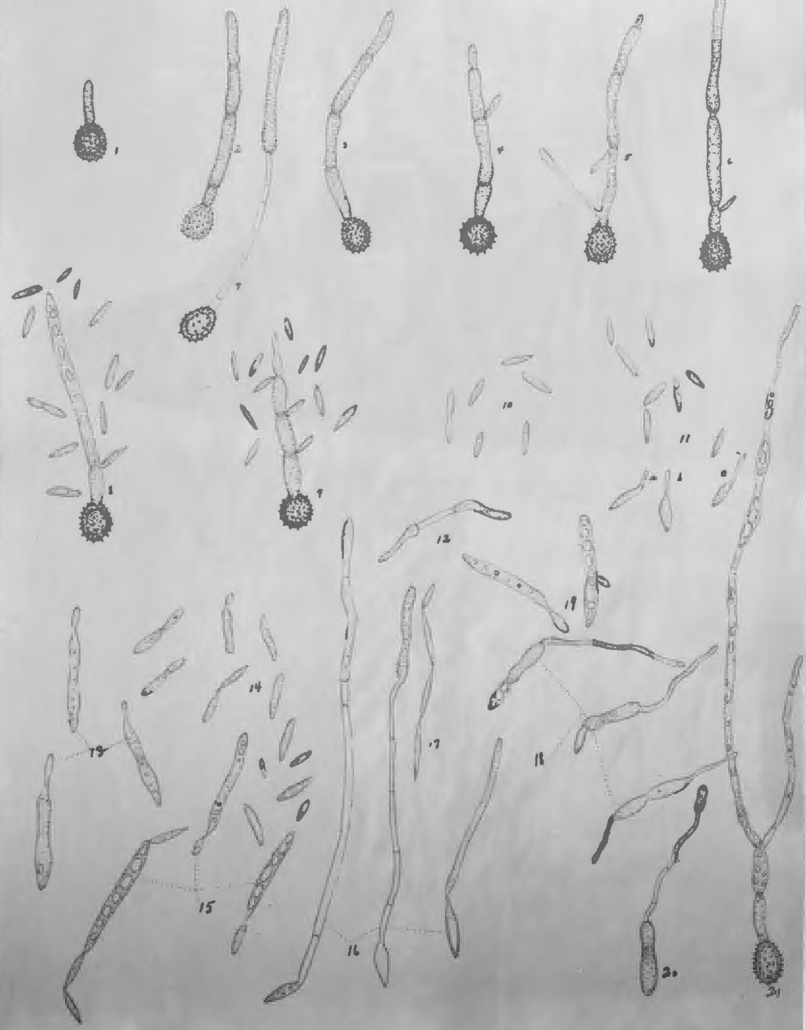

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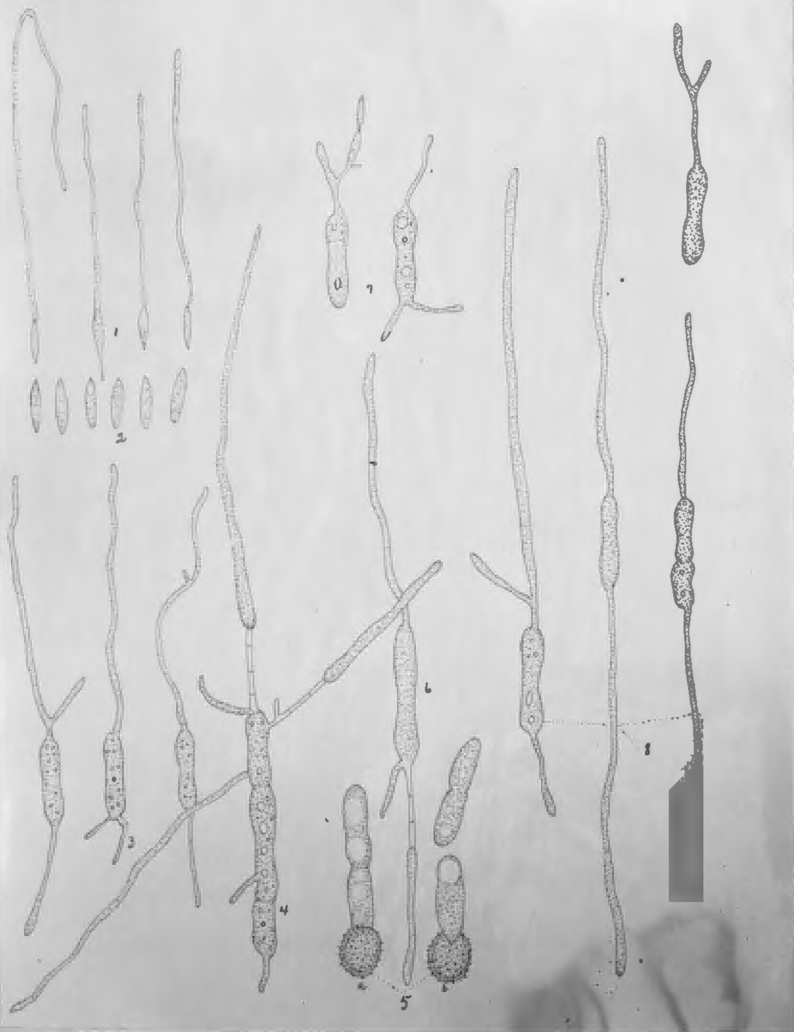
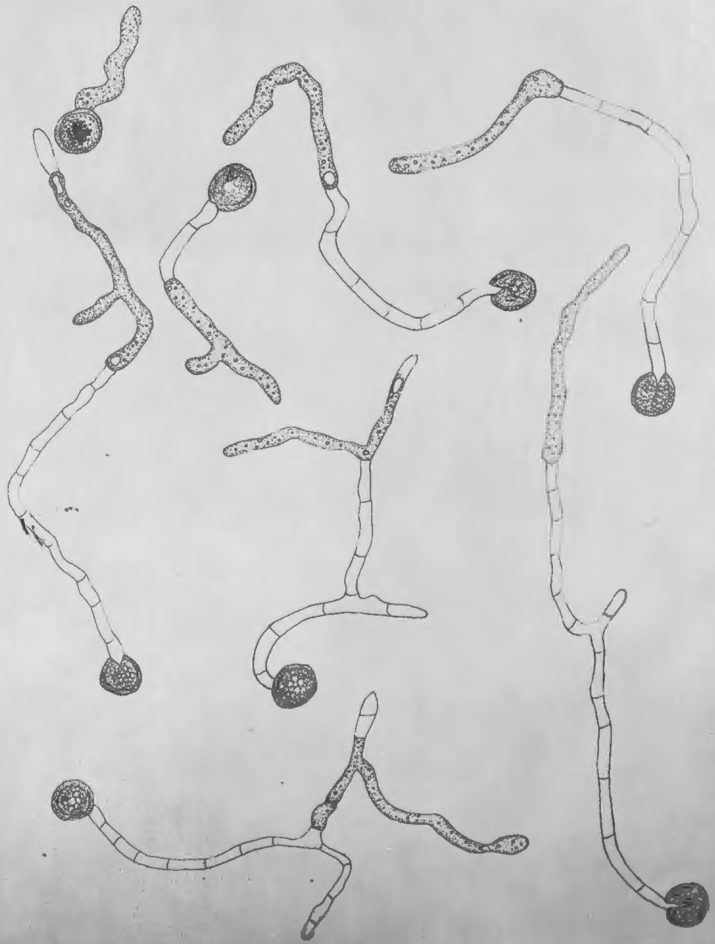


Plate XXII.

Plate XXIII.



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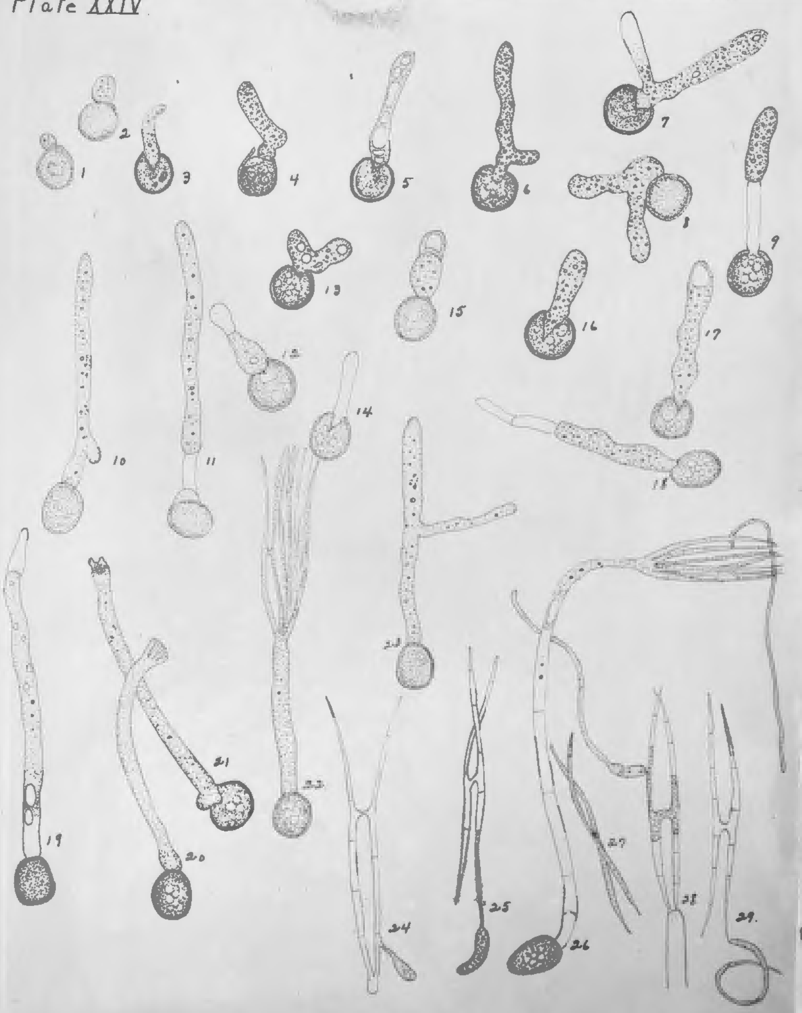

Plate XXV.