NUCLEAR PHENOMENA IN CERTAIN STAGES IN THE DEVELOPMENT OF THE SMUTS.

WITH PLATES VIII AND IX.

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The economic significance of the smuts, together with the uncertainty as to their relationships, has made them almost constantly objects of investigation both from the practical and the more purely theoretical standpoint. The older literature is cited fully in papers by Tulasne[1] and DeBary.[2] The germination of the smut spores of various species in water was first accurately observed and figured by Tulasne, DeBary, and the latter's pupil, Fischer von Waldheim,[3] whose excellent paper developed the knowledge of the spore formation and germination for a considerable series of forms into practically the condition in which it remains today. Brefeld[4] has continued and extended our knowledge of the germination of the spores in artificial media. His work is practically exhaustive so far as the external morphology of the forms is concerned, and his vast series of plates illustrating the stages in germination and the saprophytic growth of all the principal genera and species are models of completeness.

All the authors mentioned and many others have observed and

[1] Tulasne, "Memoire sur les Ustilaginées comparées aux Uredinées," *Ann. d. Sc. Nat.*, Ser. 3, VII, 1847; and "Memoire sur les Uredinées et les Ustilaginées." *Ann. d. Sc. Nat.*, Ser. 4, II.

[2] DeBary, *Untersuchungen über d. Brandpilze.* (Berlin, 1853.)

[3] Fischer von Waldheim, "Beiträge zur Biol. u. Entw. d. Ustilagineen." *Jahrbücher für Wiss. Botanik*, VII, 1869.

[4] Brefeld, *Botanische Untersuchungen über Hefenpilze.* (Leipzig, 1883); and *Untersuchungen aus dem Gesammtgebiete d. Mykologie*, XII, Heft, "Hemibasidii." (Münster, 1895.)

described the so-called copulation of the sporida, in which two cells become connected by a tube which joins them in pairs very much as are the conjugating cells of *Spirogyra.* DeBary[1] first pointed out the analogy between this union and the sexual fusions in the Conjugatae, and later[2] he presented the evidence more fully as to whether such fusions are sexual. As favoring this view he points out: First, the almost invariable occurrence of the pairing under the normal conditions of germination, i. e., germination in water. Second, the fusions are usually between two, and only two, sporidia. In the case of *Tilletia, Entyloma,* and *Urocystis,* when an odd number of sporidia are borne on the promycelium, the sporidia almost without exception fuse in pairs, and the odd one is left out and does not fuse with either of the adjacent pairs, although such a union would be very easy. This shows that a change has taken place in the fused pairs which makes a second union difficult or impossible. Most authors since DeBary's earlier paper appeared, have accepted the view that the sporidial fusions are sexual unions.

Brefeld[3] opposes this view and maintains that such fusing of cells is mere cell *fusion* in the sense in which Unger used the term. According to Brefeld the essential character in a sexual union of cells lies in the fact, that the conjugating gametes are incapable of further vegetative growth by division, but become capable of further development as a result of their union. He finds that the smut sporidia are capable of unlimited development without fusing so long as appropriate nutriment is supplied, and hence concludes that the fusions are purely vegetative phenomena accompanying, perhaps resulting from, the starvation of the sporidia. Such fusions are to be compared to the unions formed between the germ tubes of various spores or the hyphae of mycelia, such as will be described more fully below.

Dangeard[4] has more recently studied the nuclear phenomena

[1] *Beitr.* Ser. V, pp. 126-127, and *Morph. Phys., und Biol. d. Pilze, Mycetozoa, u. Bacteria,* pp. 181-184.

[2] DeBary, *Beitr. z. Morph. der Pilze,* I, Ser., "Protomyces and Physoderma."

[3] *Bot. Untersuchungen über Hefenpilze,* Hft. V. pp. 33, 49-50, 175.

[4] Dangeard, "Recherches histol. sur la famille des Ustilaginées." *Le Botaniste,* Ser. III., pp. 240, 268; and *Le Botaniste,* Ser. 4, p. 12.

in a number of forms and has come to some very interesting conclusions as to the sexual reproduction of the group. He finds that in the genera investigated, *Entyloma*, *Ustilago*, and *Urocystis*, the young spore contains two nuclei which later fuse so that the ripe spore contains a single nucleus. This fusion is considered as the equivalent of the conjugation of male and female pronuclei and the smut spore as really an oöspore formed in an oögone. On this ground Dangeard contends that the doctrine of DeBary as to the sexual nature of the conidial fusions which occur later must be abandoned. In the germination of *Ustilago* in water the oöspore nucleus passes into the germ tube and divides, cross partitions are formed, and a two, three or four celled promycelium results. In *Urocystis* and *Tilletia* eight nuclei are regularly formed in the promycelium and pass into the sporidia which are apical in these forms. The nuclei show a membrane with double contour, a nucleole which is very large, and between the two a hyaloplasm more or less charged with chromatin. In the promycelia of *Tilletia caries* some figures were seen which suggested stages in the indirect division of the nuclei. Each sporidium contains normally one nucleus. The secondary sporidia contain two nuclei in *Tilletia*, indicating in them, as the author considers, a return to the condition of ordinary vegetative cells. Dangeard observed the fusion of the sporidia of *Tilletia* in pairs and offers the suggestion, that in the passage of the nuclei from the promycelium to the sporidia an unequal distribution of nuclei may occur, by which one sporidium may obtain two nuclei and another none at all. The fusion tubes then permit a re-establishment of equilibrium by the wandering of nuclei from the sporidia that have two nuclei into those that have none. He finds nuclei in the fusion tubes under conditions which suggest this view.

DeBary proposed the term *promycelium* for the germ tube pushed out by the germinating smut spores, and I shall retain the term although Brefeld has introduced the word *hemibasidium* for the same structure in the effort to show a relationship between the Ustilagineae and the true Basidiomycetes. DeBary proposed also the term sporidium for the spores born on the promycelia. Brefeld very justly, however, points out

that these spores differ in no essential particular from other conidia produced by abstriction, and I shall use this latter term.

While working with Prof. Brefeld at Münster in the summer of 1896, I began some studies on methods of fixing and staining the nuclei of the smuts. Prof. Brefeld's experience in growing these organisms in culture media was of the greatest value to me in enabling me to obtain abundant material in all stages of development. Aside from the work of Dangeard the nuclear phenomena in the smuts have been very little studied and further contributions along this line are certainly very much needed.

I have used especially as types material of *U. antherarum* (Fries), *U. scabiosa*, *Sow. U. maydis* (D. C.), and *U. carbo* (Tul.). Material of the first was obtained from *Lychnis alba*, the commoner form on *Saponaria* being found less favorable for the study of the conidial fusions, since its conidia do not unite so readily as do those coming from the other host. The earlier stages of the spore formation were studied however in sections of the Saponaria anthers. It is easy to determine that Dangeard is right in claiming the presence regularly of two nuclei in the young spores.

In considerably older spores but a single nucleus is to be found, and the ripe spores just prior to germination are also uninucleated. Whether this spore nucleus is really formed by fusion of the two nuclei present in the young spore is a difficult question.

Material fixed in Flemming's chrom-osmium-acetic solution, weaker formula, and stained with the triple stain, shows the nuclei as very minute deeply stained bodies. At this stage it is difficult to make out nuclear structures; but later in the promycelia it is easy to recognize in successful preparations a red stained nucleole and blue chromatin net. Dangeard's description of these nuclei as consisting of a nucleole with a surrounding hyaloplasm more or less charged with chromatin and the whole enclosed in a vesicle with double contour should not be taken as indicating any essential difference between the structure of these nuclei and those of the higher plants, since

nucleole, chromatin, and membrane are perfectly distinct in practically all cases. The main difference between my observations and Dangeard's lies in his failure to observe in all cases a chromatin net between the nucleole and membrane. The point is of importance since it has been and still is in some quarters[1] the tendency to either deny the existence of nuclei in the fungi or ascribe to them a structure simpler than that of the nuclei of the higher plants and animals. These nuclei of the smuts on the contrary show the same essential differentiations in structure which are found throughout the higher plants. The simplicity of form and life history of these plants is in no degree paralleled by a corresponding condition in their visible protoplasmic structure.

For the study of the germinating spores and conidia cultures were made in beerwort on the slide or in watch crystals. For fixing and staining the following method was worked out by Mr. D. G. Fairchild and myself and has proved applicable and advantageous for the preparation of a variety of one-celled or few-celled organisms which can be had in great abundance. The spores are sown in the beerwort in sufficient abundance so as to form a thick precipitate on the cover glass when conidia are formed. If the later stages of conidial budding and fusion are desired it is necessary to sow relatively few spores, since they multiply with great rapidity. If the germination stages of the spores themselves are desired a sufficient number of spores should be sown to render the drop turbid at once, since in this case there will be no increase in numbers. When the culture has reached the desired age or stage in development, a drop turbid with spores or conidia is drawn up into a fine capillary tube and is then blown gently out into a larger drop of the fixing solution on a fresh slide.

The fixing solution is in this fashion considerably diluted, but owing to the extreme delicacy of the promycelia and conidia they require only very dilute fixing solutions. In case of more resistant bodies the solution would have to be made proportionately stronger to allow of dilution in the manner indicated. The

[1] Holtermann, *Mykologische Untersuchungen aus den Tropen.* (Berlin, 1897.)

anther smut is perfectly killed and fixed by using the weaker Flemming solution with an exposure of from fifteen minutes to an hour. Longer exposures produce no apparent effect. The most delicate cells are neither shrunken nor plasmolyzed by this treatment and are hardly visibly different than in the living condition.

A slide is now covered with a film of albumen fixative as is customary in attaching serial sections. A drop of the fixed material without any washing is drawn up into a capillary tube, touched lightly and quickly to the surface of the prepared slide, and a minute droplet of the fixing solution with the contained cells is thus deposited on the albumen film. The spores settle on the albumen which is at the same time at least partially coagulated by the action of the fixing solution. They are thus held fast, and in the same fashion a series of droplets may be distributed over an area which can be conveniently enclosed by the cover glass. From the appearance of the slide when it is successful the process may be compared to stippling and may be known by that name. The slide should be left exposed for a few moments to remove excess of moisture by evaporation, though it should by no means be allowed to become dry. The preparation can then be passed rapidly through the graded alcohols, whereby the albumen is still more firmly set.

After this treatment the preparation is ready for any desired method of staining on the slide and can be treated in all respects as a slide with attached ribbons of sections. Flemming's triple stain was used in making the preparations of smuts to be described below. The point for care in the above process is in depositing the droplets on the albumen. If the droplets are too large the albumen is washed away and the spores are not attached. The finer the droplets the more evenly the spores can be spread on the slide and the more certain it is that each will be attached. Still it is inevitable that many are lost, and the method is only to be recommended in cases where an abundance of material in every desired stage is obtainable. I have used it in making preparations of yeast and swarm spores with great success. It is also far superior to the ordinary method for attaching bacteria to the slide by drying or heat coagulation, es-

pecially when it is desired to preserve the interior structure of the bacterial cells. As Fisher[1] has recently pointed out, bacteria mounted according to current methods are likely to be plasmolyzed and frequently distorted.

The disadvantage of the presence of the albumen film in staining is very slight. If it has not been made too thick it is always decolorized in washing out stains before the cells are. In the case of heavy coarse spores I have sometimes found it necessary to drop 80 per cent. alcohol on the slide at once after stippling it with the spore containing droplets, in order to complete at once the coagulation of the albumen and prevent the possibility of the spores being washed away in the weaker grades of alcohol. This latter modification can of course be resorted to only in cases where the cells are not injured by the comparatively sudden transition from an aqueous solution to 80 per cent. alcohol. I find, however, that spores while still in such fixing solutions as Flemming's, are much more resistant and rigid than after washing in water, and will generally stand rapid transitions into alcohol with no change.

Washing out the acid fixing solution, which is such an important feature in fixing blocks of tissue, can here be entirely omitted. The cells are so small that they are sufficiently washed in passing through the grade alcohols.

If we turn now to the nuclear phenomena in the germinating spore, we find that in the case of *U. scabiosa* the promycelium pushes out without nuclear divisions having yet taken place in the spore. The nucleus wanders out of the spore and is to be found in the promycelium when it has reached one-third its mature length (Fig. 1).

When the promycelium is full grown the nucleus lies near its middle. It can be very easily studied at this stage and, when prepared with Flemming's triple stain, shows a sharply differentiated, blue stained chromatin net lying in a clear nuclear sap, a red stained nucleole, and a bounding membrane. The nucleus now divides. The figure is too minute for study of the process

[1] "Die Plasmolyse der Bakterien," *Sitzb. d. kgl. Sächs. Ges.-Wiss., Math. Nat. Cl.*, 1891, and "Unters. ü. Bakt.," *Jahrb. f. Wiss. Bot.*, XXVII.

of spindle formation, etc.; but the equatorial plate stage is very distinct and shows a sharply pointed bipolar spindle, whose fibres end in deep staining granules at the poles (Fig. 2). No polar radiations at this stage have been observed. The chromosomes are rather densely massed at the equator and are probably eight or ten in number. Frequently in the neighborhood of the spindle the nucleole can be seen, generally somewhat reduced in size but still a dense red staining granule.

In the dispirem stage the daughter nuclei appear merely as dense flattened disks of chromatin still connected by the old spindle fibres (Fig. 3); as is the case at this stage in the division of the ascus nuclei, as I have already described it for *Peziza Stevensoniana.*[1] The daughter nuclei become free from each other and wander to the opposite ends of the promycelium before they have fully completed the reconstruction stages (Fig. 4). Here they soon divide again, and the four nuclei become distributed at equal intervals in the promycelium, and three cross walls are built forming a typical four-celled promycelium. Budding then begins (Figs. 6 and 7); the conidia drop off and enter on a yeast-like period of growth, as has already been fully described by Brefeld.

The above account of *U. scabiosa* represents a case where a typical four-celled promycelium is formed, such as Brefeld regards as a typical hemibasidium. The spores produce regularly only a single promycelium and all subsequent conidial growth originates from this one conidiophore.

In some species however a series of promycelia are budded off from a single spore and these may be two or three or one celled. A type which produces series of three celled promycelia is *U. antherarum* (Fr.), growing in the anthers of many Caryophyllaceae, and for which the nuclear phenomena in spore formation have been referred to above. This is the fungus which develops its spores in the anthers of the host plant, thereby emasculating them and apparently availing itself of the means for the scattering of its spores which the host plant intended for pollen distribution.

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[1] *Ber. d. deutsch. Bot. Ges.*, XII, 1895.

The germination of the spores and fusion of the conidia of the anther smut have been very fully described by Brefeld.[1] The spores germinate in a few hours in beerwort. The single spore nucleus divides and one of the two daughter nuclei wanders into the germ tube (Figs. 8 and 9). The other remains in the spore and develops no further during the formation of the first promycelium. For demonstrating the nuclei in the thick walled spores at this stage, a methyl green stain was found preferable to the triple stain of Flemming. A one per cent. solution was used with exposure for a few minutes. Brefeld reports that the spores when germinated in water produce series of promycelia; but that when grown in nutrient solutions they produce but one promycelium, which, however, produces a very large number of conidia. In the most of my cultures, however, which were made in rather dilute beerwort, series of promycelia were thrown off, all of which produced abundant conidial buds.

When the promycelium has reached about two-thirds of its adult length its nucleus divides, and a cross wall is at once built between the two daughter nuclei (Fig. 10). As the promycelium continues to develop the nucleus of the distal cell again divides, and a cell wall is built between the two nuclei, the apical cell so cut off being generally smaller than the one below it (Fig. 11). Conidia are now budded off in abundance from all three cells, and the promycelium itself soon drops off from the spore which immediately produces a second promycelium at the same spot. This may be followed by a third, and so on. How many such promycelia can be formed from a single spore, I have not attempted to determine. Doubtless the spore nourishes itself from the beerwort as do the promycelia in producing the conidia. Each so produced promycelium continues to bud off conidia indefinitely or as long as the medium contains sufficient nutriment.

After falling off from the promycelium the conidia bud like yeast cells, forming small colonies (Figs. 11, 12, and 13), as do the conidia of most of the smuts. By transferring the conidia and promycelia to fresh beerwort the process can be kept up indefinitely. Each conidium is uninucleate and this nucleus di-

[1] Loc. cit., pp. 36–54.

vides to furnish nuclei for the daughter buds. The resting nuclei here also show the characteristic elements, nucleole, chromatin mass, and nuclear membrane.

The division is a typical karyokinetic division, although the conditions of size, etc., make a study of the details of the process very difficult. The equatorial plate stage is well defined (Fig. 14). The spindle is sharply bipolar though no polar asters can be made out. The nucleole can frequently be seen as a bright red granule somewhere in the neighborhood of the spindle (Figs. 14, 16). The separation of the chromosomes and their withdrawal to the poles is shown in Fig. 15, and the young daughter nuclei appearing as dense lumps of chromatin connected by the fibres of the old spindle, in Fig. 16. It is interesting that even in such minute nuclei the typical stages of nuclear division as found in other plants are all readily to be identified. The similarity of the nuclear divisions in these simpler plants with those seen in the higher forms is in striking contrast with the relative simplicity of their external morphology and life histories.

It is quite possible that these parasitic fungi are degenerate in their vegetative structure as a result of their parasitic mode of life. In that case, however, the nuclear divisions being entirely reproductive processes would have naturally remained at least at the stage of development they had reached at the time the plant began its parasitic habit, since reproduction is quite as important for the parasite as for the independent organism, though it can dispense to a considerable degree with complexity of vegetative development.

If such conidial cultures of the anther smut be continued for several days and are then allowed to starve by failure to transfer them to fresh culture media, a very large per cent. of the spores will fuse in pairs by means of tubes pushed out and growing together at their tips. This fusion is accompanied by a very considerable increase in volume, as seen by comparing Figs. 12 and 17. The tubes grow out from adjacent cells and are apparently directed by chemotactic stimuli, so that their ends meet exactly. The end walls are dissolved, and the tubes fuse firmly and evenly together so that the points of union cannot

be distinguished when the process is complete, except for a slight enlargement of the tube diameter. The tubes may be long or short according as the conidia are more or less widely separated in the culture. They may be either straight or curved as in Fig. 18. Cells that do not become connected thus in pairs remain small, and their protoplasm frequently undergoes changes which cause it to stain as a homogeneous mass. The behavior of the promycelia which have been carried along in the cultures and have continued to bud is also noteworthy. The distal of the three cells of the promycelium is generally the smaller. When starvation intervenes the proximal larger pair of cells put out tubes from their adjacent ends which fuse and connect the two in the same fashion as the conidial pairs are connected. The fused pair now also enlarge greatly and their adjacent ends become rounded out by increased turgor. The cell wall splits and the two cells become free from each other except for the fusion tube (Fig. 19). In this condition they frequently become inclined at an angle to each other. Whether this change in their relative positions is due to special tensions in the walls of the fusion tube itself, or merely to chance as they float in the culture medium, is not easy to say. The distal cell is thus left unpaired, and much more generally than is the case with the similarly situated conidia, it undergoes a speedy disorganization. Its protoplasmic contents are aggregated in deep staining masses (Fig. 19), and ultimately the wall collapses. In some cases, however, where a conidium is lying near such a promycelium the distal promycelial cell forms a fusion tube with the conidium, and thus provided the pair enlarge and continue in as thriving a condition as the two lower cells (Fig. 20).

The resemblance of this whole process of cell fusion to a sexual conjugation is apparent. Externally it is like what happens in the formation of a zygospore from the contents of two Spirogyra cells. There is, however, here no migration of protoplasm from one cell to the other, and what is still more significant, no fusion of nuclei. The nuclei remain in their respective cells throughout the entire process without division or visible interchange of nuclear substance. It appears like sexual fusion but no fertilized egg in the ordinary sense of the term is

formed. In this particular it is similar to the conjugation of such infusorians as *Paramoecium*, where the two conjugating individuals both maintain their individuality and continue to divide after the fusion as before. In *Paramoecium*, however, a complicated system of nuclear changes has taken place, resulting in the loss by disintegration of certain daughter nuclei and the providing of each *Paramoecium* with a new micro- and macronucleus to which both individuals have contributed. The occurrence of the fusion after a prolonged period of vegetative activity in the yeast budding and the reduced vitality of individuals which do not succeed in fusing, is another point of resemblance between the process here and in the infusorians, where Maupas has shown that after a number of ordinary vegetative divisions, somewhat definite for each species, the individuals die unless conjugation takes place. Maupas[1] also finds that the conjugation can only occur when starvation threatens, just as is the case with the smut conidia. In the case of the latter, however, fusion is at the most only necessary to render the individuals a little more resistant to a period of reduced nutrition, and as long as the food supply is abundant they show no diminution of vitality; while the infusorians, in spite of abundant food, inevitably die of "senile degeneration" unless conjugation occurs after a certain number of generations.

When fresh beerwort is supplied to the cultures containing fused pairs they begin budding off conidia as before. (Fig. 22.) The process begins frequently by the formation of a rudimentary germ tube as if a mycelium were to be formed. Each spore pushes out in a short tube of practically the same diameter as itself. Cross walls are then put in, cutting off one or two cells from the end of this tube, and these cells then produce conidia by budding. This budding is well figured by Brefeld.[2] In making fixed and stained preparations the conidia are generally broken off, leaving the spore pairs with the germ tubes alone.

Sometimes only one of the two spores germinates (Fig. 21), though as a rule both do. When only one germinates the nucleus

[1] E. Maupas, "Le rejeunissement karyogamique chez les cilies." *Arch. de Zool. Exper. et génér.* 2e. Ser., Vol. VII.

[2] Loc. cit., Tafel.

of the other remains quiescent, as seen in Fig. 21, thus indicating clearly its independent individuality. In the case figured the sterile spore is somewhat smaller than the other and its nucleus is also smaller. In the germinating spore the nucleus divides to furnish nuclei for the cells cut off from the germ tube. One nucleus always remains in the spore itself. Brefeld reports[1] that he finds no difference between the fused and unfused conidia in their ability to live through a starvation period. In my own cultures, however, a marked difference was always noticeable in the per cent. of paired and unpaired cells which survived after a period of two or three days of starvation.

There is no evidence that a thicker wall is formed about the conidia after fusing in pairs. So far as they show greater ability to live through unfavorable conditions it is apparently due to some change in the protoplasm. Exceptionally germination occurs from the fusion tube. In this case nuclei are seen still present in the two spores, and it is uncertain which of them divided to form the nucleus for the conidium. The whole fusion process may be summarized as follows:

1. In cultures several days old when starvation threatens, the conidia put out tubes which fuse end to end, thus establishing protoplasmic continuity between the two cells.

2. No nuclear fusions or visible interchange of nuclear substance accompanies this cell fusion.

3. The cells enlarge rapidly in size and show increased protoplasmic content, indicating a stimulation of anabolic processes as a result of the fusion.

4. Fusion also regularly occurs between the basal cells of the promycelia under the same conditions.

5. Conidia which have not fused in pairs and the end cells of promycelia which have not fused with adjacent conidia, are somewhat less resistant to prolonged starvation than are the fused pairs.

6. The germination of the fused pairs is directly by budding, or a short one to three celled germ tube is produced which buds out in conidia.

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[1] Loc. cit., pp. 48–49.

Before discussing further the significance of such fusions, it will be interesting to bring together other facts regarding cell fusions where no fertilization in the ordinary sense of the term can be assumed to exist. Observations of this sort are recorded quite abundantly in the literature, and it is of interest to bring them together for purposes of comparison and the determination of their significance in the light of modern theories of sexuality.

One of the familiar cases is that of fusion of the germ tubes of spores so that several spores combine at once in forming a mycelium. Reinke's figure,[1] in which the germinating conidia of Nectria solani are shown uniting their germ tubes in a common system, is a familiar example. The behavior of the nuclei here is unknown and should be investigated, but there is certainly no reason for supposing that this is a sexual union. The fusion results in a larger germ tube than could be produced by any one of the spores singly, and hence the chances that the young mycelium may reach a favorable substratum for its development may be supposed to be increased. It is evident that in this case the fusion was accomplished without especial reduction in volume of the units which combine and indicates clearly the possibility of this sort of aggregation on the part of independent cell units. They are, though each is capable of producing an entire mycelium independently, able to combine, giving up their independent individuality in order to produce a single larger mycelium, which may have a better chance of reaching a substratum favorable for further development. Whether this fusion is customary in the development of germinating spores, or whether it only occurs when the nutrient medium in which germination occurs is poor in nutriment for the fungus, should be investigated more fully. It is also unknown whether the mature mycelium produced from such a series of spores is larger and stronger than that produced from a single spore, the conditions of nutrition being the same. We can simply say that the process seems to show clearly the possibility of a combination of cells for the sake of the advantage of size in reaching a source of food.

[1] DeBary *Morph., Phys., und Biol. d. Pilze, Mycetozoa, u. Bacteria*, p. 2.

The case described by Osterhout[1] of the combination of the four tetraspores of *Rhabdonia* in their germination to form a single vegetative body may perhaps be placed in this same category, although the tetraspores do not actually fuse.

A much more striking case of fusion of germ tubes where no strictly sexual significance in the phenomenon is possible, has been worked but by Woronin and Nawaschin.[2] The case is that of *Sclerotinia heteroica*, which is a heteroecious ascomycete producing conidia on *Vaccinium uliginosum* and *sclerotia* in the fruits of *Ledum palustre*. The ascospores of the fungus infect the leaves of *Vaccinium uliginosum* and produce a mycelium which develops a rich, conidial fructification. The conidia from the leaves of the Vaccinium fall on the stigmas of the flowers of *Ledum palustre* at the time of anthesis, the germ tube penetrates the whole length of the style and develops a mycelium in the ovary. As the latter grows, it is mummified by the mycelium and becomes a sclerotium, which passes the winter in a resting condition and in the following May germinates and produces a long stalked peziza cup. The asci of this peziza discharge their spores in the air and they are carried to the young leaves of *V. uliginosum*, infecting them and producing again the conidial fructification. The peculiar fusions referred to occur in the infection of the ovary by the conidia through the stigma. The authors find that a number of the conidia which have fallen on the stigma combine their germ tubes by fusion in order to penetrate through the style to the ovary with its abundant food material below. The conidia lie on the stigma like pollen grains and several, as many as five or six, push out germ tubes which, instead of growing independently downward into the subtratum, converge towards each other, probably by means of mutual chemotactic stimulation, and become fused into a single larger tube which then proceeds downward through the style. Here we have very plainly a fusion of individual cells with loss of their independence for the sake of producing a larger body, a larger germ tube, for traversing the

[1] Osterhout (W. J. V.), *Annals of Botany*, 1896.

[2] Sclerotinia heteroica *Zeitschr. f. Pflanzenkrankheiten*, Hft. 3 u. 4, 1896.

relatively considerable distance before it reaches the appropriate subtratum for its further development. Whether the tube of a single spore would be incapable of reaching the ovary and producing the infection has not been determined experimentally, but it is plain that the certainty of infection taking place when once the spores are on the stigma is enhanced by the fusion process described. It would be interesting also to know whether the germ tubes are capable of drawing nutrition from the tissues of the style. The nuclear phenomena in connection with this fusion have not been studied, but in any case it cannot be considered as sexual in any sense of the word. It is a case of protoplasmic fusion where the advantage to be gained is simply the increased size of the resulting body. It belongs in the same category with the case of Nectria, and here the object of the fusion is much more clearly indicated by the conditions under which it occurs.

In two other related species of Sclerotinia the same process had been still earlier described by Woronin,[1] namely, in *S. padi*, whose spores fall on the stigma of *Prunus padus* and penetrate the host plant by means of a similar compound germ tube; and also in *S. aucupariae* parasitic on the mountain ash, which infects the berries of the latter by a compound germ tube from the stigma through the style. On the other hand, in *S. megalospora* which produces sclerotia in the fruits of *V. uliginosum* a single spore on the stigma can produce a germ tube long enough to reach the ovary and fusions of germ tubes in this species have not been observed.[2] The spores of this species of *Sclerotinia* are larger than those of the other three mentioned, but it would be hasty to assume that this visible difference in size is the direct cause of the difference in behavior of the species in germination. The length of the germ tube is not directly proportional to the visible size of the spores as can be shown in many cases.

There are numerous accounts in the literature of the anastomosing of adjacent branches of a mycelium by means of

[1] Woronin, "Die Sclerotienkrankheit der gemeinen Traubenkirsche und der Eberesche." *Mem. de l'Acad. imp. des Sci. de St. Petersbourg.* 1895; pp. 11–12, 17.

[2] Woronin u. Nawaschin, loc. cit., p. 11.

tubes which grow out from one branch and fuse at their ends with an adjacent branch. Such fusions are not to be considered as simply due to contact of two filaments that happen to cross each other's path of growth. I have had abundant opportunity of observing the phenomenon in mycelia of *Rhyparobius* grown in a decoction of dung on the slide, and it is plain that many filaments cross and are in contact without fusing, while in other cases a branch will grow out in the shortest path possible to an adjacent branch and fuse with it. Sometimes two tubes grow out and meet and fuse at their tips just like the conjugating tubes of Spirogyra cells. The anastomosing is most common in the peripheral parts of a mycelium and may serve to secure an even distribution of food materials through the whole by a shorter route than would be otherwise possible. Certainly the mycelium is made a more definite unity by means of such anastomosing, but its advantage is by no means clear.

Further examples are given by Woronin[1] and by Woronin and Nawaschin[2] of anastomosing in hyphae developed from the germinating conidia of the above mentioned Sclerotinia species when grown in plum juice. The young germ tubes, the older mycelial branches, and the germinating spores themselves may all become connected by fusion tubes which are developed in the greatest profusion without reference to the food supply or other noticeable external factors.

Very recently the spore fusions in *Protomyces macrosporus* have been described and figured by C. M. L. Popta.[3] The spores fuse in this case soon after they have been expelled from the spore case and the formation of the fusion tube and the appearance of the fused pairs is very much the same as in the anther smut. In stained preparations of the spores before fusion four to seven nuclei are found, and after fusion also each cell of the pair shows from four to seven nuclei. The author concludes that there is no evidence that nuclear fusions follow the cell fusion and is apparently inclined to the opposite view. In living material highly refractive bodies were observed in

[1] Woronin, loc. cit., p. 11.

[2] Woronin u. Nawaschin, loc. cit., p. 10.

[3] "Beiträge zur Kenntniss der Hemiasci," *Flora*, Hft. 1, 1899.

the fused spores which the author suspects might be the nuclei. In two cases one of these refractive bodies was seen in the fusion tube but prolonged observation revealed no change in its position. Popta says nothing of changes in the spore pairs which follow fusion. De Bary[1], however, has recorded that after fusion the pairs enlarge very considerably as is the case in the anther smut. According to De Bary also when the fused pairs germinate but a single tube is pushed out and the entire protoplasmic content of the pair passes out into the young mycelium thus formed. The germination regularly occurs on the surface of a host plant and the germ tube at once penetrates the tissues of the host plant and continues its vegetative existence there.

The nuclear phenomena during these germination stages should be investigated before we can be entirely certain that a sexual fusion of nuclei does not occur at any stage of the process; though the phenomena are very similar to those in Sclerotinia, where the number of spores which combine is so large and variable as to practically exclude the assumption of sexuality.

Further in this connection should be mentioned Hoffman's[2] clamp connections between adjacent cells of the mycelia of the *Basidiomycetes*. The more exact investigation of these structures is to be credited to Prof. Brefeld.[3] He finds that in the mycelium of *Coprinus* a tube grows out from the end of one cell and curves in toward the lateral wall of the next adjacent cell, with which it comes in contact and finally fuses, forming thus an open passage between the two cells. Later a transverse wall is built at the base of the fusion tube, so that the continuous passage way formed between the two cells is again interrupted. Brefeld thinks the structure has something to do with circulation of food materials in the mycelium. Its resemblance to the fusion of the cells of the promycelia in the smuts has already been pointed out.

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[1] *Beitr. zur Morph. u. Phys. der Pilze*, I.

[2] Hoffman, *Bot. Zeitung*, 1856; p. 156. Also De Bary, *Morph., Phys., u. Biol. der Pilze, Bakt., und Mycetozoa*, pp. 2–3.

[3] Brefeld, *Unters. über Schimmelpilze*, III, p. 17.

All the cases of union so far discussed are between cells which are either themselves coenocytic or belong to organisms which in some stages of their development form coenocytic cells. The fusing conidia of the smuts are uninucleate. The fusing spores o *Protomyces* contain each several nuclei. The tendency among the fungi to form multinucleated cells may be another expression of some fundamental quality of their protoplasm which also leads to the cell fusions which have been described. How a binucleated or several nucleated cell arising by fusion would differ from a binucleated or several nucleated cell which became so by internal nuclear divisions, is not certain, especially when the fusing cells are separated in descent by only one or a few cell divisions.

Oltmanns' recent researches on the nuclear phenomena in the sexual reproduction of the red algae, have furnished a very interesting case of cell fusions without nuclear fusions. They are the cell fusions which Schmitz regarded as secondary fertilizations. According to Oltmanns, in the forms where these secondary fusions occur, Dudresnaya purpurifera for example, the sexual fusion of energides — spermatium and egg with their nuclei — takes place in the carpogon. From the carpogon grow out typically three branches ("oöblastema threads" of Schmitz, "carpogenous filaments" of Oltmanns). The fertilized egg nucleus divides, and daughter nuclei are carried along in the carpogenous filaments. The carpogenous filaments fuse with ordinary vegetative auxiliary cells of the branch which bears the carpogon, but no nuclear fusions occur. The sporogenous energide simply uses the material of the cells with which it fuses for its own further vegetative development. After these fusions the carpogenous filament proceeds still further to fuse with special auxiliary cells on the ends of branches in the various parts of the plant. Here again no nuclear fusions occur. The nuclei of the carpogenous and auxiliary cells repel rather than attract each other. In the fusion cell thus formed the sporogenous nucleus divides; one daughter nucleus passes on with the further growth of the sporogenous filament, and the other remains in the fusion cell, appropriates its protoplasm, and by subsequent

[1] "Zur Entwicklungsgeschichte der Florideen," *Flora*, 1898.

divisions accompanied by cell divisions forms the mass of carpospores. The original nucleus of the auxiliary cell gradually becomes disorganized and disappears. The whole process consists in the parasitic development of the nuclei derived from the fertilized egg in the auxiliary cells of the mother plant. It is analogous to the parasitic development of the sporophyte on the gametophyte in the mosses. The fusions of sporogenous (oöblastema) filaments with the auxiliary cells is purely a nutritive process, and adds thus a most interesting further type of cell fusion to those already enumerated. The whole process of the presumably chemotactic attraction of the cells for each other, the breaking down of the cell walls, and the union of the cytoplasmic masses, is carried out solely for the purpose of supplying the sexually produced energide with material for the production of a mass of carpospores. It is a case of parasitism in a sense; and yet the cell fusion is in no respect like the penetration of a host plant cell by a haustorium or a swarm spore, as it occurs in the case of the parasitic fungi. The haustorium maintains its own bounding membrane and simply devours the killed and liquefied substance of the host cell by absorption. The unions of the sporogenous and auxiliary cells is a real fusion. The fused cells have a common plasma membrane. The cell wall also, which encloses the fusion cell, as Oltmanns points out, is in part the wall of the auxiliary cell and in part the wall of the sporogenous filament.

These investigations of Oltmanns must be considered as of the greatest importance, not only in bringing clearness out of the chaos which has hitherto existed in our notions of sexuality in the red algae, but also because they bring into sharp contrast the sexual fusion of cells and nuclei and cell fusions which have a purely vegetative and still well defined significance. The independence of the phenomena of nuclear and cell fusions is clearly brought out and the suggestion given, that while each may be of great importance to the organism, their significance may well be quite distinct. These secondary cell fusions have at least a nutritive function and may have others.

In this connection the view of Iwanzoff[1] that normal conju-

[1] N. Iwanzoff, "Physiologische Bedeutung u. s. w." *Bull. Soc. Imp.*, 1898.

gation is a mutual devouring of each other by egg and sperm is interesting and perhaps gains an added element of probability. That this does not, however, afford a complete explanation of cell fusions is shown by the case of the conidial fusions of the anther smut, where neither cell devours the other but each maintains its individuality and germinates independently. In this case we must assume that the fusion produces some change in the condition of the protoplasm whereby its metabolism is increased; and, perhaps also, it becomes more resistant to unfavorable external conditions.

For completeness may be mentioned the fusion of cells end to end to form the vessels, tracheae, of the higher plants and the fusion to form laticiferous ducts in certain cases. These cases are however quite different from the others mentioned, in that they occur in cells not destined to further development by division, and in the first case at least are accompanied by the death of the cells.

In still a further and quite interesting category may be mentioned the union of the swarms spores of slime moulds to form plasmodia. Here the union consists in the formation of a larger amoeboid mass with a common plasma membrane by the union of numbers of smaller amoeboid cells. It is in its origin, doubtless, a preparation for spore formation and has been developed from the habit of the Acrasieae to come together to form stalked spore fruits, although in these latter forms the swarm spores pass through their entire vegetative existence as distinct bodies. Plasmodium formation would then be an occurrence at an earlier stage and more completely of the aggregation of cells which in *Dictyostelium*, for example, occurs at the time of uiting. What the advantage of this union is cannot be pointed out with certainty, except that it brings and keeps the cells together so as to prevent their becoming too widely scattered before the time of fruiting. It perhaps also secures a more even distribution of food among all the cells that are later to combine to a single fruit body.

If we look now for the significance of these various types of cell fusions, it seems plain that in none of them do we have anything that is comparable to sexual fusions. The nuclear fusion

which Hertwig and Strasburger have pronounced the essential feature in sexual fusions in both plants and animals is lacking. None the less we have, at least in the pairing of the conidia of the anther smut, a phenomenon which shows some of the physiological results which accompany a true sexual fusion, namely, increased metabolism and increased capacity to resist unfavorable external conditions. And in all the cases mentioned an improved physiological condition may be fairly assumed as a result of merely cytoplasmic fusions. How it is that a cell finds itself in better condition as a result of increasing its mass by fusion with a second cell, is not clear except where the increased size is a necessity in order to reach a certain position necessary for the further development of the organism, as in the case of the germ tubes of *Nectria* or *Sclerotinia* described above. Such an advantage is not, however, present, in the fusions of the anther smut. If reduction of volume and consequently of exposed surface for osmosis followed the fusion, as is the case in so many true fertilizations, an advantage might be assumed to exist in this diminution of surface contact with unfavorable surroundings and the greater density of the protoplasmic mass; but in the anther smut fusion is followed by a very notable increase in the size of the fused cells. It is impossible at present to say wherein the significance of this fusion consists. It may be that the phenomena are to be interpreted as a primitive type of sexual union, where the process of fusion has not yet gone so far as to include a union of the nuclei and a loss of individuality of the gametes; or it may be a degenerate form of sexuality in which the nuclear union has been lost and the cytoplasmic union retained.

There can be no doubt, however, that such cases show clearly that there is an advantage to the one celled organism in a cytoplasmic union, even without nuclear fusion. The importance of this cytoplasmic union in the cases of true fertilization where the male cell consists of little but the nucleus, as in the mosses and ferns, may be much reduced. The existence of such cases, however, whether primitive or degenerate, is strongly in favor of the view that sexual reproduction may have originated in fusions of a simpler type than are found in most plants and an-

imals today, and in which neither nuclear fusions nor loss of individuality of the fusing gametes was present. Such cases would furnish an interesting connecting link between ordinary asexual reproduction by division and the sexual fusion of nuclei, with the resulting necessity of chromosome reduction and so forth.

On the much discussed question as to whether the cytoplasm is concerned in the transmission of hereditary characters in true sexual fusions, such cases of cell fusions, showing that merely cytoplasmic unions have a distinct value for the organism, make it probable that that advantage is a prominent feature in the results of fusion of equal gametes in *Spirogyra* and other cases where large amounts of cytoplasm unite in the process of fertilization. In higher organisms, as already noted, where the amount of cytoplasm present in the male gamete has been reduced to a minimum, the significance of the non-nuclear elements in the fusing cells may be correspondingly diminished.

Summarizing the types and apparent effects of cell fusions where no nuclear fusions occur, we can note the following:—

1. Cases where fusion results in increased size thus enabling a germ tube to reach the substratum most favorable for its further development: *Sclerotinia;* possibly *Nectria.*

2. Cases where fusion provides for more ready and equal distribution of food materials in a mycelium: *Rhyparobius* and many others; also possibly the clamp fusions of *Basidiomycetes.*

3. Cases where fusion leads to growth in size of the fused cells, perhaps also making them more resistant to unfavorable external conditions: Anther smut, *Protomyces.*

4. Cases where the fusion is largely at least a nutritive phenomenon, the protoplasm of one energide passing entirely under the control of the other, and results in the disorganization of the nucleus of the former: Secondary fusions in the red algae according to Oltmanns.

Madison, Wis., December, 1898.

EXPLANATION OF FIGURES.

All figures drawn with aid of Abbe camera lucida and with Zeiss apochromatic objective 2 m.m., aperture 1.40. Figs. 1–10, 12, and 17–22 with compensating ocular 12, the others with compensating ocular 18.

PLATE VIII.

Figures 1–7, *Ustilago scabiosae.*

Fig. 1. Promycelium with resting nucleus.

Fig. 2. —— with nucleus in equatorial plate stage of division, nucleole lying near one end of spindle.

Fig. 3. —— with daughter nuclei in dispirem stage, connected by spindle fibres.

Fig. 4. —— with two daughter nuclei not fully developed but widely separated.

Fig. 5. —— with four resting nuclei.

Fig. 6. Four celled promycelium with conidia.

Fig. 7. Upper cell of four celled promycelium. Nucleus in equatorial plate stage.

Figures 8–22, *Ustilago antherarum.*

Fig. 8. Germinating spore uninucleate.

Fig. 9. Germinating spore after first nuclear division.

Fig. 10. Promycelium with first septum.

Fig. 11. Conidium budding like yeast.

PLATE IX.

Fig. 12. Three-celled yeast-like colony.

Fig. 13. Four-celled yeast-like colony.

Fig. 14. Conidium with nucleus in equatorial plate stage.

Fig. 15. Conidium with nucleus in metaphase.

Fig. 16. Conidium with nucleus in dispirem stage, spindle still present.

Fig. 17. Conidia fused in pairs and swollen.

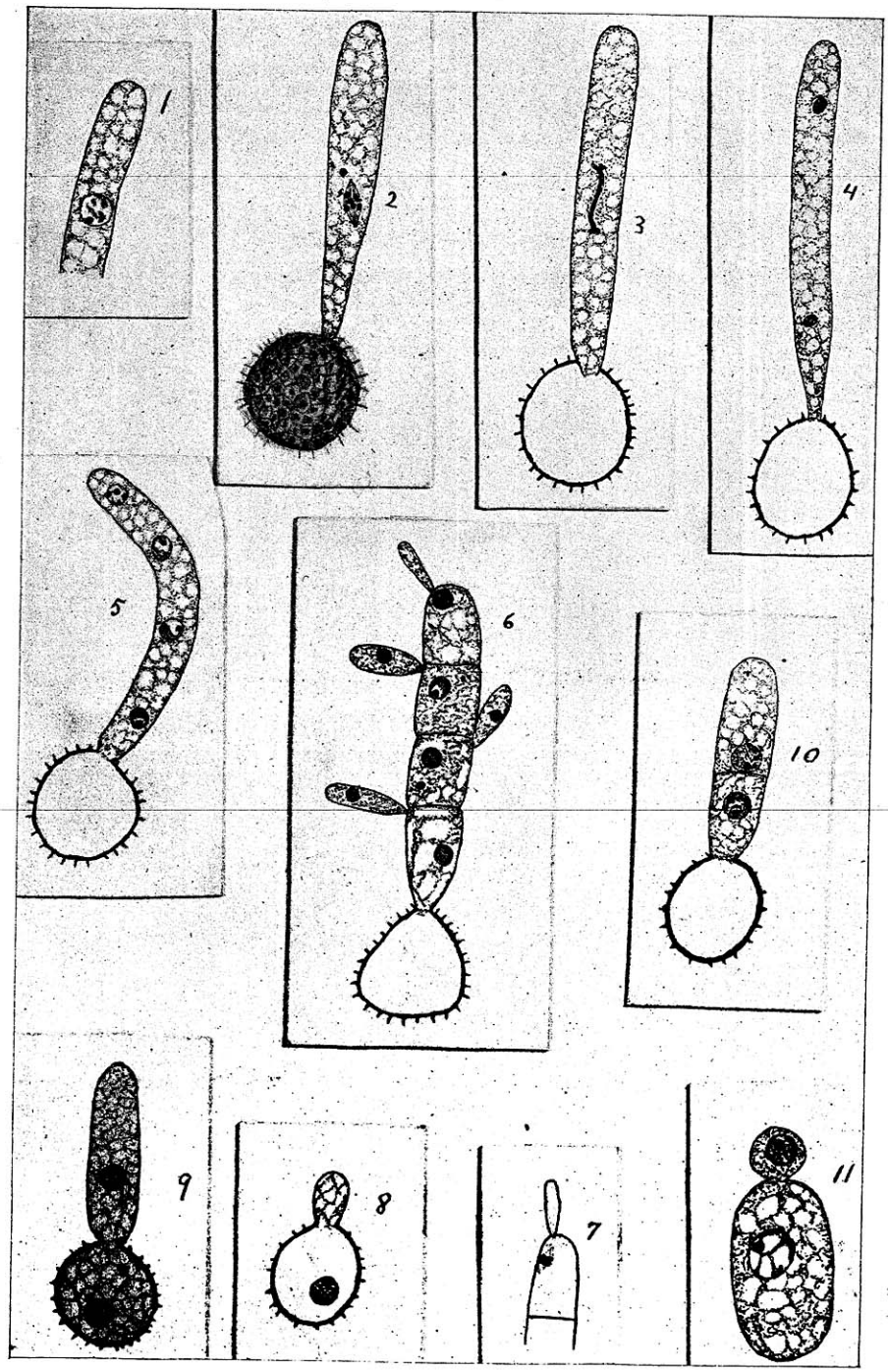
Fig. 18. Conidia fused in pairs, curved fusion tube.

Fig. 19. Promycelium cells with fusion tube. Apical cell in process of disorganization.

Fig. 20. Promycelium with apical cell fused with adjacent conidium; fusion tube of lower pair lies behind them.

Fig. 21. Fused pair with one germinating in tube.

Fig. 22. Fused pair with conidial buds.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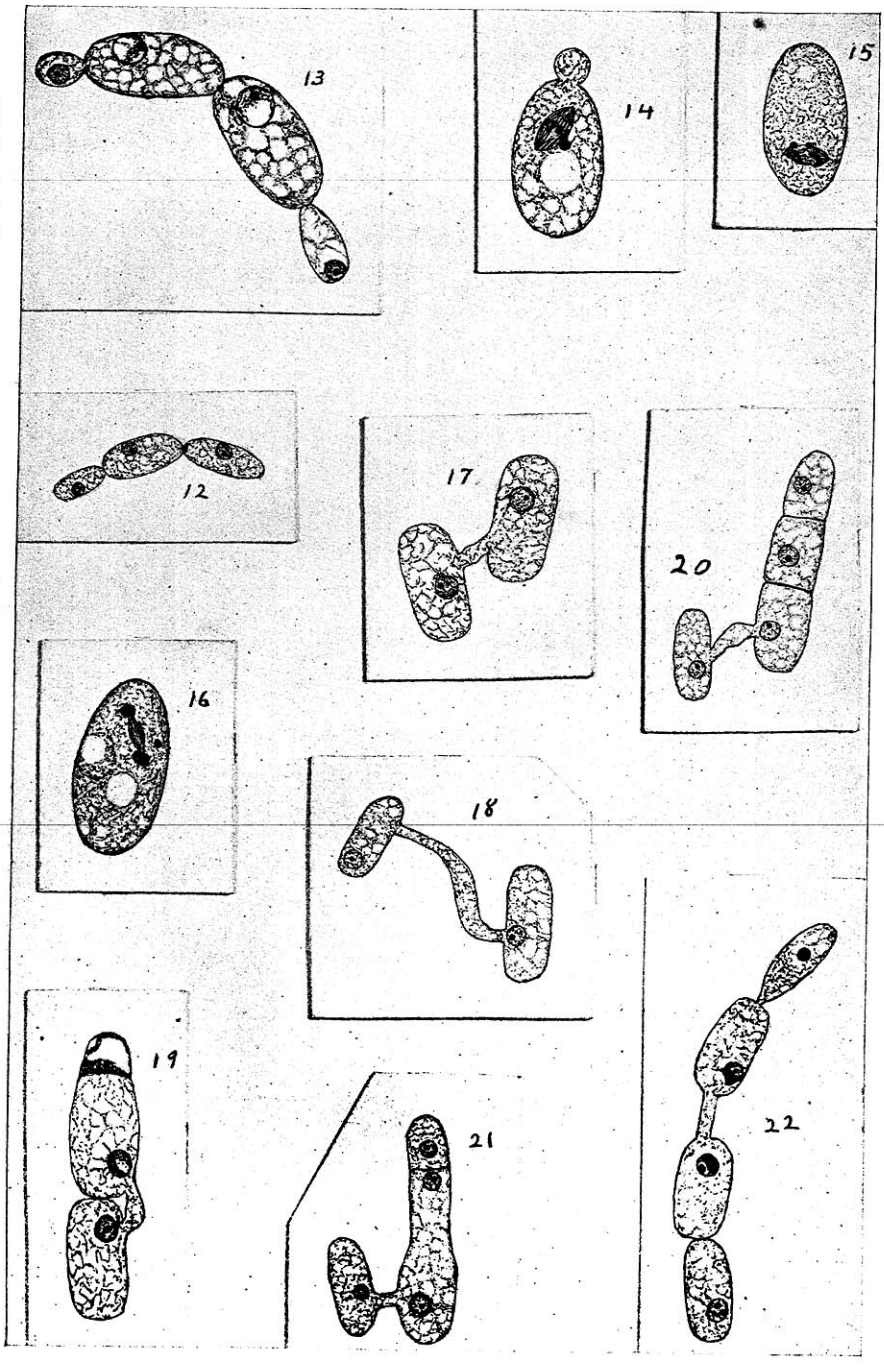
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