T H E S I S

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on

THE LIFE-HISTORY and CYTOLOGY of DIDYMIUM NIGRIPES.

BY

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I N T R O D U C T I O N .

Since 1860, in which year DE BARY published his great work 'Die Mycetozoen', the investigation of the life history of members of the Mycetozoa has aroused a considerable amount of interest, and a great deal of important research has been carried out in this connection. This group of organisms is particularly interesting because it lies on the border line between plant and animal kingdoms, and it is very possible that a detailed investigation of several species of the Mycetozoa might be of considerable assistance in elucidating certain obscure points in the life histories of higher members of both the great natural groups. The term 'Mycetozoa', which we owe to DE BARY, will be used throughout, in preference to the older term 'Myxogastres' invented by FRIES, (51 p.2.), and that of Myxomycete first employed by LINK (51.p.2.).

'Mycetozoon', or 'fungus-like animal', is a very appropriate description of a member of the group, since, during part of its life history it exhibits distinctly animal-like characters, and the/

the individuals move rapidly by means of flagella, whilst later, during the development of the sporangium, a plant-like form is assumed. The combination of plant and animal characters has given rise to much discussion as to the position of the Mycetozoa in plant or animal kingdom, and the group has been claimed by both zoologists and botanists.

Many of the more recent investigators have confined their attentions to the important parasitic sub-group of the Mycetozoa, the 'Plasmodiophoraceae' chiefly on account of the serious nature of the diseases caused by two members of the group, Spongospora subterranea on potato and Plasmodiophora brassicae on Brassica, but no connected life history of these organisms has appeared. Most of the investigators who have studied members of the other sub-groups of the Mycetozoa have described isolated stages in the life history of the organism, though JAHN (27) in 1908 published a fairly full account of the life history of Ceratiomyxa, and WILSON and CADMAN (71) in 1928, described in detail the life history and cytology of Reticularia Lycoperdon.

Reticularia Lycoperdon is a member of the sub-group 'Endosporeae" of the Mycetozoa, and is a wood/

wood-inhabiting species. The plasmodium lives within the tissues of decaying timber, and only comes to the surface for spore formation. It was thought that it would be of interest to study in detail a species of the same sub-group which possesses a plasmodium creeping superficially over dead and decaying organic material. The purpose of the present investigation is to publish a full life history, particularly in regard to its cytology, of such a species, Didymium nigripes being the one chosen.

REVIEW/

REVIEW of LITERATURE.

It is not intended to give here a complete summary of the very extensive literature dealing with the Mycetozoa. STURGIS, (68), in 1912, compiled a list including all papers on the subject published between 1875 and 1912, and a very comprehensive bibliography is attached to the section dealing with these organisms in RABENHORST'S Kryptogamenflora, (51.) but, unfortunately, this mentions no literature later than 1911. The more important investigations which have appeared since that date and before 1926, are summarised in WILSON and CADMAN'S paper on Reticularia Lycoperdon (71.) It will be sufficient therefore to give a brief résumé of the chief literature dealing with the Endosporeae, the group of the Mycetozoa to which Didymium nigripes belongs, and include in this account a consideration of several papers which have been published since 1926.

DE BARY, who was the first to give a detailed description of the life-history of the Mycetozoa, made very careful investigations of living cultures of a large number of species and published his results in 1860. (1.) He came to the conclusion that the course of events in the life-history was as follows. On germination the spores of the Mycetozoa give/

give rise to an actively swimming structure, which he termed a 'Schwärmer' or swarm cell. The swarm cell divides several times and then loses its flagellum and becomes transformed into an amoeba. The amoebae either by individual growth or by fusion of two or more individuals, he was not sure which, give rise to sarcode strands, which are multinucleate structures and these, after a period of development, form the sporangia. He described in great detail each stage in the life history and also stated that under unfavourable conditions, for instance lack of moisture, the sarcode strand may break up into a number of small fragments each of which develops a distinct wall and thus is rendered resistant until conditions become favourable once more. On the addition of water the wall ruptures and the contents emerge as a sarcode strand and resume active life. DE BARY (2) in two later publications in 1864 and 1887 (3) not only supplemented the work he had already done, but summarised the more important conclusions reached by other investigators, in particular WIGAND, CIENKOWSKI and STRASBURGER.

WIGAND (70) whose paper on the genera Trichia and Arcyria appeared in 1863, examined in detail the structure of the mature fruit body in these two groups, confirming the description by DE BARY and others that it consists of a peridium or skin/

skin which encloses a large number of spores lying free within the meshes of a thread like capillitium. As he was only dealing with the mature sporangium he was unable to form any opinion as to how the stalk, peridium, capillitium and spores were formed, but he corrected an erroneous conclusion arrived at by several previous investigators that the spores arise from the capillitial threads, and stated emphatically that, from the first, they lie freely within the meshes of the capillitium.

The work of CIENKOWSKI, which was embodied in two papers, (10) and (11), both published in 1863, was of an exceedingly accurate description, especially when one considers the somewhat primitive methods of research in use at that early period. As a result of his first series of investigations (10) he confirmed very largely the observations of previous workers, especially those of DE BARY, whose term 'sarcode - strand' he replaced by the more modern 'plasmodium'. He came to the conclusion that the plasmodium is a large amoeba-like body with no definite wall, but possessing on the exterior a hyaline layer, and consisting within of a granular, very fluid substance. This differs slightly from the description of DE BARY, who regarded the plasmodium as a large bag with many branches, and with a very distinct wall. CIENKOWSKI found that within the plasmodium, as well as/

as well as in the interior of the resting cells (sclerotia) and myxamoebae, contractile vacuoles are present. He also stated that plasmodia and amoebae are capable of engulfing and digesting foreign bodies, and that plasmodia increase in size in two ways, firstly by the digestion of foreign organisms, and secondly by fusion with myxamoebae; two plasmodia of different genera will not fuse.

In his second publication (11) CIENKOWSKI described in great detail the formation and structure of the plasmodium in three different species of the Mycetozoa. He found that the plasmodium is formed by the fusion of two or more amoebae, and watched this process under the microscope several times. He applied the term 'myxamoeba' apparently to the young plasmodium and said that the large plasmodium arises by the fusion of a number of these. The term 'amoeba' was kept for the structure which arises from the swarm cell by the withdrawal of the flagellum, and which can readily develop a flagellum and become a swarm cell again. The myxamoeba, he affirmed, can be distinguished from the amoeba by its apparent lack of a nucleus, its larger size and the greater number of vacuoles present. CIENKOWSKI also described three resting stages: 1) Microcysts. 2) Thickwalled cysts, 3) Resting cells. The two latter had already been discovered by DE BARY. The microcysts/

microcysts are formed by swarm cells which, under unfavourable conditions, round off and may or may not form a wall; in Didymium no wall is formed. On germination these give rise to swarm cells. The thick walled cyst is the resting stage of the myxamoeba, and in this case a very definite wall is formed. The resting cells had already been described by DE BARY.

STRASBURGER, who examined the developing sporangia of Trichia fallax (63) was the first investigator to make use of the more modern methods of fixation and staining. He published his results in 1884, and came to the following conclusions. The wall of the sporangium is laid down very early and was present in the youngest stages examined. It consists of two portions, an outer, transparent, colourless layer, which exhibits distinct radial striation as well as tangential stratification, and an inner brown layer in which tangential stratification alone is present. The threads of the capillitum are formed much later, their walls being laid down on the sides of elongated vacuoles in the protoplasm. Some time after the capillitium is complete an almost simultaneous karyokinetic division of the nuclei takes place and this is followed immediately by spore formation. The karyokinetic division is essential to increase the number of nuclei/

nuclei present and to render them small enough for the individual spores. The young spores are formed by the laying down of cell plates cutting out a polygonal area round each nucleus.

A.LISTER was the first in England to make an intensive study of the Mycetozoa and he examined the group from several points of view. His greatest work was the compilation of a complete classification, and this forms the bulk of his Monograph, the first edition of which appeared in 1894. The Monograph was revised and extended by his daughter, MISS G. LISTER, in 1925.[38] In his three papers[33] published in 1888 and[34] and[35] which both appeared in 1890 LISTER gives the results of a very careful examination of the feeding habits of the swarm cells and plasmodia of several species. He found that the swarm cells of all the species investigated can absorb and digest bacteria, and by the introduction of very large bacilli he was enabled to follow the entire process. He discovered that there was a considerable difference in the behaviour of the swarm cells of the various species towards inanimate solid particles, such as grains of carmine. In some species these were greedily engulfed,whilst in others, though efforts were made to flow round the particles, this actually was never accomplished. He described the behaviour of the plasmodium particularly in the

case of Badhamia utricularis, and the results obtained may be summarised as follows. The plasmodium can engulf and digest solid food particles if these be of a suitable kind. Fungal filaments form a particularly favourable food supply, but the plasmodium has a remarkable power of selection and will only digest the hyphae belonging to a limited number of species. Starch granules cannot be digested until they have been heated in water and have swelled slightly. The digestive enzyme is present in all parts of the plasmodium, and undigested detritus is ejected at the surface through the pulsating vacuoles.

In his fourth paper published in 1893, (36) LISTER continued the cytological investigations commenced by STRASBURGER and described the division of the nuclei in swarm cells, plasmodia and developing sporangia of a number of species. He stated that in the swarm cells the nuclei divide by a process of karyokinesis quite as complicated as that found in the higher plants and not somewhat simplified as indicated by STRASBURGER, but that in the plasmodium direct division occurs. In the sporangium karyokinetic division takes place just before spore formation and LISTER noted that in some species nuclear division is completed before cleavage of the protoplasm/

protoplasm to form the spores commences, whilst in others division and cleavage are progressing simultaneously. In his last paper, in 1901 (37) the results are given of certain experiments regarding the germination of the spores of several species of the Mycetozoa, and the observation is made that wetting, drying and rewetting has a stimulating effect on the spores causing them to germinate when they otherwise refuse to do so.

LISTER was assisted by his son, J.J.LISTER (40) in the prosecution of his cytological investigations. The latter found two cases of karyokinesis in the plasmodium of Badhamia utricularis, and so was enabled to modify his father's belief that only direct division of the nuclei in the plasmodium takes place.

DANGEARD in 1890 (14) examined Spumaria alba and described the structure of the plasmodium and the nuclei present in plasmodium and spores. He found that two types of nuclei are present. Nuclei of the first type are poor in chromatin, staining almost homogeneously throughout and possessing a very small nucleolus. Nuclei of this kind are found in the spores. The second type of nucleus is rich in chromatin and stains so deeply that a nucleolus cannot be distinguished. These are found in the young/

young sporangium before spore formation. The plasmodium possesses nuclei which are intermediate between the two types. DANGEARD found the significance of the different types of nuclei in the fact that the plasmodium, just before spore formation, has reached the height of its development and its nuclei are so rich in food material that abundant chromatin can be manufactured and this must be expelled, giving nuclei of the first type, before the spores are formed.

The investigations of ROSEN (53) published in 1893, were concerned with types of nuclei, nuclear division and spore formation in Fuligo septica. He stated that no division of nuclei, direct or karyokinetic, takes place in the plasmodium and that the number of nuclei present is an indication of the number of amoebae which fused to form the plasmodium. He is in agreement with DANGEARD in describing two types of nuclei in the plasmodium, one with a distinct nucleolus and one staining homogeneously and, like the latter, found that those which stain homogeneously predominate in the young sporangium some time before spore formation. He was of the opinion that the deeply staining/

staining granules which fill the nuclei of the homogeneous type are of great importance in wall formation, as those nuclei in close proximity to the wall of the sporangium, and to the developing threads of the capillitium lose their granular contents and become nuclei of the first type, and this also happens in the case of the nucleus in the ripe spore. He regarded the homogeneous nuclei as the source of the microsomes described by STRASBURGER and he agreed with the latter that the spores are initially polyhedral structures cut out by cell plates.

HARPER examined the development of the sporangium in several species of the Mycetozoa and published three papers on the subject. In the first of these, which appeared in 1900, (21) he described the development of the sporangium of Fuligo varians. He agreed with STRASBURGER that the capillitium is laid down on the wall of vacuoles though he was not quite sure whether deposition primarily occurs in the form of microsomes. He failed to find the two types of nuclei described by DANGEARD and ROSEN and was of the opinion that these are due to inequalities in fixation. He agreed with LISTER that the karyokinetic division of the nuclei in the young sporangium is as complicated as that found in the higher/

higher plants, and not of a much simplified type as stated by ROSEN, and was sure that the last named missed several important stages in the process. HARPER described spore formation as the final result of a process of cleavage which commences at the surface of the sporangium and progresses inwards. Whilst this is taking place the nuclei divide almost simultaneously. In the first stages of cleavage, which result in the division of the protoplasm into masses containing a number of nuclei, the cleavage furrows run indiscriminately through the sporangium, but in the later stages, when the young spores are finally cut out, the nucleus apparently exerts some influence on the direction of the final cleavage lines. This differs markedly from the previous descriptions by STRASBURGER and ROSEN that the young spores are cut out simultaneously into polyhedral structures by cell plates.

In his second paper, published in collaboration with DODGE in 1914 (22) HARPER was concerned with the development of sporangia of more advanced types such as Trichia and Hemiarcyria, in which a very highly organised capillitium is present, the walls of which are thickened in various ways. He found that the/

the formation of the capillitium is initiated as a result of the gradual loss of water by the young sporangium which gives rise to a series of vacuoles ramifying in all directions through the protoplasm. Deeply staining granules appear on the walls of the vacuoles which he believed to be too large and too irregularly scattered to be the microsomes of STRASBURGER. Connected with these granules are radiating lines comparable with the astral rays of centrosomes and these HARPER believed, indicate lines of flow by which material passes from nuclei arranged in a row along the vacuole to build the wall of the capillitial thread. He stated that in the earlier stages of development the capillitium of Trichia is a connected reticulum, which breaks up later to form the typical elaters of the group.

The species which HARPER discussed in his third paper, also published in 1914 (23) is Didymium melanospermum, and here he was concerned entirely with the cleavage of the protoplasm into uninucleate spores. He stated that the results obtained agree with his previous assertion that cleavage is a progressive process due to gradual loss of moisture. He found that in Didymium the process first commences at/

at the periphery of the sporangium and works inwards and a little later it is also initiated on the surface of the columella, working from thence outwards. The formation of the capillitium is not described. Three types of nuclei are figured, varying in size and structure, but HARPER comes to no conclusion concerning their significance.

The work of BISBY (4) in 1914 was on the same lines as that of HARPER. BISBY investigated the formation of the capillitium and sporangial wall in Physarella mirabilis and Stemonitis fusca, and stated that the capillitium is laid down in both species on the walls of tubular invaginations from wall and columella of the sporangium. He was not clear as to how the process of the formation of the wall of the capillitial thread is actually carried out, but merely stated that the wall gradually increases in thickness. Cleavage follows the lines of the capillitium threads and is initiated through the shrinkage of the adjoining protoplasm from the surface of the thread. A simultaneous karyokinetic division of nuclei occurs during cleavage. The formation of the sporangial wall in both cases, is due to protoplasmic secretion. A much thicker wall is/

is formed in Physarella than in Stemonitis, because in the former cleavage commences a considerable time after wall formation, whilst in the latter wall formation and cleavage commence almost simultaneously.

The most extensive investigations in regard to the life history and cytology of the Mycetozoa, have been made by JAHN and published in a series of papers from 1904 to 1928. In his first paper in 1904 (24) he described the nuclear division and flagellum formation of the swarm cells of Stemonitis fusca. (49) He agreed with PLENGE'S description of the structure of a Mycetozoan swarm cell which states that it is a pear shaped body with a nucleus at the anterior end connected with a deeply staining granule, the blepharoplast, from which the flagellum springs, by a cone shaped structure called by PLENGE the 'Verbindungstück' and by JAHN the 'Geisselglocke'. JAHN found that the swarm cell divides karyokinetically, the division being intranuclear and two deeply staining centrosomes being present. After the completion of nuclear division the pointed ends of the spindle and the centrosomes remain, the former becoming the 'Verbindungstücken' and the latter functioning as the blepharoplasts of the/

the daughter swarm-cells. Blepharoplast and centrosome are therefore homologous.

In his second paper, published in 1905 (25) JAHN gave the results of many experiments on the germination of numerous species of the Mycetozoa. He came to the conclusion that germination is not a matter of simple osmosis but that it is dependent upon the presence of a substance, probably an enzyme, which he terms 'Awakening-stuff'. The awakening-stuff is more or less closely associated with material, called by JAHN 'Mother substance'. The 'awakening material' must be split off from the 'Mother substance' before germination can take place and the more closely the two are combined the more difficult is it to get the spores to germinate. This differs in the various species, so that the spores of some species germinate with great readiness, whilst those of others will hardly germinate at all. The splitting off of the 'Awakening material' is favoured by subjection to a comparatively high temperature, by alternate wetting and drying and by the presence of maltose. JAHN described two types of germination in the Endosporeae. In the first, the Reticularia type, the spore contents emerge as an amoeba and remain quiescent for/

for some time before forming a flagellum. In the second, the Didymium type, the spore contents divide before germination and emerge as young swarm cells with the flagella in process of formation.

The next two papers of JAHN which are concerned with the Endosporeae, (26) and (28) are the most important of the series as they give the first description of sexual fusion in that group. In the first of these, which appeared in 1907, he came to the conclusion that throughout most of its life history the organism contains the haploid or gametophytic number of chromosomes, and the diploid condition is only present for a very short time just before spore formation. The nuclei fuse in pairs during the development of the young sporangium and almost immediately divide again to form the nuclei of the young spores. He regarded this division as the heterotype division, but failed to find the succeeding homotype division, and concluded that this must be deferred until the first division of the germinating spore. He found several stages in the heterotype division, including synapsis and diakinesis, and stated that eight double chromosomes are present. JAHN compared his results with those obtained in Ceratiomyxa, (27) a member of the Exosporeae in/

in which he was able to find heterotype and homotype divisions closely following each other. The work of HELEN KRANZLIN,(31) a pupil of JAHN'S, may be considered at this point as she investigated the sexual process in Trichia and Arcyria, and found that in these genera also, the nuclear fusion occurs in the young sporangium. In the reduction division she counted eight double chromosomes. KRANZLIN described in great detail the formation of the sporangial stalk, stating that a large amount of degenerating protoplasm enters into its composition. She also examined the wall of the sporangium, and found that in Arcyria the membrane is usually more delicate than in Trichia. Her investigations of the nuclear phenomena led her to the conclusion that the division of the nuclei in the plasmodium is always karyokinetic, and she never found the direct type figured by LISTER. In regard to the formation of the elaters, KRANZLIN makes the somewhat surprising statement that they are of nuclear origin, arising from the transformation of the centrosomes into vacuoles, which elongate and form the ground work of the future elaters.(22) HARPER found the astral rays figured by KRANZLIN, but he failed to connect them with centrosomes.

In/

In JAHN'S second paper on nuclear fusion, published in 1911, he corrected the description he gave in the first, concerning the point in the life history at which the sexual process takes place. He came to the conclusion that he was wrong in stating that the fusion of nuclei occurs in the young sporangium and said that the amoebae, which arise from the swarm cells by the loss of the flagellum, act as gametes and fuse in pairs giving rise to uninucleate plasmodia. The plasmodia increase in size by the karyokinetic division of their nuclei, and also by engulfing and digesting unfused amoebae and other solid food material. They may fuse also with other plasmodia which they encounter accidentally, but this is merely a fusion of protoplasm and not of nuclei. He found divisions in the mature plasmodium, and was able to count the chromosomes, proving that the diploid number is present. He confirmed his previous statement that the heterotype and homotype divisions are separated by spore formation. He was of the opinion that in Physarum didermoides, at any rate, the swarm cell loses its flagellum at the first nuclear division, but that the amoebae produced can divide several times before they function as gametes and fuse. (39) MISS G. LISTER later/

later examined JAHN'S stained preparations and confirmed his conclusions concerning the sexual process.

In JAHN'S next publication in 1919 (29) he gave some very interesting information concerning what may be termed the senile decay of the plasmodium of Badhamia utricularis. He found that the plasmodium will not live for ever without encystment or sporangium formation. The plasmodium kept under conditions which do not favour encystment or sporangium formation, gradually becomes more vigorous until it reaches a maximum after which the vital processes slow down until about three and a half years after its birth death results. The life of the plasmodium is of the same length whether in the active or latent condition, for sclerotia kept longer than three and a half years will not germinate. He belived that encystment or sporangium formation are necessary for the 'rejuvenation' of the individual.

JAHN'S last paper published in 1928 (30) deals with a new classification of the Mycetozoa. He considered that grouping according to the colour of the spore, as adopted by LISTER, is an unsatisfactory method, and based his system upon the type of/

of germination of the spores, the form of capillitium and the origin of the stalk. He described four types of germination. In the first, the spore contents emerge as an amoeba which gradually transforms itself into a swarm cell, in the second, a fully formed swarm cell slips out of the spore, in the third, two swarm cells are present on germination, and in the fourth a swarm cell and a granule of slime are discharged from the spore. He believes that the first type is the most primitive and is present in those species which form an aethalium. He also stated that the capillitium may be absent, a pseudocapillitium may be present, or a true capillitium may be formed. The stalk may be formed by the lower portion of the sporangium collapsing into folds, or it may actually be built within the interior of the sporangium.

During the last thirty years a considerable amount of investigation has been carried out on various species of the Endosporeae by two continental workers, PINOY and SKUPIENSKI. PINOY has published a series of six papers, the first three of which deal exclusively with the important role played by bacteria in the germination of the spore and/

and development of the plasmodium [43]. [44] and [45]. These three publications, the first of which appeared in 1902 and the second and third in 1907 may be summarised as follows. The presence of bacteria of a suitable species is essential for the germination of the spores of the Mycetozoa. PINOY believed that the bacteria must have some action on the spore coat which renders its rupture possible and so permits the contents to emerge. This theory of germination differs markedly from that of JAHN [25], who believed that the rupture of the coat can be explained entirely by the process of osmosis. It also differs from that of CONSTANTINEANU [12] who stated that the chemical characters of the nutrient solution are the chief factors in causing germination. The bacteria also form the main source of food for the plasmodium. He was able to isolate a plasmodium entirely free from bacteria, and found that it soon perished unless suitable bacteria were added.

PINOY [46] next turned his attention to an interesting discovery he had made that in Didymium nigripes three types of plasmodia are present. The one most usually obtained is greyish white in colour and readily forms fructifications. Of the remaining two one is orange yellow and the other/

other violet-black. Neither of these could be induced to develop fructifications, but they always formed sclerotia. If the sclerotia from orange-yellow and violet-black plasmodia be allowed to mix, a greyish-white plasmodium results which does form sporangia. He deduced from this that sexual differentiation occurs during spore formation giving rise to + and - spores which in turn germinate giving + and - amoebae and these form + and - plasmodia. + and - spores or + and - sclerotia must be mixed before a (±) plasmodium can be formed. In PINOY'S next paper (47) in 1915 he changed his opinion that the different colours of the plasmodia denote sex and stated that the colour of the plasmodium is due to the presence of bacteria, and can vary with the species present; for instance the plasmodium of Didymium nigripes is white with Bacillus subtilis and yellow with Bacillus luteus. He also stressed again the importance of bacteria as a food supply for the growing plasmodium. In his last paper (48) PINOY summarised the work he had done, still maintaining his belief in the necessity of the presence of bacteria during the process of germination, and in the existence of three kinds of plasmodia, only one of which, being the result of fusion between + and/

and - amoebae, is able to form sporangia.

The investigations of SKUPIENSKI are embodied in a series of papers published between 1918 and 1929. In these he dealt with the life history of two species belonging to the Endosporeae Didymium nigripes and D. difforme and found that there are striking differences between them. The results of his cytological examination of the first named are given in three publications (54), (55), (57). He stated that the spore of D. nigripes germinates giving rise to an amoeba which is soon transformed into a zoospore. The zoospores divide longitudinally several times and then withdraw their flagella and become amoebae once more. The amoebae can continue to divide, the number of divisions for each mother zoospore being four or five. The amoebae next group themselves in pairs and fuse, but there are apparently + and - amoebae and a + amoeba will only fuse with a - one. The plasmodia formed as a result of the fusion can coalesce with each other and they can also engulf and digest amoebae which have not fused. By using monospore cultures he was able to prove that each spore is capable of producing both + and - amoebae. In a paper published in 1919 (56) he gave an account of the effect of the nutrient medium on the/

the development of Didymium nigripes, particularly in regard to the form of the sporangia.

SKUPIENSKI'S examination of Didymium difforme,[59] and[60] led him to the conclusion that the main differences in the life-history of this species when compared with that of D. nigripes are as follows. The first division often takes place inside the spore-coat, two protoplasmic masses emerging which soon become zoospores. He described the division of the zoospore very fully in the case of D. difforme, stating that he found centrosome-like bodies present, though he was rather inclined to consider them as differing from true centrosomes because they appear quite irregularly and do not accompany the division in every case. After several divisions each zoospore encysts for 12 hours before it becomes an amoeba. The amoebae continue to divide and finally fuse in any number to form a zygote. The zygote encysts for some time and then emerges as a plurinucleate plasmodium. Neither period of encystment is mentioned in a more recent paper dealing with the same species[61]. He found that nuclear fusion occurs in the mature plasmodium before spore-formation. Monospore cultures show that not only are there + and - amoebae but + and - spores as single/

single spore cultures never give rise to sporangia.

In his last publication (62) SKUPIENSKI described some very interesting experiments in the use of intravital stains, particularly neutral red, during the life-history of Didymium nigripes. By the use of such stains he established the fact that there are three types of vacuoles found at various stages in the life-history.

a) 'Vacuoles élémentaires' present in spores, swarm-cells, myxamoebae and plasmodia. These behave like vacuoles in the cells of the higher plants.

b) 'Vacuoles pulsatiles', present in swarm-cells and plasmodia.

c) 'Vacuoles digestives', found in the larger plasmodia.

He discovered that the stalk and head of the young sporangium differ markedly in their reaction to the various stains, and concluded that the stalk consists merely of the outer layer of protoplasm of the plasmodium, the inner portion emerging to form the head.

The investigations of SKUPIENSKI have been very adversely criticised by BUCHET and a short but spirited discussion arose between the two which has been published in the following series of papers (7), (8) and (58).

The life-history and Cytology of Reticularia Lycoperdon by WILSON and CADMAN (71) published in/

in 1928 gives a very full cytological account of the organism from the germination of the spore to the formation of the ripe spore. They found that germination takes place very rapidly giving rise to an amoeboid structure which gradually becomes a flagellate swarm-cell. During the amoeboid stage the blepharoplast originates in the nucleus and passes out to the periphery of the cell. The swarm-cell may divide several times, the division of the nucleus being preceded by a division of the blepharoplast, the two daughter blepharoplasts functioning as centrosomes. Four chromosomes are present and the division is extra-nuclear. After several divisions the swarm-cells may function as gametes and fuse in pairs, cell fusion being followed by nuclear fusion. On the conclusion of cell fusion the pair of gametes may coalesce with five or six unfused swarm-cells, the nuclei of the latter being digested after nuclear fusion of the gametes is completed. Division of the nuclei in the young plasmodia was observed, and it was found to be intra-nuclear, no centrosomes being present and the chromosomes eight in number. The young plasmodia can engulf and digest unfused swarm-cells, and they probably coalesce with each other. In the developing sporangium, considered/

considered to be a simple structure and not an aethalium composed of fused sporangia, large tracts of protoplasm with the nuclei associated degenerate forming the wall and pseudo-capillitium. Typical heterotype and homotype divisions occur, both of which are intra-nuclear, without centrosomes and with four chromosomes. The homotype division is complete before cleavage of the protoplasm to form the spores commences. The formation of the spore wall is fully described.

GILBERT, (18) and (20) has recently investigated the feeding habits of the swarm cells of several species of the Endosporeae. He found that the swarm cells, when in the amoeboid condition, are capable of ingesting solid food particles such as fungal spores of many species of the Agaricaceae. Those species of the Mycetozoa, such as Didymium nigripes, in which the swarm-cells spend a considerable period in the amoeboid condition are capable of ingestion in a marked degree, whilst in those, such as Reticularia Lycoperdon, in which the swarm cells are nearly always actively swimming, little ingestion occurs. He also described the germination of a large number of species, (19) and stated that there are two ways/

ways in which this may take place, one in which the swarm-cell escapes through a deep wedge-shaped rupture in the spore wall, and the other in which the swarm-cell emerges through a jagged aperture. In the first type he believed that germination is due entirely to osmotic action, whilst in the second softening of the spore wall at one particular spot by enzyme action also plays a part.

Still more recently CAYLEY (9) published the results of observations on living cultures of several species of the genus Didymium. She stressed particularly the importance of an adequate supply of moisture during the early stages in the life history and stated that the swarm cell stage in Didymium difforme is entirely dependent on the presence of water or a suitable nutrient liquid. On the other hand sporangial development usually takes place in the absence of water and is accompanied by loss of water from the surface of the young sporangium. In this she agreed with HARPER (23). She found that the spores of Didymium difforme are bisexual and that fusion between motile gametes takes place and so differed from SKUPIENSKI. She discussed very fully the present state of our knowledge in regard to nuclear behaviour and sex segregation in the Mycetozoa/

Mycetozoa and stated that, from her own observations the sexes are separated at the first division of the young swarm-cell, which she considered to be the second meiotic division.

METHODS/

METHODS.

I. PREPARATION of CULTURES for OBSERVATION.

Didymium nigripes is very readily grown on a culture medium in the laboratory. The material for this work was obtained from a number of beet 'seeds' which had been used to carry out a germination test. A fortnight after the 'seeds' had been placed in the germination tray it was noticed that two distinct types of plasmodia were creeping over the surface of the damp blotting paper. One type of plasmodium was pale brown in colour and formed a very coarse network; the other was pale yellow and formed a much finer network than the first. The plasmodia were kept under close observation for three to four weeks and at the end of this period the pale brown one gave rise to the small, round, stalked sporangia of Didymium nigripes var. xanthopus, whilst the yellow one formed the sessile, irregularly shaped plasmodiocarps of Didymium difforme. The two species were very readily separated in culture. Both Didymium nigripes and Didymium difforme develop equally well when grown on artificial media so that it was a matter of indifference as to which should be selected as the subject/

subject of investigation, but finally Didymium nigripes var. xanthopus was chosen.

Trial was made of a large number of artificial media in order to establish which was most favourable for the full development of the organism throughout its entire life-history. Amongst the various media employed, the more favourable were found to be :-

1. Tap water.
2. Rain water.
3. Maize decoction.
4. Hay infusion.
5. Carrot decoction.

Germination took place in all these liquids, but was most abundant in tap water, though it was quite good in rain water and in carrot decoction. For the complete investigation of the life-history from germination to spore formation, carrot decoction made up and used as described below was decidedly the most successful. The carrot solution was made up as advised by SKUPIENSKI. Fifty grammes of raw carrot were chopped up and placed in a flask with 500 cc. of tap water. The decoction was boiled gently for twenty minutes, a plug of cotton wool being placed lightly in the mouth of the flask. The solution was then cooled, filtered, made up with tap water to 500 cc. and autoclaved for twenty minutes at one atmosphere. The carrot decoction prepared as described above/

above was employed:-

1) As a solid medium on which to obtain a constant and abundant supply of sporangia for the purposes of the investigation.

2) As a liquid medium for the detailed study of the various stages in the life-history.

When the decoction was to be used as a solid medium 2½ gms. of agar-agar powder were added to each 100 cc. and autoclaved and sloped in the usual way. Before infection a few drops of sterile water were placed in the foot of the test-tube as an abundant supply of moisture is essential for the successful development of the earlier stages in the life-history. Usually 1 sporangium of Didymium nigripes was added to each test-tube. After infection the cultures were kept in the dark during their entire development until the initiation of spore formation, and they were only brought to the light at comparatively lengthy intervals for the purposes of examination. SKUPIENSKI (61) states, and the present investigations are in accord with his conclusion, that light has a deterrent effect on the development of the earlier stages in the life-history whilst it favours spore formation.

The question of a suitable moisture content was found to be very important. If the cultures became too dry during the early stages of the/

the life-history, the plasmodium very soon dried up and further development ceased until a fresh supply of liquid was added. On the other hand, if the plasmodium had reached a suitable stage in growth, spore formation might be initiated and hastened by exposing the cultures to drier conditions. By keeping the cultures in closed boxes an adequate moisture content was ensured. It was found, however, that if the cultures were kept in the boxes for any length of time after spore-formation, the spores lost their power of germination within two or three months. The most recent cultures have been brought out into the light as soon as sporangia have appeared, and placed on the bench in the laboratory, where they dry out rapidly. The power of germination is retained much more satisfactorily by spores formed under such conditions. Possibly this may be due to the fact that, if the ripe spores are kept under moist conditions, bacteria, which are always present in the cultures, readily attack and destroy them. It has also been noticed that, under very moist conditions, the ripe spores germinate, giving rise to a second, and even a third crop of sporangia. It is possible that, in some cultures the conditions might be sufficiently moist to permit germination, but/

but not to ensure the satisfactory development of the earlier stages in the life-history and so no plasmodia are formed. In such cultures, which have been frequently encountered, the sporangia are filled with empty spore-cases, and spores capable of germination are few and far between.

Successful cultures were also grown in petri-dishes on a thin layer of carrot-agar. A small amount of sterile water was added, and each culture was infected with one sporangium of Didymium nigripes. The cultures were arranged in an oblique position, in a large glass crystallising dish, in the bottom of which several layers of damp filter-paper had been placed. The whole was covered with a bell-jar to ensure that the conditions might be kept sufficiently moist to ensure germination and the satisfactory development of the earlier stages in the life-history. Sporangia are produced very rapidly in these cultures, and, as the petri-dishes dry out readily when the bell-jar is removed, the spores germinate well and retain their power of germination satisfactorily. The petri-dish cultures are very useful when photographs of plasmodia and developing sporangia are desired. They also form a convenient method of obtaining fresh crops of sporangia in a comparatively/

comparatively short time, as the life-history from germination to spore-formation is passed through within two or three weeks, whereas in test-tube cultures it takes from four to six weeks.

For the detailed investigation of the earlier stages in the life-history, it was necessary to employ liquid cultures. The carrot decoction, prepared as described above, was used, 1cc being diluted with 7 cc of sterile tap-water. For observation of the living organism, drop cultures were put up, a few spores being added to each. In order to obtain material for cytological investigation the spores were germinated in small petri-dishes. 10 cc. of the diluted carrot decoction were placed in a petri-dish whose capacity was about 20 c.c. To the liquid were added one dozen sporangia which were broken up against the sides of the dish in order to separate the spores. The liquid was then air-pumped to ensure that the spores were thoroughly wetted. The cultures thus prepared were placed in the dark and a few drops were pipetted off at frequent intervals for observation under the microscope.

II. STAINED and MOUNTED PREPARATIONS.

In order to prepare stained and mounted preparations for detailed cytological investigation two methods were made use of. In the first place that advised by BLACKMANN (5) was adopted. When by microscopical examination, it was found that one of the petri-dish cultures had reached the desired stage, 10c.c. of FLEMMING'S fixative made up according to the strong formula was added. The dish was left standing in the dark for twenty-four hours, and the fixative was then centrifuged off and water poured in to take its place. The water was changed about six times during the next twenty-four hours and the culture containing the fixed and killed swarm-cells, amoebae and plasmodia was taken up through the alcohols to Absolute Alcohol, remaining twenty-four hours in Methylated Spirit and twenty four hours in Absolute Alcohol. The centrifuge was employed between each change. After the Absolute Alcohol had been centrifuged off a 10% solution of Cedar Oil in Absolute Alcohol was added, the swarm cells, amoebae, or plasmodia well shaken up with it and the whole poured into a small petri-dish/

dish, and left in an incubator at 30 C until all the Absolute Alcohol had evaporated. The Cedar Oil and its contents were poured into a small tube, corked, and kept till required. BLACKMANN states that material thus prepared can be kept indefinitely and certainly delicate swarm cells and amoebae still retain the most minute details of their structure after several years immersion in Cedar Oil.

Mounted slides were prepared from the Cedar Oil material as follows. A well cleaned slide was treated with egg-albumen and a drop of the Cedar Oil containing the organisms was placed in the centre of the slide and spread out slightly with the rounded end of a glass rod. The slide was placed in a petri-dish to keep it free from dust and left for at least two days so that the swarm-cells, amoebae, or plasmodia might have plenty of time to settle down into the egg-albumen. The slide was next canted by raising one end on another slide and Absolute Alcohol allowed to run gently down over it from a pipette at the top. This washed off the Cedar Oil and left the organisms firmly embedded in the egg-albumen. The slide could then be stained and mounted in Canada Balsam in the usual way.

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The method just described was found to work admirably in the case of Reticularia Lycoperdon and also in the earlier stages of the life history in Didymium nigripes, but it was not satisfactory when investigating the development of the plasmodium in the latter species. Probably the plasmodia of Didymium are more delicate than those of Reticularia and so are more readily destroyed by the rather violent centrifuging to which they have to be subjected. A much simpler method, therefore, was adopted. Drop cultures, in which No.1 coverslips were used, were put up. When they had reached the desired stage of development, the coverslip was removed, the drop allowed to dry somewhat, and a drop of FLEMMING'S strong fixative added. The coverslip was then placed in a petri-dish, the foot of which had been covered with damp filter-paper, and left for twenty-four hours. At the end of that time the fixative was removed by thorough washing, six changes of water being used. Each lot of water was very carefully pipetted off before the next was added, everything being done to disturb the coverslip as little as possible. The preparation was then stained and mounted in the usual way. It was found that a large number of the organisms adhered to the coverslip/

coverslip and that quite successful preparations could be made by this comparatively simple method.

The development of the sporangium and the nuclear phenomena which precede spore formation were investigated in smear preparations or in microtome sections. Smears were made at hourly intervals as soon as the sporangia had definitely formed. This was continued until the spores were obviously ripe. The smears were prepared on egg-albumened slides, fixed with FLEMMING'S strong fixative, taken up through the alcohols to Methylated Spirit and stored in it until it was convenient to stain them, though this should not be delayed longer than is absolutely necessary. Some slides were stored five weeks in Methylated Spirit without any bad effects.

On the whole microtomed preparations were much more satisfactory for investigation than were the smears, but the latter were useful in confirming the results obtained from the microtomed preparations The smears, especially if they were prepared by placing a young sporangium on an egg-albumened slide and pressing it down gently into the albumen with a coverslip, gave very reliable information concerning the relative development of head and stalk.

Sporangia/

Sporangia to be used for microtomed preparations were fixed at hourly intervals in FLEMMING'S strong fixative. The fixative was allowed to act for twenty-four hours and the sporangia were then washed in frequent changes of water during the ensuing twenty-four hours. The material was taken up through the alcohols in the usual manner. Some of the sporangia were transferred gradually from the Absolute Alcohol to Xylol and then embedded in wax with a melting point of 48°C. Treatment with Xylol rendered the sporangia exceedingly brittle and the microtomed sections obtained were most unsatisfactory. A new method was tried with fresh sporangia and this proved so successful that it has been adopted throughout the investigation. Instead of transferring the sporangia from Absolute Alcohol to Xylol, Cedar Oil was gradually run in down the side of the tube so that it formed a layer at the foot. The material floated at the junction of the two liquids for some time, but gradually began to sink as the Absolute Alcohol in its tissues was replaced by the Cedar Oil. When the sporangia had sunk to the foot of the tubes and small bubbles had ceased to stream from them, the mixture of Absolute Alcohol/

Alcohol and Cedar Oil was decanted off and replaced with pure Cedar Oil. The material was allowed to remain in the Cedar Oil until it was quite transparent, usually after two days, the oil was then poured off and Xylol added in its place. This transference was effected because it was found that Cedar Oil is very difficult to replace with wax and the substitution of Xylol for the oil resulted in much more efficient embedding. Wax was gradually added until the mixture of Xylol and Cedar Oil was about 50/50, the tube was placed in an incubator about 30 C until all the Xylol had been driven off, and the material was then transferred to pure wax of 48 C melting point and embedded in the usual way.

Trial was made of a number of stains, but it was found that the most satisfactory results were obtained with HEIDENHAIN'S iron haematoxylin and FLEMMING'S triple (orange G, safranin and gentian violet) stain. Iron haematoxylin stained such minute structures as blepharoplasts and chromosomes very brilliantly indeed, but it was considered that the triple stain was the more reliable and preparations stained with it were invaluable in confirming results obtained with iron haematoxylin.

The/

The triple stain was decidely more satisfactory for staining smear preparations. Iron haematoxylin stained such dense preparations much too deeply and it was difficult to interpret the results obtained. Even the triple stain was often too deep to be satisfactory and in a few cases a more transparent stain such as picro-carmine was used with some success. Iron haematoxylin was always used to stain the coverslip preparations, as the triple stain process is so lengthy that many of the organisms become detached before it can be completed.

Considerable difficulty was experienced in arriving at the appropriate times to leave the slides in each stain. It is well known that material after fixation in a solution containing Chromic Acid is difficult to stain, and this seems to be intensified in the case of the Mycetozoa. It was found that preparations had to be left for lengthy periods in several of the stains in order to obtain satisfactory results. As a guide the following times may be suggested.

(1) In FLEMMING'S triple stain.

Safranin	2 days
Gentian Violet	12 hours
Orange G	1 minute
Clove Oil	10 minutes.

(2) In iron haematoxylin and orange G.

Iron Alum	1 hour
Haematoxylin	2 hours
Iron Alum	3 minutes
Orange G	1 minute.

3) Picro carmine was used as a fixative and stain combined and was allowed to act for ten minutes.

Special acid free balsam made up with benzol instead of Xylol was used in mounting the preparations to ensure that the stains should not fade.

All the measurements were made with a Leitz micrometer screw eyepiece.

THE/

THE LIFE HISTORY OF DIDYMIUM NIGRIPES.

I. CARROT-AGAR CULTURES.

Efforts were made to obtain mono-spore cultures, but up to the present these have not been successful. The difficulty encountered in the case of Reticularia Lycoperdon (71,p.585) was again experienced. It was found, in the case of Reticularia that none of the single spores isolated were capable of germination, and, though germination did occur when three spores were present the resulting swarm cells never developed flagella, soon rounded off, and were finally swamped and destroyed by bacteria. A very simple method has recently been described by CALEY (9,p.236) by means of which she was enabled to obtain mono-spore cultures of several species of the genus Didymium. The author intends to repeat her endeavours to isolate and germinate single spores using the method and apparatus employed by CALEY.

Several methods of obtaining cultures of Didymium free from bacteria were investigated but none of them gave satisfactory results. Sterilizing the spores in ·01% Mercuric Chloride was tried, but though/

though the solution was only just strong enough to kill the bacteria, germination of the spores was prevented. The latest method suggested by SKUPIENSKI (61) still remains to be tested. He kept cultures of Didymium nigripes four years and found, at the end of that period, that the spores still germinated readily while all the bacteria had perished, for sub-cultures were quite free from contamination. The oldest cultures the author has at present are three years old and these are still heavily infected and the percentage germination of the spores is low. It was found that the most successful cultures were obtained by sub-culturing until very few, if any, bacteria were present.

In carrot-agar slopes the first sign of growth visible to the unaided eye is the appearance, five or six days after germination, of a large plasmodium which creeps away from the moisture at the base of the test-tube on to the solid agar medium The plasmodium very rapidly increases in size as it creeps over the substratum, and continues to grow until it covers the entire surface of the medium. It is creamy-yellow in colour and of a creamy consistency, but the colour is not constant and varies with the medium used. Several cultures were grown/

grown on pieces of sterile carrot, and it was found that when the plasmodium crept off the medium on to the glass of the test-tube it was of exactly the same colour as the piece of carrot. Observation under the microscope showed that the acquired colour was due to the presence of numerous carrot cells in the streaming protoplasm of the plasmodium. Growth continues, if conditions are favourable, for two or three weeks, and then the plasmodium often creeps on to the sides of the test-tube or up to the thinner and drier portions of the agar slope. This creeping continues for one or two weeks, the plasmodium passing on to the glass of the test-tube and back on to the surface of the agar several times, and also back and forward from the thick, moister portion of the slope to the thin, drier region. During this period of growth the plasmodium has the power of dissolving and making use of the carrot-agar as food, for as it creeps it leaves deep channels cut into the surface of the medium. This may possibly be due to the presence of an enzyme in the plasmodium, which has the power of acting on the carrot-agar, converting it into a liquid which can then be absorbed.

About four or five weeks after germination the plasmodium prepares for spore formation. The exact/

exact time at which sporangium development is initiated is quite indefinite and varies from between four to six weeks after germination. In petri dish carrot-agar cultures, as stated before, this period is considerably shortened, ripe spores being obtained in a fortnight.

The plasmodium may pass through the initial stages in its preparation for spore formation within twenty-four hours, or these may be spread over two or three days. The first indication is that the feathery appearance of the plasmodium gradually disappears, all the protoplasm being withdrawn into the conspicuous veins present, thus rendering them even more distinct. As the protoplasm is withdrawn it leaves behind it trails of debris which are often accompanied by the deeply hollowed furrows in the surface of the medium mentioned above. As will be seen later, microscopical investigation shows that the protoplasm of the plasmodium is divided into an outer, non-motile layer, and an inner portion, in which the protoplasm is in active motion. Within the outer layer the inner portion creeps along to the tips of the veins from whence it emerges as a number of distinct, rounded masses, each of which eventually gives rise to a sporangium.

The/

The rounded masses gradually become more exactly spherical in shape, and paler in colour until they are almost white, the change in colour probably being due to the fact that, as the protoplasm is aggregated to form the young sporangia, it leaves behind it any impurities. As the sporangial heads become more spherical they rise up from the surface of the medium, leaving behind them distinct stalks which, from the first, appear to be very dark brown or black in colour, though in rare cases, perhaps owing to a certain type of bacillus present, they are distinctly red. The stalks, which are from 1 mm. to 1.5 mm. in height, are apparently built up comparatively rapidly behind the young sporangium as it rises from the surface of the nutrient medium. The spherical heads are, at first, quite soft and creamy in consistency and are about .8 mm. in diameter, but very soon a definite skin forms over the surface, and the sporangium as a whole commences to darken, becoming pale pink then reddish-brown, dark-brown, and finally almost black in colour. The black colour denotes that the spores are quite ripe. During the formation of the spores the sporangium gradually becomes smaller until it is only .7 mm. in diameter. (PL. XIX FIGS. 90 and 91)

As/

As the sporangia darken a white powder of crystals of calcium carbonate forms over the surface. This powdery coating varies very much on the different sporangia. It is much denser on those formed in the upper portion of the test-tube where the conditions are dry, than on those in the lower portion where conditions are much moister; in fact, when conditions are very moist, the sporangia may remain quite black, little or no deposit of calcium carbonate being formed. On examination under the microscope the crystals are found to be of the stellate type, which is the characteristic feature of the Didymiaceae. The sporangia with a dense coating of lime are distinctly smaller in diameter, about ·5 mm., than those with a scanty deposit which are usually ·75 mm. in diameter.

The following figures give some idea of the variations in time to be expected throughout the development of the organism.

(1) TEST TUBE.

Cultures infected.	28th Nov. 1928.
Small plasmodia.	4th to 6th Dec. 1928.
Large plasmodia.	24th to 30th Dec. 1928.
Sporangia.	31st Dec. 1928 to 6th Jan. 1929.

(2)/

(2) TEST TUBE.

Cultures infected.	23rd Feb. 1929.
Small plasmodia.	2nd to 8th March 1929.
Large plasmodia	20th March to 8th April 1929.
Sporangia	12th to 19th April 1929.

These cultures were exceptionally tardy in development, and it may be that the exceedingly cold period in the spring may have been responsible, even though the culture were kept in a laboratory heated to 60°F. for most of the time.

(3) TEST TUBE.

Cultures infected	4th April 1929.
Small plasmodia	8th to 12th April 1929.
Large plasmodia	16th to 20th April 1929.
Sporangia	26th April to 2nd May 1929.

(4) IN PETRI DISH AGAR CULTURES.

Cultures infected	4th April 1929.
Small plasmodia	8th April 1929.
Large plasmodia	10th to 12th April 1929.
Sporangia	17th to 19th April 1929.

II. CARROT DECOCTION DROP CULTURES.

Carrot decoction drop cultures are a means by which the life history can be investigated in much greater detail than in test-tube cultures because the organisms can be kept under constant microscopic observation.

(a) GERMINATION.

Very soon after its immersion in carrot decoction the spore commences to swell, and within one or two hours a distinct rupture appears in the spore-coat. No further change can be detected for a further period of one or two hours. At the end of that time the contents of the spore emerge through the rupture formed. The process is not the same in every case but may take place in one or other of two ways. In some cases the contents of the spore emerge as a single amoeboid mass of protoplasm, which becomes rounded or oval and lies quiescent beside the empty case for five minutes to ten minutes. Very soon afterwards the rounded mass comences to elongate, and within half an hour to an hour gradually becomes constricted in the centre and is pinched off into two roughly triangular portions. This process of pinching off takes from one/

one quarter of an hour to half an hour; that is from the time at which the constriction is first noted, to the completion of division. Sometimes before the constriction has become obvious the two ends of the elongated mass are seen to be pointed and from each point a flagellum soon grows out. By the time the division is complete and the daughter cells have been formed, the flagella have reached a considerable length and the young swarm-cells very soon swim off actively. The times taken for the various stages in the process just described vary considerably, and the figures given are only indications of what is to be expected; they differ for every example investigated.

In the second method in which the spore contents emerge division has been completed within the spore-case resulting in the formation of two swarm-cells which pass out through the rupture in the wall one after the other, and already often possess distinct flagella. The flagella, which, on the first appearance of the cells are usually very small stumps, rapidly increase in length and the two young swarm-cells soon swim off actively. Again the times taken during the various stages vary/

vary considerably, but generally the swarm-cells swim away within half an hour of their emergence from the spore.

In the first method discussed the emergence of the spore-contents usually takes place within one to two hours after the rupture in the spore-coat is first observed.

This, as might be expected, is earlier than in the second process described, in which it is usually two to three hours after the rupture appears before the swarm-cells pass out through the fissure.

The percentage germination of the spores varies considerably in the various cultures. As stated previously it is highest in those cultures in which the sporangia had been dried comparatively rapidly after spore-formation. For this reason spores from petri dish cultures were used almost entirely in infecting the drop cultures.

(b) THE MATURE SWARM-CELL AND THE MYXAMOEBA.

The mature swarm-cell is distinctly pear-shaped and has a pulsating vacuole at the posterior end. Its food supply is derived very largely from the nutrient solution, though at times numerous pseudopodia/

pseudopodia are extruded from the posterior end, and bacteria have been seen attached to them. In cultures in which the swarm-cells are numerous bacteria are usually very few in number. This is probably due to the fact that the swarm-cells only flourish when few bacteria are present, but it is also possible that the bacteria do form a subsidiary food supply, particularly as the swarm-cell stage draws to a close.

A very striking feature of the living swarm-cell is the presence of a number of highly refractive granules in the general protoplasm. They are rendered more conspicuous because of their marked Brownian movement, which is lost on the death of the cell. The loss of movement is probably due to the fact that at death, the protoplasm becomes coagulated and the granules are embedded in the solid mass. These granules are present throughout all stages of the life of the organism but they are most conspicuous in the actively swimming swarm-cell. In the ungerminated spore they are not seen, because of the thick wall and they are obscured in the plasmodium by the presence of a large quantity of solid ingesta. They stain red/

red with Sudan III and are soluble in ether. They are identical, therefore, in their reactions with the granules observed in Reticularia, which it was decided were lipoid in nature, and the result of cell metabolism. Similar granules have been described by GUILLIERMOND in Leptomitus and other fungi and termed by him microsomes. VON WILLER also observed such granules in Amoeba proteus. Fuligo varians and Plasmodiophora brassicae and states that they are present throughout the life history of Fuligo, being very obvious in the swarm-cells and amoebae, and dividing before cell-division. VON WILLER compared them with the mitochondria of various authors and gives many references. COWDRY has described mitochondria in the plasmodia of several of the Mycetozoa, which resemble the granules observed in Didymium very closely and LEWITSKY (32) has recently published a paper on the condriosomes of the Myxomycetes. On the whole the impression was given that at no time are these granules so conspicuous and so numerous in the case of Didymium as they are in Reticularia. A full list of references on the subject is given in the paper on Reticularia.

Reticularia.

The swarm-cell swims about actively for some time, and there is considerable evidence, supported by the investigation of stained and mounted preparations, that at least one further division takes place within the next one or two hours. It is unlikely that there are many subsequent divisions of the swarm-cells as the number does not increase to any marked extent. The active swarm-cell stage usually persists for a comparatively short period, five to twenty-four hours at the most, though isolated swimming forms may be found a fortnight after germination.

In those cultures which are progressing normally, very few actively swimming swarm-cells are found twenty-four hours after germination. In many cases the flagellum has been withdrawn and the swarm-cell has become amoeboid, though a number of distinctly amoeboid swarm-cells may be seen which still possess the flagellum. In the latter the flagellum moves very slowly to and fro, and appears to have little to do with the movement of the cell. When the swarm-cell is about to withdraw its flagellum, the movements of the latter become/

become very sluggish, but the swarm-cell itself develops marked amoeboid movement constantly extruding pseudopodia from its posterior end. Gradually the typical, elongated form is lost, and the swarm-cell becomes shorter, in some cases being distinctly broader than it is long. The flagellum is now slowly withdrawn, until it has entirely disappeared and the swarm-cell moves only in an amoeboid manner. It is at this time that the swarm-cell engulfs and digests bacteria most readily; indeed bacteria have often been seen attached to the pseudopodia.

The actively swimming condition of the swarm-cell may persist for quite a long time, but its duration depends very largely on external factors, such as the presence of moisture and of an adequate supply of food in liquid form. As stated above, few actively swimming forms were found in a normal drop-culture twenty-four hours after germination. If, however, a drop of water, or a drop of fresh culture solution was added, most of the amoeboid structures extruded their flagella again, became pear-shaped and swam about actively for some hours/

hours. Twenty-four hours later the swarm-cells had become amoeboid once more, and again many of them could be restored to the flagellate condition by the addition of fresh culture solution. The number which remained amoeboid, however, appeared to be greater than on the previous day. It was found that, on each succeeding day, the number of amoeboid forms which were capable of returning to the flagellate condition diminished until about a fortnight after germination, very few actively swimming swarm-cells could be obtained.

The above observations led to the conclusion, confirmed by the stained preparations, that the amoeboid structures present in a normal culture two or three days old are of two types. They may be amoeboid swarm-cells capable of returning to the flagellate condition, or they may be permanently amoeboid. To the latter the term "myxamoeba", has been applied. As it is impossible to distinguish between amoeboid swarm-cells and myxamoebae in living cultures, except by their behaviour when fresh culture solution is added, the explanation of the term "myxamoeba" and a comparison between the two may appropriately be held over/

over until after the description of the stained and mounted preparations.

Divisions were carefully sought for in normal cultures two or three days old, but in no case have they been found. It must be made quite clear that by a normal culture is meant one which is allowed to develop without the addition of water or fresh nutrient solution, the moisture conditions being kept as constant as possible. In such cultures as stated above, very few swimming forms are present two days after germination. It appears, then, that after the first twenty-four hours few divisions take place, and it is very probable that the myxamoeba never divides. This supposition is supported by an experiment of SKUPIENSKI (61, p 312). He kept an amoeboid structure, which may have been a myxamoeba, for three months without division taking place, and finally it rounded off and disintegrated without further development.

(c) THE MICROCYSTS OF CIENKOWSKI.

Microcysts have been described and figured by DE BARY, CIENKOWSKI and other investigators, and they were searched for, by the present author, in cultures/

cultures which were not progressing satisfactorily due to lack of moisture or lack of an adequate food supply. It was observed that, in such cultures, many rounded forms are present which are peculiarly refractive in appearance, probably owing to their spherical condition. Stained and mounted preparations show that these structures possess no definite wall. They are capable of returning immediately to the actively swimming condition should fresh culture solution be added within twenty-four hours after they have rounded off, but should this addition be too long delayed they disintegrate and are devoured by the bacteria present. It was noticed that a number of amoeboid forms remained amongst the rounded structures, and it appears that it is the active swarm-cell which rounds up most rapidly. The amoeboid condition, as is well known, is adapted to drier conditions.

The rounded structures described above are very probably comparable with the microcysts described by CIENKOWSKI and later by DE BARY. The former states that they may or may not have a definite wall, and the latter examined in detail the germination of microcysts in Didymium difforme. He found that they germinate after having been dried for two/

two months and that no wall is present. The microcyst, in the case of Didymium nigripes also has no wall and cannot be compared with a true resting-stage, for it is unable to resist unfavourable conditions for any length of time. It is merely a rounding off of the swarm-cell before disintegration, which may be arrested if favourable conditions are restored with sufficient rapidity. It appears to be unnecessary, therefore, to apply a special term, such as 'Microcyst', to this stage, at least in the case of Didymium nigripes.

(d) THE FUSION OF MYXAMOEBAE AND THE FORMATION OF THE PLASMODIUM.

In cultures about three days old it was observed that, in several cases, there was a tendency for some of the amoeboid forms to group themselves in pairs; in fact, on one occasion a bridge of protoplasm was observed between the two. It was not possible to determine in living culture, whether the amoeboid structures were amoeboid swarm-cells or myxamoebae. Investigation of stained and mounted preparations proved that they were myxamoebae. The myxamoebae obviously function as gametes and fuse in pairs/

pairs, though it has not been possible to follow the entire process in any one case. Apparently fusion is initiated by the extrusion of a pseudopodium from one or more probably both of the gametes. Fusion of the pseudopodia takes place and a bridge of protoplasm is formed between the two which gradually decreases in size until the gametes have been drawn together. This is followed by fusion of the entire protoplasm of the gametes. The fusion of the nuclei which follows can only be described from stained preparations. The result of fusion is the formation of a zygote which initiates the plasmodial stage, and may be regarded as a uninucleate plasmodium. In the living condition it is difficult to distinguish between myxamoebae, amoeboid swarm-cells and uninucleate plasmodia. There is very little difference in size, and the streaming movement of the protoplasm, which is so characteristic of the older plasmodium, cannot be detected.

Four days after germination, in very favourable cultures, amoeboid structures, which are distinctly larger than the surrounding myxamoebae and swarm-cells, have been observed. They are very probably young plasmodia which contain more than one/

one nucleus. These somewhat larger plasmodia are present in greater number, and have increased in size in cultures five days old. In the largest of them the streaming movement of the protoplasm can be detected with difficulty, particularly round the edge of the structure. The larger plasmodia are readily distinguished, not only on account of their size, but also because many of them have obviously engulfed myxamoebae and swarm-cells which can be seen very clearly in the interior of large vacuoles. Some of the plasmodia have engulfed five or six myxamoebae or swarm-cells and this accounts for their comparatively rapid increase in size, in many cases their diameter being four or five times that of the myxamoeba. The process of engulfing can be followed in the living cultures. When a young plasmodium encounters a swarm-cell or an unfused myxamoeba it apparently exerts some influence which causes them to adhere more or less firmly to some part of its protoplasm. The plasmodium then extrudes a pseudopodium which gradually elongates until it surrounds the doomed organism. The latter may be observed for some time as a highly refractive body in the tissue of the plasmodium, but it slowly disappears as digestion/

digestion progresses.

A very striking appearance has been observed in cultures six to seven days old. Suddenly, within six to twelve hours, a small number of very large plasmodia, readily detected under low powers of the microscope, arises. Their protoplasm is distinctly granular in appearance, and its streaming movement is very obvious. In the stream are carried along spores, unfused myxamoebae and swarm-cells. For some time the rapid appearance of the large plasmodia could not be explained, but finally a very successful culture was obtained in which they were detected actually in the process of formation. One of the somewhat larger plasmodia seen in cultures five days old, apparently acts as a centre of attraction for the smaller, generally uninucleate plasmodia in its neighbourhood. These gather round in large numbers, and their protoplasm coalesces with that of the larger, central plasmodium. In this way a very big structure is rapidly formed. The number of large plasmodia which arise in this way, varied considerably in the different cultures observed, but usually five or six are present. During the next two or three days they creep about and/

and coalesce with each other until about fourteen days after germination usually only one very large plasmodium remains, which is visible to the naked eye. The process by which this large plasmodium is formed, and the fact that the smaller plasmodia have vanished as a result, support the statement made by several previous investigators, that the larger plasmodia are not entirely the result of nuclear division and consequent increase in the amount of their own protoplasm, but also owe their size very largely to fusion with smaller plasmodia.

In the large plasmodium, the formation of which has just been described, the streaming movement of the protoplasm so frequently observed by previous investigators, is very distinct, and may be studied readily, even under the lower powers of the microscope. The movement, which is rhythmic in nature, was timed in a number of cases, and it was found that the protoplasm flowed forwards for 1 minute 15 seconds ceased for 10 seconds and then flowed backwards for 1 minute 10 seconds. It can be very clearly seen that the protoplasm is divided into two portions, a comparatively narrow outer layer, which is quite hyaline in appearance, and an inner, granular portion, in/

in which the streaming movement is obvious. In the stream of protoplasm much solid material is carried along, consisting mainly of spore-cases, ungerminated spores, rounded swarm-cells and unfused myxamoebae. This leads to a consideration of the source from which the developing plasmodium derives its food supply. Much of its food is engulfed in the solid form and digested. Any solid material which has been engulfed and cannot be digested, such as ungerminated Didymium spores, spore-cases, and the spores of many fungi, is carried along in the stream of protoplasm, and finally extruded at the surface of the plasmodium. That food material may also be absorbed in the liquid form is shown by a consideration of the description of the test-tube cultures given above, where it was stated that the plasmodium can eat into the solid carrot-agar, and it was suggested that an enzyme acted on the carrot-agar converting it into a liquid, which could then be absorbed. The principal food supply of the young plasmodium which is only four or five times the size of the myxamoeba is certainly obtained by engulfing and digesting swarm-cells and unfused myxamoebae.

(e)/

(e) THE PRESENCE OF VACUOLES.

Vacuoles are very readily observed in the living condition; indeed they are often much more obvious in the living organism than in stained and mounted preparations. As stated by SKUPIENSKI (62), the presence of an intravital stain is most useful in an examination of the vacuoles. Methylene blue was the stain most frequently employed, but SKUPIENSKI (62, p. 207) has found that neutral red gives more striking results. The present author agrees with SKUPIENSKI that there are three types of vacuole present. Firstly, there are pulsating vacuoles. One of these is found in each actively swimming swarm-cell, amoeboid swarm-cell, and myxamoeba. They are not obvious in the plasmodia, though various authors have stated that they are present in some number, and that, by means of them, waste products are discharged. Secondly, there are vacuoles which may be compared with the "vacuoles élementaires" of SKUPIENSKI (62, p. 207). These may be present in the spore, and are found at all stages in the life-history, their number and size depending on the condition of the organism. For example, it was observed that rounded swarm-cells often/

often became very vacuolate, the number and size of the vacuoles present varying considerably. Thirdly, there are digestive vacuoles which are found in amoeboid swarm-cells and myxamoebae, but are much more obvious in the plasmodium. They are most striking in young plasmodia present in cultures five days old, which have engulfed five or six swarm-cells or myxamoebae.

SKUPIENSKI (54) has stated that the number of the vacuoles present is an aid in distinguishing between myxamoebae and uninucleate plasmodia. He found that the latter tended to have a larger number of vacuoles present than the former. The present author has found that this is not a reliable distinction. In stained and mounted preparations may be found myxamoebae with one to three vacuoles present, and plasmodia with the same number. If, however, an engulfed swarm-cell or myxamoeba is seen in one of the vacuoles, this is a definite proof that the structure under observation is a plasmodium.

(f) THE FORMATION OF THE SPORANGIUM.

The large plasmodium described above continues to creep about for some time in the drop of nutrient material and then in many cases, it rounds off/

off, or ceases to develop further, falling a prey to the bacteria present. In one culture an abortive attempt at spore formation was made, probably because the drop had dried up somewhat. The plasmodium, which was an exceptionally large one to have been formed in drop culture, gradually withdrew its pseudopodia and contracted, its protoplasm becoming much denser. The division of the protoplasm into an outer non-motile layer, an inner actively motile portion was very obvious. Gradually the inner portion became aggregated towards one end forming a rounded structure and leaving behind the outer layer as a stalk. The active protoplasm underwent no further development, and typical spores were not formed. In several cultures the plasmodium succeeded in creeping out of the drop of liquid on to the coverslip, and in such cases normal sporangia were formed, as described for the test-tube cultures.

Though drop cultures are very satisfactory for the observation of the earlier stages in the life-history, it is obvious that they are not favourable for the continued development of large plasmodia, and the initiation of spore-formation. Numerous investigators have found that plasmodia and sporangia require somewhat dry conditions for their development/

development, and this accounts for the lack of success in drop-cultures. CAYLEY (9, p.235) states that the plasmodium of Didymium nigripes must reach a certain size before spore-formation can take place, and the required dimensions are probably rarely attained in small drop-cultures.

III./

III. THE LIFE-HISTORY FOLLOWED IN STAINED AND MOUNTED PREPARATIONS.

The detailed life-history and cytological investigation of Didymium nigripes can only be followed by the careful examination of a series of stained and mounted preparations, amd a full description of the results obtained in this way will now be given.

(a) SPORE STRUCTURE, DIVISION OF SPORE CONTENTS, AND SPORE GERMINATION.

The spore of Didymium nigripes is rounded to oval in shape, and approximately 10 to 12 μ in diameter. (Pl.I. FIG.I) The spore membrane is comparatively thin, consisting of a single dark coloured layer, 2 μ in width, and not of two layers as stated in LISTER'S monograph (38,p.XV). The membrane is provided with a number of spines, 5 μ long, which are regularly arranged over the entire surface. The formation and structure of the wall will be more fully described when dealing with sporangium and spore formation, but it may be stated here that ZOPF'S (72,p.53) statement, that the membrane consists of a substance akin to cellulose, was confirmed.

The/

The iodine and sulphuric acid test was applied, and the typical violet reaction of cellulose obtained.

The protoplasm of the ripe spore is granular in appearance, the granules forming a coarse network. In the spore which has just been placed in water, the protoplasm does not reach to the wall, the spaces between forming a colourless, fairly regular layer within the spore coat. This is the condition shown in FIG 1., drawn from a preparation of unstained spores in dilute glycerine, and it may account for the fact that the spore coat has previously been described as possessing two layers, the outer deeply staining and the inner quite hyaline. In the protoplasm are embedded a few large granules which stain comparatively readily with HEIDENHAIN'S iron haematoxylin, but very poorly with FLEMMING'S triple stain. These are probably of a lipoid nature, and are similar to those seen in the motile swarm-cell in the living condition. The nucleus, which is spherical in shape and 2.5 to 3μ in diameter, is situated approximately in the centre of the spore, and stains rather deeply. The nucleolus, about 1μ in diameter, is placed in the centre of the nucleus and stains very deeply indeed with both safranin and gentian/

gentian violet. It probably contains nearly all the chromatin material of the nucleus, though a small amount may be spread along the distinct nuclear reticulum. The nucleolus, therefore, agrees with that described in Reticularia (71, p.562), and in the case of Didymium also may be regarded as a true karyosome.

From an examination of stained and mounted preparations it was found that the protoplasm of the ripe spore, after immersion in water, or culture solution gradually swells up until it reaches the wall, and the hyaline layer described above entirely disappears, FIG. 2. The protoplasm continues to swell until it has ruptured the spore coat FIG. 3, and then commences to retract again. Meantime the nucleolus starts to divide, first into two, FIG. 3. and finally into five or six fragments FIG. 4. In the figure the impression is given that one of these portions, even before it has separated from the remainder of the nucleolus, is moving towards the nuclear membrane. This granule, or one very similar, is next seen on the outer surface of the nuclear membrane FIG. 5. No stage has yet been found corresponding to that discovered and figured in Reticularia (71.Pl.I FIG.10), in which the granule is actually seen in its passage through the nuclear membrane, but/

but it probably passes from the nucleus in the same way in Didymium. At a later stage the granule obviously functions as a centrosome and it will be referred to as such in future. The centrosome next passes out from the nucleus until it may reach a point midway between the nuclear membrane and the spore wall, though, in some cases, it remains much nearer to the nucleus. (FIG. 6.) Behind it appears a cone-shaped, faintly striate structure, with its broad end situated on the nuclear membrane. This structure forms a connection between the nucleus and the centrosome, and is obviously similar in origin and function to the "Verbindungstück" of Plenge, (71, p.587) which was described fully in Reticularia and will be referred to subsequently by this name. In FIG.6. it is clearly shown that the "Verbindungstück" has a very distinct boundary, but that the striations within this boundary are faintly visible. The nuclear membrane, where it adjoins the "Verbindungstück", is obviously thinner than it is elsewhere. Usually four fragments of the nucleolus are still visible at this stage, within the nucleus.

The centrosome very soon divides and the two/

two portions move apart leaving between them a distinct thread, the centrodesmose, decribed and figured in Reticularia (71, p.566), and also in certain flagellates. In the stage shown in Pl. II FIG.7 the centrosomes have moved only a short distance apart, and the "Verbindungstück" has widened out considerably. The nuclear membrane is stained deeply, and is now quite as thick where it adjoins the "Verbindungstuck" as it is elsewhere. The spore shown in the figure is remarkable in that rupture of the spore coat has apparently just taken place, at a comparatively late stage, and contraction of the protoplasm has not yet commenced. Five nuclear fragments are figured, but, in other cases, four are found much more frequently.

The stages described above were obtained in stained and mounted preparations of spores one-and-a half hours after they had been placed in a nutrient solution. The subsequent course of the division was followed in preparations of spores which had been immersed in culture solution for three-and-a-half hours.

The centrosomes contiue to move apart, and as/

as they do so the "Verbindungstuck" divides, one portion remaining attached to each centrosome. As the centrodesmose grows in length the two portions of the "Verbindungstück" appear to be drawn apart over the surface of the nuclear membrane until they, with their accompanying centrosomes, have reached opposite sides of the nucleus, where they probably play an important part in the formation of the spindle. In FIG. 8 a stage is shown in which the centrosomes have not quite reached the opposite sides of the nucleus. In this figure is seen an indication that the centrodesmose has a share in building up the spindle, just as it had in Reticularia. It is commencing to divide along its length, and will probably give rise to a number of spindle fibres in this way. In the cell drawn in FIG. 9 the spindle is fully formed, and the centrosomes are quite opposite each other.

Meantime changes have been taking place within the nucleus itself. The fragments formed when the nucleolus broke up before the centrosome emerged do not unite again to form a single structure. That four of these fragments should remain, at least in most cases, after the centrosome has left the nucleus,

suggests/

suggests that they function directly as chromosomes. In FIG. 8, which depicts the prophase of the nuclear division, they are seen clearly, and give the impression that they have become much larger and stain more deeply; four obviously, are present. In any individuals in which more than four fragments are present, the smaller ones probably coalesce together until the chromosomes are formed. At this stage, usually, the nuclear membrane has almost disappeared and stains very faintly indeed; the chromosomes have not yet attained a position on the nuclear spindle. Prophase is probably passed through rapidly as very few examples have been seen. Metaphase, likewise, may be of short duration, for no cases of it in this first division within the spore wall have yet been found. The next stage observed, anaphase, is shown in FIG. 9. The chromosomes, at this time, are exceedingly distinct, and their number, size and shape, are clearly seen. They are oval in shape, and are arranged on a narrow, rounded spindle. The four more lightly shaded chromosomes are not in the same plane as those which are more deeply shaded. On focussing very carefully it is seen that all the chromosomes stain in exactly the same manner and the difference in/

in shading is only meant to indicate a difference in position and not in density of staining. The fact that the upper end of the spindle is distinctly curved gives one the impression that the whole cell may be curved in the same plane, and, in consequence, one may be getting a rather foreshortened view of both cell and spindle.

The chromosomes are still a considerable distance from the poles of the spindle when telophase is initiated FIG. 10. They now commence to fuse together to form a homogeneous mass, but in the figure they still retain their identity, though their outline is becoming distinctly blurred, an indication that the process of fusion is about to begin. In FIG 11, which shows a very late telophase, fusion of the chromosomes has been completed, and the two homogeneous masses which result, have already assumed the oval shape of the daughter nuclei. In this figure it is also seen that the centrosomes are both some distance from the periphery of the cell, and that the central portion of the spindle, though still distinct, is obviously narrower. One has the impression that the cell is curved, with the two ends pointing towards the observer, so that if it had been viewed at right angles to the plane in which/

which it was drawn, the shape would not have appeared to be oval, but more like the letter C. The two homogeneous structures very soon give rise to the typical daughter nuclei, with nuclear membrane, alveolar nuclear protoplasm and large, central nucleolus. In FIG. 12 is shown a stage towards the conclusion of this process. The nuclei are quite distinct, and possess an obvious nuclear membrane; the network of the nuclear protoplasm is very clear, and the nucleoli have almost assumed their typical rounded form though, in the lower one particularly, there is still very decided irregularity of outline. Remnants of the spindle can be seen faintly between the two daughter nuclei. The centrosmes have almost reached the periphery of the cell. In FIG. 13 is shown a somewhat later stage, in which the nucleoli of the daughter cells have lost their irregular outline and have attained the typical rounded form. From the position of the centrosomes, which have now reached the periphery of the cell, it appears that the cell is bent in a C shaped manner, the two ends pointing towards the observer. No traces of the spindle can be detected.

During nuclear division the size of the protoplast,/

protoplast, or mass of protoplasm within the spore wall, changes very markedly. As described previously, the contents of the spore swell considerably when placed in a suitable solution, and the spore coat is ruptured. As soon as this has been accomplished the protoplast commences to contract, and by the time nuclear division has been completed it is very much smaller than the surrounding spore case, being about 6μ in diameter as compared with 9μ, the diameter at the time of the rupture of the spore coat. Such marked contraction during division is not usual, and may be accompanied by the loss of a comparatively large amount of water. Certainly the protoplast appears to round up into a more compact sphere, and consequently becomes somewhat denser in structure. The great difference in size at the two stages is accentuated by the fact that the circumference of the spore case tends to increase. If one compares Pl.I. FIG.3. with Pl.II.FIG.12. it is seen that in the latter figure the spines on the spore coat are distinctly further apart, and this is an accurate representation of the condition in the individuals drawn. Apparently the coat of the ripe spore is in a state of tension, and when this is removed by its rupture the coat/

coat elongates along its circumference and the spines spring apart.

Nuclear division is soon followed by cell division, and this is probably an exceedingly rapid process as only two examples have been found in which the cell is actually dividing. Apparently the spherical protoplast commences to elongate, until it becomes distinctly oval in shape. This elongation may begin during the later stages of nuclear division and the protoplast may also become somewhat curved like the letter C. Indications of this are seen in Pl.II.FIG .12, and in Pl.III,FIG.13. A constriction then appears in the centre of the protoplast FIG.14. and this deepens FIG.15,until the daughter cells have been pinched apart. In FIG.16. a stage is shown in which cell division has been completed. The two daughter cells are triangular in shape, their nuclei are spherical with a large, deeply staining nucleolus, and the 'Verbindungstück', in each case,is very distinct. They may now be regarded as young swarm-cells and they usually emerge from the spore-coat in this condition. FIG.17. is a drawing, showing the final act of germination in progress. The upper swarm-cell, which is triangular in form, has just emerged, whilst/

whilst the lower one is passing through the rupture in the spore coat, and is much elongated in consequence.

The process of nuclear division within the spore, followed by cell division and spore germination, is usually as described above, but this is not always the case. There is tremendous variation in the times at which the succeeding stages take place. For instance, though in quite a number of cases the spore contents emerge four to five hours after the spores were immersed in the nutrient solution, germination may continue for at least twenty-four hours, and GILBERT (20a, p.424) found that in some cases spores of Didymium nigripes germinated a week after sowing. It is obvious from an investigation of the stained and mounted preparations, in conjunction with observation of the living cultures, that the spore contents do not always pass out from the spore coat in the same condition. Usually, as described above, two young swarm-cells emerge, which may have very short flagella already present. In quite a number of cases, however, the spore contents slip out through the rupture before cell division takes place; indeed in one case FIG.18. the protoplast has emerged during the later stages of nuclear division. The cell figured/

figured is amoeboid in shape and the nuclear spindle is distinctly curved, probably an indication that it was laid down within the spore coat. The nuclear division is in the condition of early anaphase, and the chromosomes, four in number, are clearly shown. This is an unusually early stage for the emergence of the protoplast. In Pl.IV. FIG.I9. is shown a more usual condition. The protoplast has just emerged, and it is seen that nuclear division has been completed, whilst cell division has not yet commenced. Though the centrosomes are shown sone distance from the edge of the cell they have probably reached the periphery, as both of them are at a higher focus than the main body of the two nuclei. If cell division is to occur between the two nuclei, resulting in the formation of two swarm-cells, the nuclei would appear to be very unusually placed relative to the 'Verbindungstücken'. As stated above, the blepharoplasts are not in focus in the same plane as the nucleus, and this may account, in some measure, for the strange appearance. The protoplast has just passed through the rupture in the spore coat, and may have been somewhat distorted in the process. As the protoplast is a very amoeboid structure it does not appear to be a matter of great consequence what/

what position the nuclei have assumed at the stage shown. Four vacuoles are present, which will probably fuse in pairs to form the pulsating vacuoles of the daughter swarm-cells. In FIG. 20. the cell drawn has almost emerged from the spore coat. Nuclear division has been completed, but the daughter nuclei are still in process of reconstruction. Beaking has taken place at each end of the cell, indicating the typical swarm-cell shape which will be assumed by the daughter cells. The cell shown in FIG. 21. is lying quite close to the spore coat from which it has just emerged. The exact condition of the protoplast when it passed through the rupture in the coat cannot be determined, but at the moment when it was killed and fixed cell division was about to begin. A construction has appeared round the centre and the two ends have become beaked. Short flagella have commenced to grow from the blepharoplasts. Several vacuoles are present, and these will ultimately disappear, or two of them may remain to form the pulsating vacuoles of the daughter swarm-cells. The flagellum of the young swarm-cell grows rapidly, and it soon attains the adult form.

(b)//

(b) THE MATURE SWARM-CELL.

The mature swarm-cell Fig. 22 is pear-shaped, tapering from a breadth of 4 to 5 μ at its broadest portion towards the posterior end, to a point at the anterior end. It is 12 to 16 μ in length and possesses a flagellum which is from two to three times the length of the cell itself. The flagellum arises from the blepharoplast, a body less than ·5 μ in diameter, which occupies the pointed anterior end of the swarm-cell and stains deeply with Flemming's triple stain and also with iron haematoxylin. Situated a little way behind the blepharoplast is the nucleus; it is usually spherical in form, but may be slightly elongated in the direction of the long axis of the cell. The nucleus is approximately 2·5 to 3·5 μ in diameter, and at this point the swarm-cell is very little more, so that there is only an exceedingly narrow layer of protoplasm between the cell wall and the nuclear membrane. The nucleus, with its central nucleolus which is 1 μ in diameter, is exactly similar in structure and in staining properties to that found in the spore before germination. The 'Verbindungstück', previously mentioned, connects the blepharoplast with the nucleus, and/

and shows up very clearly in the mature swarm-cell. The 'Verbindungstück' is cone-shaped, with a definite limiting boundary, and its internal structure has a striate appearance, giving the impression that it consists of a number of delicate fibrillae which radiate out from the blepharoplast and terminate on the nuclear membrane. The nature of the fibrillae is discussed in the paper on Reticularia, 71, p. 564. The rhizoplast, which is a more deeply staining fibril connecting the blepharoplast with a more or less obvious granule on the nuclear membrane, has not been seen very clearly in Didymium. This structure was described and figured in Reticularia, in which species, on account of the larger proportion of actively swimming swarm-cells present in a culture at the same time, there is a much more favourable opportunity of observing it.

The general protoplasm of the swarm-cell is distinctly alveolar in structure. It is less deeply staining than is that in the ungerminated spore, probably because the swarm-cell is elongated, and hence its protoplasm is more diffused than is that of the rounded spore. The lipoid granules observed in the living swarm-cell are not so obvious in the stained/

stained condition in Didymium, as they were in Reticularia, even with iron haematoxylin; indeed, in most cases, the stain has been entirely removed from them by washing in iron alum. FIG. 23 is a drawing of a swarm-cell in which the lipoid granules show comparatively clearly, but even in this example they are very lightly stained. In FIGS. 22 and 23 the large pulsating vacuole present at the posterior end of each swarm-cell is clearly shown.

The swarm-cell of Didymium, as stated in the description of the living cultures, spends a comparatively short period in the swimming condition, and very soon becomes amoeboid, FIG.24. It will be seen from this figure that the swarm-cell possesses seven vacuoles. This is quite a usual occurrence, for in the amoeboid condition, more than one vacuole is frequently present, though, in many cases, the single pulsating vacuole alone may be observed. The flagellum has been withdrawn to some extent, for it is only about one-third the length of the swarm-cell. As stated in the description of the living cultures, the flagellum may be entirely withdrawn and extruded once more, should fresh culture solution be added. The amoeboid condition of the swarm-cell is a much more/

more definite feature in Didymium than it was in Recticularia. Indeed, under normal conditions, the swarm-cell probably spends a much longer period in the amoeboid condition than it does in the actively swimming state. Two or three days after germination the swarm-cell enters upon the series of changes which result in its transformation into a myxamoeba.

(c) DIVISIONS OF THE SWARM-CELL AFTER GERMINATION.

The swarm-cell probably divides three or four times during the first twenty-four hours after germination. No divisions of the swarm-cell have been observed in stained and mounted preparations of cultures more than twenty-four hours old; in fact the stages in division now to be described were obtained from preparations of cultures three-and-a-half hours after germination. In some cases the second division may follow very rapidly after the first, indeed Pl.V.FIG.25 shows that it may actually take place within the spore coat itself. In the figure four young swarm-cells are seen, their exceptionally small size suggesting that there have been two rapidly succeeding divisions. Three of the swarm-cells have attained the typical elongated form, whilst the fourth/

fourth is still rounded. The blepharoplast in each case is quite distinct, but the flagella have not yet commenced to grow out. GILBERT (19, p. 350.) states that in several species of the Calcarineae, the sub-order of the Mycetozoa to which Didymium belongs, the spore may give rise to from one to four swarm-cells. He does not actually describe any species of the genus Didymium, but obviously Didymium nigripes falls into line with the members of the genera he mentions. The present author has never seen four swarm-cells emerging from one spore case, in the living condition, but the stage shown in FIG.25. proves conclusively that this may occasionally take place.

The division of the swarm-cell is preceded by the division of the blepharoplast and of the nucleus. In the case of the second division the blepharoplast not infrequently divides before a flagellum has grown out from it and therefore, strictly speaking, before it has functioned as a blepharoplast at all. The cell shown in FIG.26. was obviously the second to emerge from the spore coat, and the daughter centrosome of the first division has divided again even before it has reached the periphery of the cell. The centrodesmose is seen as an indistinct line between the two portions of the centrosome. The 'Verbindungstück/

'Verbindungstück' also has divided. It is almost certain that the cell under consideration is a daughter-cell, and not the original, undivided protoplast of the spore. If it had been the latter the nucleolus would have consisted of at least four fragments, and not of one large, deeply staining body. The cell is probably too large to have resulted from a second division within the spore coat. It is practically certain, therefore, that we are dealing here with a very early stage in the second division of the swarm-cell. The next stage obtained is shown in FIG. 27, and it is obviously a late prophase. Remnants of the nuclear cytoplasm are still present, and the chromosomes, which are four in number, have not yet reached their position on the fully formed spindle. The cell is distinctly amoeboid in shape. FIG. 28. is a drawing of metaphase, in which the four chromosomes have attained their position on the nuclear spindle, but have not yet commenced to divide. In the next figure FIG.29. is shown anaphase. The chromosomes have divided and the daughter chromosomes have moved some distance towards the poles of the spindle. The cell has become rather elongated, and a constriction is commencing to form round the centre.

It/

It is interesting to compare FIG.29. with Pl.II. FIG.9, which depicts a similar stage in the first division within the spore coat, and one is impressed immediately with the great difference in the type of spindle and of chromosome. In the first division the spindle is narrow, 2μ in width, and probably rounded, as indicated by the arrangement of the chromosomes in a circle, whilst the chromosomes themselves are distinctly oval in shape. In the second division, outside the spore coat, the spindle is somewhat broader, 3μ in width and the equator forms a very flattened oval, for the chromosomes, which are spherical in shape, are arranged approximately in a straight line. These obvious distinctions add weight to the view that one is dealing with two separate divisions, and not alternative stages in one and the same division. There is usually no danger of confusing the corresponding stages in the two nuclear divisions, for the first nuclear division is almost always completed within the spore coat and it is only cell division that occasionally takes place outside. In the second division nuclear and cell division generally occur outside the spore coat, and only very rarely/

rarely within it. In the exceptional case shown in Pl.III.FIG. 18. which is considered to be a stage in the first nuclear division, the narrow width of the spindle, and its bent shape support this view. The shape of the cell, also, is not typical of that found at a corresponding stage in the second nuclear division. At late anaphase of the second division, the cell would probably have become elongated, and very likely have shown some indication of a constriction round the centre. In FIG.18. the cell is distinctly amoeboid and shows no signs of becoming elongated.

Telophase of the second division very soon succeeds anaphase. In the stage shown in Pl.V.FIG.30 the daughter nuclei are about to be reconstructed, but the chromosomes are still distinct. It is obvious that the ends of the spindle will remain as the 'Verbindungstücken' of the daughter cells, and that the centrosomes, from which the flagella have commenced to grow, have assumed the function of blepharoplasts. A deep constriction has appeared in the centre of the cell, between the daughter nuclei. The stage shown in FIG.30 can be readily distinguished from the stage in the first division illustrated in/

in Pl. IV. FIG.21. In the first division, cell division and the growth of the flagella do not commence until nuclear division is completed and the daughter nuclei have been reconstructed. In FIG.30. the flagella have commenced to grow whilst the nuclear division is still in the condition of telophase No later stages in the process have been obtained as yet, but the daughter nuclei apparently complete their reconstruction and, simultaneously, the constriction between the two young cells deepens until, finally, they are separated from each other. The flagella shown in FIG.30 are very short stumps, but they will increase rapidly in length whilst the daughter swarm-cells are elongating and assuming the typical pear like shape of the mature swarm-cell.

(d) THE MYXAMOEBA.

A myxamoeba cannot be distinguished from an amoeboid swarm-cell in living culture, unless the flagellum of the latter is visible. That the flagellum is not always present is readily observed from an examination of stained and mounted preparations. Before going any further it will be advisable to make quite clear what the present author means by an amoeboid/

amoeboid swarm-cell and a myxamoeba. The amoeboid swarm-cell is one which has ceased to swim actively by means of its flagellum, and frequently extrudes pseudopodia from any part of its surface. The flagellum may sometimes be seen moving slowly to and fro. In many cases it is absent but may grow out again should the cultural conditions be changed. In a swarm-cell which has withdrawn its flagellum the 'Verbindungstück' and blepharoplast are still present, and can be seen in stained and mounted preparations, Pl.VI. FIG. 31. Such a cell is capable of extruding its flagellum again and will still be regarded as an amoeboid swarm-cell. In the myxamoeba, on the other hand, flagellum, blepharoplast and 'Verbindungstück' have disappeared, and the cell possesses a nucleus, approximately centrally placed, which has no connection with the periphery. (Pl.VII. FIG 39. and 41.) The myxamoeba, as far as can be seen, is incapable of producing a flagellum once more, and so is essentially different from the amoeboid swarm-cell.

The transformation of the swarm-cell into a myxamoeba is a very definite process, and much light is thrown on it by the investigation of stained and/

and mounted preparations. In the initial stages the swarm-cell probably becomes much shorter and rounder and the flagellum is gradually withdrawn. Such a condition is seen in Pl.VI. FIG. 32. The flagellum is quite short, and the nucleus is beaked slightly towards the blepharoplast. The swarm-cell finally becomes quite spherical and the flagellum, now a mere stump, is present at one point on its circumference, FIG. 33. The flagellum disappears, and in FIG. 34. is seen the next stage, a rounded cell with a very definite blepharoplast on the circumference connected with the nucleus by the 'Verbindungstück'. The blepharoplast soon leaves the periphery of the cell and gradually passes in towards the nucleus. In FIG. 35. it is seen just within the cell, in FIG. 36. it is about halfway towards the nucleus and in Pl. VII. FIG. 37 it has reached the nuclear membrane. The subsequent fate of the blepharoplast cannot be followed with certainty. It is last seen clearly as a small granule on the nuclear membrane, and one cannot be sure whether it actually enters the nucleus or disintegrates and is absorbed on its surface. A certain amount of evidence has been obtained in support of the latter hypothesis. One example has been found, FIG./

FIG.38, in which the blepharoplast has apparently disappeared, but a very slight thickening appears at one point on the nuclear membrane. This might possibly be a remnant of the blepharoplast before it finally disappears. The blepharoplast is absent in the myxamoeba, FIG.39, and does not appear again until it arises in the nucleus before the first division of the protoplast within the spore coat.

With the withdrawal and absorption of the blepharoplast is correlated the disappearance of the 'Verbindungstück'. This structure appeared in a rather obscure manner behind the centrosome of the first division within the spore coat as it passed outwards from the nucleus, and it disappears again as mysteriously during the passage of the blepharoplast towards the nuclear membrane.

A point of great interest is the beaking of the nucleus towards the blepharoplast which is clearly seen in Pl.VI. FIGS. 34 and 35. This was observed and figured on several occasions during the life history of Reticularia (71) and is always associated with some period of great activity in the life of the blepharoplast.

The series of changes which mark the transformation of the swarm-cell into a myxamoeba have been/

been illustrated by drawings of rather rounded cells. The cell, however, may become very amoeboid at any stage, and parallel series of sketches might be made in which the cells are distinctly irregular in shape. FIG. 31 might very possibly be a stage comparable with FIG. 34, the only difference being that in the ~~latter~~ former the cell shown is very amoeboid. The cells figured in both these cases would probably have been capable of growing their flagella again had cultural conditions been altered. In Pl. VII. FIG. 40, is shown a stage which can be compared with Pl.VI. FIG. 36, in which the blepharoplast is at a point halfway between the periphery of the cell and the nuclear membrane, the cell in Pl. VII. FIG. 40. again being very amoeboid. It will be observed that one vacuole is present, which is probably the pulsating vacuole seen in the living condition. FIG. 41. is a drawing of a myxamoeba which is much more definitely amoeboid than is that shown in FIG. 39. In the myxamoeba figured in FIG. 41. three vacuoles are present, one of which contains a small flagellate in process of digestion.

The structure of the definite myxamoeba, as illustrated in FIG. 41. may now be described in detail/

detail. The shape of course, varies considerably with the activity of the amoeboid movement of the cell, but the myxamoeba is usually longer, about 11 μ than it is broad, approximately 8 μ. The protoplasm is quite similar in structure to that found in the swarm-cell, and embedded in it is a small number of the lipoid granules already described. The nucleus, 2.5 μ in diameter, is centrally placed, and the nucleolus, 1 μ in diameter, is large and deeply staining. A single pulsating vacuole is present, and several non-pulsating vacuoles appear from time to time. In the latter bacteria and flagellates are occasionally seen in process of digestion. The presence of solid ingesta in the vacuoles is so rarely observed that probably the myxamoeba, like the actively motile swarm-cell, derives a considerable portion of its food supply from the surrounding nutrient solution.

No divisions of the myxamoeba have been found in stained and mounted preparations, and, as suggested in the description of the living cultures, it is very probable that none take place. This absence of division may be correlated with the fact that the blepharoplast disappears during the transformation of the swarm-cell into the myxamoeba.

The/

The myxamoeboid stage can probably persist for a very lengthy period, as stated in the description of the living cultures, but normally the myxamoebae function almost immediately as gametes. Stages in the transformation of the swarm-cell into the myxamoeba are found in greatest abundance in preparations from cultures three or four days old, and it is in the same cultures that stages in the fusion of the myxamoebae are observed most commonly. This indicates that the myxamoeba is able to function as a gamete almost as soon as it has been formed.

(e) FUSION OF MYXAMOEBAE.

Indications that the myxamoebae fuse in pairs were obtained in the living cultures, but an examination of the stained and mounted preparations proves definitely that this is the case, and a series of stages in the process has been figured. Two myxamoebae about to fuse approach each other, and a pseudopodium is extruded from each of them. The two pseudopodia fuse at their tips, thus forming a very narrow bridge of protoplasm between the myxamoebae, Pl. VII. FIG. 42. This bridge gradually contracts, Pl. VIII. FIGS. 43 and 44, until it becomes very much/

much broader and shorter, FIG. 45. In the cell to the left shown in FIG. 45. a vacuole is present containing a very small inclusion, probably a bacterium. During these early stages in fusion both the gametes are distinctly amoeboid in shape. In FIG. 46. cell fusion has gone a step further and the bridge between the gametes has almost disappeared, though there is still a slight constriction present. Both the gametes appear to be unusually large, but this may be because their protoplasm is not so dense as usual, so they may be spread out more than is normally the case. In the upper gamete a large vacuole is present. FIG. 47 shows a stage in which the constriction has disappeared entirely and cell fusion has been completed. Again a single large vacuole is present, and this time it is situated between the two nuclei, suggesting that it may have arisen from the fusion of the two pulsating vacuoles belonging to the fusing gametes. The cell figured is oval in shape.

Cell fusion is rapidly followed by nuclear fusion. In FIG. 46 the nuclei of the gametes are quite far apart, in FIG. 47 they are approaching each other, and in FIG. 48 they are close to each other.

The/

The cell in the last figure possesses four vacuoles. No stage was obtained in which the nuclei were actually touching each other, before the nuclear membranes commenced to break down. In Pl.IX. FIG. 49 the membranes between the two nuclei have disappeared and the nuclei have fused together, though there is still a constriction between them. The two nucleoli are quite distinct in FIG. 49, but they very soon approach and fuse together. In FIG. 50 they are seen in the act of fusion. In FIG. 51 the fusion of the nucleoli has been completed and the young zygote is formed. The fusion cell is frequently very amoeboid in shape, particularly as the nuclei approach the conclusion of the sexual process, and this condition is well illustrated in FIG. 50. The number of vacuoles present varies considerably during the process of fusion, for in Pl. VIII, FIG. 47 one vacuole is present, in FIG. 48 there are four, in Pl. IX. FIGS. 49 and 50 there are none, and in FIG. 51 there are two small ones and one large one. On the whole the number of vacuoles tends to increase as the zygote condition is attained.

(f) THE YOUNG PLASMODIUM AND ITS GROWTH BY NUCLEAR DIVISION AND COALESCENCE.

The zygote, the result of the sexual pro-

cess/

process, is called the plasmodium by all investigators of the Mycetozoa, and this term will be applied to it in future. Before passing on to a consideration of the means by which the plasmodium increases in size, it is necessary to give a detailed description of the young, uninucleate plasmodium, before it commences to engulf myxamoebae and swarm-cells, and to compare it with the myxamoeba. The plasmodium, FIG. 51, is amoeboid in shape, but usually rather longer 14μ than it is broad 8μ. It is, therefore, somewhat larger than the myxamoeba, but the difference in size is generally too slight to be distinguished in living culture. The general protoplasm of the plasmodium is similar in structure to that of the myxamoeba, but towards the centre it stains rather more deeply, which may be accounted for by the larger size of the plasmodium. On the whole, though this is not a reliable feature, the number of vacuoles present in the plasmodium is greater than that in the myxamoeba. In the former there are three to five, and in the latter usually one to three. The nucleus of the plasmodium is approximately central, and is from 3 to 3·5μ in diameter, distinctly larger than that of the myxamoeba. The nucleoplasm appears to/

to stain much more homogeneously, which may be due to the fact that the fusion nucleus is not yet completely reconstructed after the sexual process. There is a very obvious clear area surrounding the nucleus, and this is a further distinguishing feature between the plasmodium and the myxamoeba, as it has never been seen in the latter. The nucleolus is 1.5 μ in diameter, and so is bigger than is that in the myxamoeba. The large size of the nucleus and nucleolus in the plasmodium is, of course, due to the fact that they are the result of the fusion of two nuclei and two nucleoli, but it may also be accounted for to some extent by the considerable activity of the nucleus at this time, for it is in process of reconstruction after fusion. To sum up the main points which distinguish the young uninucleate plasmodium or zygote from the myxamoeba we have:-

1) the larger size,
2) the larger nucleus and nucleolus,
3) the homogeneous nucleoplasm, and
4) the clear area of protoplasm round the nucleus.

The distinctions just enumerated only hold for a comparatively short period just after the formation/

formation of the zygote. In many cases the young, uninucleate plasmodium very soon commences to engulf and digest myxamoebae and amoeboid swarm-cells, but a large number, apparently, do not do so, and their fate will be described a little later. It is sufficient to state here that in zygotes which do not contain inclusions the fusion nucleus gradually decreases in size until it is apparently of the same diameter as that in the myxamoeba, the nucleolus becomes smaller and the clear area round the nucleus disappears so that at this stage it is impossible to distinguish between the two.

From an investigation of living cultures, combined with an examination of stained and mounted preparations, it is quite clear that the young, uninucleate plasmodium may become multinucleate in two ways. Its own nucleus may divide or it may coalesce with other plasmodia, the nuclei of each one remaining distinct. Division of the nucleus in the plasmodium is apparently of somewhat rare occurrence, and comparatively few examples have been found. No stage in the division of the uninucleate plasmodium has been discovered and the first illustrated FIG 52 shows one with three nuclei, two of them being in process of division whilst the third is in the resting/

resting condition. Both divisions are at the stage of metaphase, a direct view of the equatorial plate being obtained in one case and a slightly oblique view in the other. Eight chromosomes are present, and the spindle gives the impression of being intranuclear, though little trace of the nuclear membrane can be seen. Centrosomes are absent, so that this division agrees completely with that found in the plasmodium of Reticularia (71,p.575). The fact that two of the nuclei are dividing whilst the third is in the resting condition may possibly be explained as follows. The plasmodium under consideration was formed by the fusion of a binucleate with a uninucleate plasmodium. The two nuclei of the binucleate plasmodium probably arose by the division of the nucleus of the zygote, so that both daughter nuclei would tend to divide simultaneously whilst the nucleus derived from the uninucleate plasmodium might divide quite independently. In FIG 53 is shown a seven-nucleate plasmodium, five of the nuclei being in process of division whilst the other two are in the resting condition. One of the dividing nuclei was not drawn because it is situated directly above the large inclusion, which is probably a myxamoeba. Three of the dividing nuclei are in the condition of metaphase with the eight chromosomes arranged/

arranged on the equator roughly in a circle. The other two are in anaphase, a rather oblique view being obtained in each case. The divisions again show signs of being intranuclear, have no centrosomes, and the spindle is about 3 μ in width. This plasmodium possibly arose as a result of the fusion of three smaller plasmodia, two of them being binucleate and the third tri-nucleate. The nuclei of one of the binucleate plasmodia are in the resting condition, those of the other are at anaphase and those of the tri-nucleate one are at metaphase.

Both cases of division described above were found in rather isolated portions of the preparation where the plasmodia had not been able to flourish so well as those in a more central position. Two examples of division were obtained, however, in plasmodia situated towards the centre of the drop-culture, and, obviously, in a much healthier condition. In the first of these the plasmodium contained about twelve nuclei, one of which was dividing and in the condition of anaphase. In the second the plasmodium was an exceptionally large one to be present in a six days old culture. It contained numerous nuclei and inclusions and was altogether in an exceedingly flourishing state. Most of the nuclei were in the resting condition/

condition, but in one portion of the plasmodium a number of them were dividing. Pl.X. 54. is a drawing of this part of the plasmodium, and it will be noticed at once that the nuclei are at very different stages in the division process. At (a) is seen a nucleus in which the chromatin is arranged on a spiral thread and is apparently separating out to form the chromosomes. The nuclear membrane is not very distinct. At (b) is an equatorial plate view of metaphase in which the eight chromosomes may be readily counted. The nuclear membrane is clearly visible and this definitely proves that the division is intranuclear. The nucleus at (c) is at very early anaphase. The chromosomes have divided and moved a short distance apart. In (d), which is a later anaphase, the daughter chromosomes have moved further apart and are arranged more or less in two circles. At (e) is probably an early telophase. The chromosomes are still quite distinct but there is a suggestion that they are beginning to fuse to form the daughter nuclei. At (f) are shown two nuclei in the resting condition, and at (g) is a vacuole containing a myxamoeba in an advanced stage of digestion. In such a large plasmodium it is naturally impossible

to/

to explain the origin of the nuclei, but it might be suggested that the dividing nuclei were present originally in one comparatively small plasmodium which fused with the large one. The fact that so many stages in division are present is rather puzzling, but perhaps the size of the large plasmodium exerts some influence which upsets the equilibrium and causes the divisions to take place in progressive waves instead of simultaneously. The presence of waves of division in mature plasmodia has been described by LISTER (38.p.XXII) and a figure is given of dividing nuclei in the plasmodium of Badhamia utricularis. The above examples of division were all found in cultures six days old. No divisions have been found as yet, in plasmodia more than a week old.

Evidence has been obtained from investigation of the stained and mounted preparations and of living cultures that coalescence with smaller plasmodia plays a much greater part in the growth of the young plasmodium than does nuclear division. As described in the living cultures, about six days after germination, the larger plasmodia present appear to exert some attractive influence on the smaller ones in their neighbourhood. These cluster round the large plasmodium and many of them coalesce with it.

An/

An example of the process is seen in FIG. 55. This figure shows a plasmodium containing nine nuclei. One of the large, deeply staining nuclei was not drawn because it is situated directly above one of the vacuoles containing an inclusion. There are obviously two different types of nuclei present, six larger ones 3 to 3·5μ in diameter, which possess very big deeply stained nucleoli 2 to 2·5μ in diameter and three smaller about 2·5μ in diameter with small nucleoli ·75μ in diameter. The six large nuclei are situated towards the centre of the plasmodium, whilst the three smaller nuclei are connected with extrusions from the main body which closely resemble pseudopodia. These are almost certainly uninucleate plasmodia which have very recently coalesced with the large plasmodium and their nuclei and nucleoli still retain the size and structure of that present in the unfused, uninucleate plasmodium.

The photograph shown in Pl.18 FIG.89 is of a rather larger plasmodium in the act of coalescence, and gives a more general view of the process. It shows very clearly that the big plasmodium is a centre of attraction for a large number of uninucleate plasmodia. It is quite impossible to state definitely whether/

whether the structures are really all uninucleate plasmodia, or whether, in some cases, they are myxamoebae, but it is more than likely that most of them, if not all, are uninucleate plasmodia. The large plasmodium contains many vacuoles, in each of which a myxamoeba in an advanced stage of digestion is present. It is very probable that the large plasmodium has engulfed all the available myxamoebae in the neighbourhood, particularly as no evidence of recent engulfing could be obtained. Pl.XI. FIG.56. is a drawing of a portion of the plasmodium photographed, but at a much greater magnification. The point of interest is that from this sketch one can obtain a rather neat series of stages in the transformation of the small nucleus into the large one. Two uninucleate plasmodia are seen at (a) which have just coalesced with the large plasmodium and contain nuclei of the small type. At (b) is seen a somewhat larger nucleus the nucleolus of which has not yet commenced to increase in size. At (c) the nucleus has attained the size of the normal large one, and the diameter of the nucleolus is now about one-third the diameter of the nucleus. At (d) the nucleolus has still further increased in size, and its/

its diameter is about one-half that of the nucleus. At (e) are seen the fully developed large nuclei with nucleoli about three-quarters the diameter of that of the nucleus. Two large vacuoles are also figured. The lower one contains two myxamoebae in an advanced stage of digestion, and in the upper one are a myxamoeba and a flagellate.

Apparently not only do uninucleate plasmodia coalesce with those which are multinucleate, but the latter coalesce with each other, for, in living drop cultures the end product is nearly always a single large plasmodium which is quite visible to the naked eye.

(g) INGESTION BY THE PLASMODIUM AND THE STRUCTURE OF THE MATURE PLASMODIUM.

In the description of the living cultures it was stated that the developing plasmodia did not rely on the liquid medium for their entire food supply but obtained much of it by engulfing and digesting swarm-cells, and perhaps more frequently, myxamoebae. The complete process may conveniently be termed ingestion, and several stages in it have been found in stained and mounted preparations. One of the earliest of these is shown in Pl.XII. FIG.57.

a/

a drawing of an eight-nucleate plasmodium. A myxamoeba is seen quite close to the plasmodium, and the latter is commencing to extrude a pseudopodium towards the former. In the bottom right-hand corner of the plasmodium appears a myxamoeba in a somewhat advanced stage of digestion, though a faint trace of its nucleus can still be distinguished; it is enclosed within a distinct vacuole. FIG. 58. which illustrates a slightly later stage, is a drawing of a plasmodium with about fourteen nuclei, only ten of which are shown. To the left of the figure a pseudopodium has grown out and nearly surrounded a myxamoeba so that the process of engulfing is almost completed. Two inclusions are present which are at a very advanced stage of digestion. In Pl. XIII. FIG. 59. is shown a four-nucleate plasmodium with three vacuoles containing inclusions. The one to the left contains one myxamoeba in which the nucleus and nucleolus are still quite distinct, and in the right-hand one are two myxamoebae in a similar condition. This is somewhat later than the stage shown in FIG. 58 for the process of engulfing has very recently been completed and digestion has hardly begun, though the nuclei and nucleoli of the two myxamoebae to the right/

right have a rather blurred appearance as if the process of disintegration had just commenced. The myxamoeba in the lower vacuole is in an advanced atage of digestion.

From the above account it will be seen that engulfing is completed before digestion commences. Digestion first affects the nucleus and nucleolus which gradually fragment and finally disappear. FIG. 60. is a drawing of a uninucleate plasmodium, the nucleus of which is ommitted as it is situated directly above the large vacuole. The vacuole contains two myxamoebae, in both of which the nucleus has undergone considerable change. In the one to the left the nucleus is represented by a number of deeply staining granules, and in the one to the right by a round, homogeneously staining structure with a more deeply staining rim. The general protoplasm of the two myxamoebae has also changed considerably, for it has lost its alveolar structure and stains homogeneously. The myxamoeba present in the lower vacuole in FIG.59. illustrates a still later stage in the process of digestion. The nucleus and nucleolus have entirely disappeared and the protoplasm is quite homogeneous in structure. The body of the inclusion gradually decreases in size as if it were being dissolved away, and/

and it finally disappears except perhaps for a finely granular residue.

In Pl.X. FIG. 55. at (a) is shown a most exceptional case of engulfing. The large digestive vacuole contains a uninucleate plasmodium which itself has engulfed and is digesting a myxamoeba. This is the only example found in Reticularia or in Didymium nigripes in which a plasmodium has been engulfed. In all other cases the diploid structure had engulfed haploid myxamoebae and swarm-cells, and this is an exceedingly interesting exception. It will be noted that the nucleus of the engulfed plasmodium, though rather distorted owing to the presence of the large vacuole containing the myxamoeba, stains quite clearly and does not give any indication that the process of digestion has commenced.

The débris left behind as a result of the process of digestion probably accounts for the markedly granular appearance of the protoplasm in the older, multinucleate plasmodium. In the protoplasm are embedded myxamoebae in process of digestion, ungerminated spores, empty spore coats and other foreign bodies. Myxamoebae, naturally, are not found in the plasmodium two to four weeks old as none are present/

present at so late a stage in the life-history. In the clearer portions of the plasmodium it can be seen that the protoplasm is alveolar in structure, though the network formed is coarser than that found in the young plasmodium which has only one to four nuclei. The ectoplasm forms a very narrow layer, staining homogeneously, over the entire surface of the plasmodium. The nuclei, apparently, change somewhat in structure as they grow older. FIG. 61 is a drawing of a very small portion of a mature plasmodium three or four days before the initiation of sporangium formation. At (a) are seen three of the typical nuclei found at this stage. The nuclear membrane is very inconspicuous and the nuclei themselves tend to be rather irregular in shape. They are about 4 to 5μ in diameter, and therefore considerably larger than those found in the young plasmodium during coalescence which were about 3μ in diameter. The nucleolus, however, has become very much smaller, being now only 1 to 1·5μ in diameter, compared with 2·5μ, the diameter of the nucleolus in the young plasmodium. The nuclei in the older plasmodium apparently tend to group themselves in threes and fours, leaving large tracts of protoplasm quite devoid of them. The piece of protoplasm drawn is crowded with inclusions, the nature of which it is impossible to determine, except at (b) where two empty spore cases are present. The plasmodium/

plasmodium is now in a condition to proceed to the last stages in the life-history, those connected with the development of the sporangium and spore formation.

(h) THE FORMATION OF THE SPORANGIUM.

A series of microtomed, stained and mounted preparations, which had been made from a very vigorous test-tube culture, illustrated particularly successfully the various stages in the development of the sporangium and the formation of the spores. Sporangia were killed and fixed at 9 a.m., 10.45 a.m. noon, 2 p.m. and 4.45 p.m. on April 19th, 1929.

Nearly all the sporangia belonging to the first set, killed and fixed at 9 a.m. already possessed a distinct head and stalk. The head was comparatively large, sometimes about 2 m.m. in diameter, and of a soft, creamy consistency very like that of the mature plasmodium; it was much lighter in colour than the latter, however, being pure white instead of a rather deep cream. The stalk was brown in colour, with a comparatively smooth surface, and triangular in shape, tapering from a broad base to a very narrow diameter at its junction with the head. Pl. XX, FIGS. 92 and 93 are photographs of a microtomed and stained section through two young sporangia at this stage. The one in FIG.92 is younger than that shown in FIG. 93 and/

and has very recently risen from the surface of the nutrient medium. From the photograph in FIG 93 it will be seen that the structure under investigation consists of three portions, the hypothallus, the stalk, and the head of the sporangium.

'Hypothallus' is the term usually applied to the degenerate protoplasm left behind when the active protoplasm emerges to form the sporangium. The degenerate protoplasm consists of a very coarse network in which no alveolar structure and no nuclei of any kind can be distinguished. It is very probable that it originally formed the outer, non-motile layer of the plasmodium. In the case of Didymium nigripes the plasmodium consists of a number of fine, anastomosing branches, each possessing an outer non-motile layer and an inner core of active protoplasm. When the inner portions are withdrawn from all the branches and massed together to emerge as the young sporangia, the outer layers are left behind and form the network of the hypothallus. (a Pl. XX FIG. 93) Pl. XIV FIG. 62 is a drawing of a small portion of the hypothallus, highly magnified, showing that a large number of inclusions are present which were left behind by the active protoplasm. The rejection of all such debris accounts for the pure white colour of the head of the young sporangium.

The/

The young sporangium in Pl.XX FIG. 92 has not yet formed a true stalk, but as the active protoplasm left the surface of the nutrient medium it pulled out the hypothallus after it, and this raised the developing head somewhat. In the sporangium in FIG. 93 a true stalk (b) has been formed which consists entirely of a large mass of protoplasm in process of degeneration. Degeneration has progressed farthest on the outside of the stalk. The protoplasm there has entirely lost its alveolar structure, and no nuclei are present, in fact it forms a homogeneous, comparatively narrow layer which shows a marked affinity for the orange G. of FLEMMING'S triple stain. Within the outermost layer is an inner layer of about the same width in which degeneration has not progressed so far. All the nuclei have disappeared, but the protoplasm retains some traces of its original, alveolar structure, and forms a very coarse network. This layer resembles the normal protoplasm in its affinity for gentian violet. The central portion of the stalk consists of a mass of protoplasm at an earlier stage in degeneration. This protoplasm has been cleaved into a number of fairly large pieces which are degenerating from the exterior inwards. Numerous nuclei are present which have changed considerably in structure from those of the normal/

normal type. They stain deeply and homogeneously with safranin and vary tremendously in shape and size, giving the impression that an enzyme is acting on them transforming them into soluble material which subsequently disappears. The protoplasm itself is coarsely alveolar in structure, with numerous vacuoles and it stains readily with gentian violet. (Pl. XIV FIG. 64) It will be seen from the photograph (Pl. XX FIG. 93) that in the case of the older sporangium also the hypothallus forms a tapering base, as if it had been pulled out when the active protoplasm left the surface of the nutrient medium.

The head of the young sporangium in FIG. 92 is smaller and more compact in structure than is that of the one in FIG. 93. The impression is given that had the younger sporangium been permitted to continue its development, it would have spread out until it resembled the older one in size and structure. An examination of the head of either sporangium under comparatively low powers of the microscope reveals the surprising fact that a large amount of the protoplasm is doomed to degenerate, whilst only a very small portion will eventually give rise to the spores. The sporogenous tract (c) forms a narrow, compact layer covering the entire surface of the head. Under the highest powers of the microscope this/

this layer is seen to consist of normal protoplasm resembling that of the plasmodium in its alveolar structure but of a somewhat denser nature, containing very few vacuoles, and with no foreign inclusions of any kind present. (Pl. XIV FIG. 63) The nuclei are very numerous, and are crowded together in every part of the layer. They are similar in size and structure to those present in the mature plasmodium, though their shape is, on the whole, rather more regularly rounded or oval, and the nuclear membrane is more distinct. The rest of the head consists entirely of degenerating protoplasm (d, Pl.XX FIG. 93) identical in structure with that found in the centre of the stalk, the only difference being that it is not cleaved into a number of pieces. A small portion of this protoplasm, with the nuclei present, is shown very highly magnified, in Pl.XIV FIG. 64. At this early stage there are no signs of the formation of the wall and capillitium.

The stalks of the sporangia belonging to the second set, killed and fixed at 10·45 a.m. were quite fully formed, being dark-brown or almost black in colour, and possessing a distinctly wrinkled surface. The heads were somewhat smaller than those of the first lot, their diameter being about 1·8 m.m. They were still pure white in colour, but of a firmer consistency/

consistency than before. PL. XXI FIG. 94 is a photograph of a microtomed section through a young sporangium at this stage. It will be seen that the degeneration of the protoplasm which forms the stalk has progressed considerably. The entire stalk, with the exception of a very small portion in the centre, now shows a distinct affinity for Orange G, and has become a more or less solid structure. The central portion still stains with gentian violet. The protoplasm of which it consists forms a coarse network, and is identical in structure with that of the second layer present in the young stalk of the sporangium described in the first set.

Very marked changes have also taken place in the young head. The degeneration of the inner portion of the protoplasm has progressed considerably and much of it, probably, has gone to aid in the formation of the stalk, leaving a cavity which may now be regarded as the columella. Just above the point at which columella and stalk meet, some of the degenerating protoplasm is still present, which resembles in structure that found in the centre of the stalk. The most surprising feature of the section under observation is the presence of a fairly broad, uniform layer (a) covering the surface of the young head and continuous with a similar layer between the sporogenous/

sporogenous tract and the columella. This layer, which stains faintly with gentian violet, exhibits a networklike structure in sections of some sporangia at this stage, but in others it appears to be almost structureless. The impression is given that it is condensing on its outer surface, for it is more solid there and stains more deeply. At several points on the surface of the sporangium and also on the surface of the columella furrows (b) varying in width and depth run off from the layer into the sporogenous tract. They are filled with material identical in structure and staining properties with that comprising the layer. No change has taken place yet in the sporogenous tract itself except towards the inner edge which abuts on the layer covering the surface of the columella. Here a small portion of the protoplasm (c) is evidently in the early stages of disintegration. It still retains its alveolar structure, but has become decidedly more vacuolate, and the nuclei present show all stages in the transformation of normal nuclei into the shapeless, homogeneously staining structures already described.

The fairly broad, structureless layer covering the surface of the sporangium is a very evanescent structure and is only present in comparatively few sporangia sectioned. It soon condenses to form the/

the dark wall of the sporangium and later a similar wall appears, arising from the condensation of the layer covering the surface of the columella. Condensation is due almost certainly to loss of moisture and this naturally first takes place over the surface of the sporangium. In Pl.XXI FIG. 95 a slightly later stage, it is seen that the wall in that region has been formed, whilst the layer covering the columella is still very broad and structureless. The wall of the sporangium is apparently an exceedingly brittle structure, and in the processes of embedding and microtoming it becomes very much broken up. Sometimes groups of small granules may be seen adhering to its surface. These consist of Calcium carbonate and dissolve readily in hydrochloric acid. They probably increase in amount as the sporangium matures but are readily detached whilst embedding and microtoming the material and so never appear in large numbers in the stained and mounted preparations.

The sporangia belonging to the third set, killed and fixed at noon, showed little change in outward appearance. The head was still pure white in colour but of a rather firmer consistency than before. Investigation of a microtomed preparation Pl. XXI FIG. 96 shows that only slight changes have proceeded internally. The stalk is identical in structure/

structure with that of a young sporangium belonging to the second set, with the exception that very little of the degenerating protoplasm previously seen at the junction between stalk and columella now remains. The columella is filled with a very indistinct network of material, probably the final remains of the huge mass of degenerating protoplasm which has gone to build up the stalk. The layer covering the surface of the columella has condensed somewhat, but it has not yet attained a solid structure similar to that exhibited by the wall of the sporangium at this stage. The furrows passing from the walls of sporangium and columella have increased in number and length, particularly in the case of those arising from the wall of the columella. In several of these it can be seen that the colourless contents previously present have condensed to form a distinct dark thread. (a) In exceptional cases the threads can be traced from the surface of the columella to the wall of the sporangium. They are very few in number and form a scanty capillitium. In some of the furrows the material is still in process of condensation and others appear to be quite empty. It is suggested that the latter indicate that the cleavage of the sporogenous protoplasm to form the spores has commenced. The degenerating protoplasm previously seen on the inner surface of the sporogenous tract has/

has decreased in amount and is now found aggregated in (comparatively) large patches, many of which are broken up considerably by the presence of conspicuous vacuoles. In several cases it was noted that nuclei about to degenerate had divided by direct division (PL.XIV FIG.65) a phenomenon frequently observed in connection with the degeneration of the tapetum in the young anther. The protoplasm and nuclei, which will directly take part in the formation of the spores still show very little change. In a few cases the nucleoli have divided into two or three portions, probably an early indication that the nuclei are about to divide.

(i) CLEAVAGE AND THE HETEROTYPE AND HOMOTYPE DIVISIONS.

In the sporangia of the fourth set, killed and fixed at 2 p.m. though to outward appearance very little change had taken place, microtomed preparations show that the internal structure has altered considerably. PL.XXII FIG. 97 is a section through a sporangium at this stage, and it will be seen that very little of the disintegrating protoplasm between stalk and columella now remains. The wall of the columella has completely condensed, resembling the wall of the sporangium itself in structure/

structure, and the capillitium is fully developed, one of the threads being shown at (a). The disintegrating protoplasm associated with the sporogenous tract has disappeared entirely in all the normal sporangia examined at this stage. In one or two abnormal cases, however, large tracts of protoplasm appear to have been cut out from the normal course of development and to be disintegrating en masse. (Pl. XXIII FIG. 98)

The most striking feature is the change which has taken place in the sporogenous protoplasm. It has become cleaved up into a number of comparatively small pieces, which are rather larger in the region of the columella but decrease in size towards the wall of the sporangium. In the sporangium photographed in Pl.23 FIG.99cleavage has not progressed quite so far as in that shown in Pl.XXII FIG. 97, comparatively large pieces of protoplasm still being present. The process of cleavage had probably commenced in the sporangia belonging to the third set, and it progresses rapidly. Unfortunately, no intermediate stages have been obtained, but the impression is given that the process closely resembles that described by HARPER (23) in Didymium melanospermum. He found that cleavage is a progressive process working/

working from the surface of the sporangium inwards. The first cleavage furrows run radially and cut the sporogenous protoplasm up into a number of cylinders. These are soon cut up into blocks by tangential furrows, and the process continues until finally uninucleate pieces result. The cleavage furrows at first run indiscriminately through the protoplasm, and it is only when the masses are of approximately two-spore capacity in size that the nuclei apparently exert some influence on the direction of the cleavage planes, for no small portion of one spore capacity in size is left without a nucleus. Cleavage, according to HARPER, is accompanied by considerable loss of water, resulting in marked shrinkage of the protoplasm, so that large spaces appear between the cleaved masses. This condition is clearly shown in the sporangium photographed in Pl. XXII FIG. 97.

Examination under the highest powers of the microscope shows, that at this stage the nuclei are all in process of division. The nuclei do not commence to divide absolutely simultaneously, for almost all the stages in division can be obtained from a single section. Pl XV, FIGS. 66 and 67 show two stages in synapsis. In FIG. 66 the thread is slender and forms a number of large loops. In FIG. 67 the thread has become considerably condensed towards/

towards one side of the nucleus, and forms a very compact structure in which it is difficult to make out any loop-like appearances.

In FIG. 68 a stage immediately preceding metaphase is shown. Synapsis has been completed, and the chromosomes have been isolated. They are four in number and are distinctly U shaped. The nuclear membrane is clearly visible so that the division is obviously intranuclear. No centrosomes are present. FIG. 69 is a drawing of metaphase, in which the spindle, about 3μ in diameter is fully formed and the chromosomes are arranged on its equator. The nuclear membrane is still quite distinct. In FIG. 70 division has reached the stage of anaphase. The U shaped chromosomes have divided and moved some considerable distance apart. The nucleus has become rather more oval in shape but the nuclear membrane is still present. In FIG. 71, which is an early telophase, the chromosomes have commenced to fuse together to form the daughter nuclei. When the typical U-shape of the chromosomes, and their reduced number, four instead of eight, is considered, it is quite obvious that one is dealing with the heterotype, and reducing division of meiosis.

The heterotype division is very rapidly followed by a typical, homotype division, the earliest/

earliest stage of which is shown in FIG. 72. Even before the daughter nuclei of the first division have been reconstructed, and while signs of the spindle fibres still remain between them the spindles of the second division have commenced to form. In PL. XVI FIG. 73 metaphase of the homotype division is seen. The chromosomes, four in number, are spherical in shape and the spindle is comparatively narrow, about 2.5μ in width. FIGS. 74 and 75 are drawings of anaphase. Even at this late stage, in many cases, a clear area is visible round the division figure which probably is a part of the nucleus, although no nuclear membrane can be distinguished. Telophase rapidly follows metaphase, and by this time the protoplasm has nearly always become cleaved up into masses of two spore capacity. The concluding stages of the homotype division will be considered, therefore, in the section dealing with spore formation.

The process of cleavage goes on simultaneously with, and is quite independent of meiosis. The first stages in the heterotype division are usually found in masses of protoplasm containing five or six nuclei, and these are rapidly cleaved up until, when late telophase is reached pieces with only one dividing nucleus present are formed. Cleavage continues/

continues with great speed and the daughter nuclei are soon separated by the furrows. (FIG. 75) This is generally accomplished by the time that the daughter nuclei have reached the condition of anaphase, and one usually finds at this stage that the protoplasm is divided up into small pieces of two spore capacity, each containing a nucleus passing through the later stages of the homotype division.

(j) SPORE FORMATION.

The various stages in spore formation were followed in a series of smears made every half-hour between 4·30 p.m. and 7·30 p.m. on May 9th, 1929. In the preparations made at 4·30 p.m. the concluding stages in the homotype division were obtained. FIG. 76 is a drawing of a small mass of protoplasm of two spore capacity, in which the dividing nucleus is at anaphase. The mass is about 14μ in diameter, and is somewhat polygonal in outline giving an impression of very recent cleavage. The chromosomes are not very distinct and cannot be counted with certainty. In FIG. 77 is shown a late telophase in which the chromosomes have completely lost their identity and fused to form the daughter nuclei. FIG. 78 is a still later telophase in which the daughter nuclei have assumed their final rounded form and only a slight indication of the spindle fibres remains.

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The final cleavage to form the uninucleate spores has commenced and a deep constriction has appeared between the daughter nuclei. In FIG. 79 a drawing of the young uninucleate spore is seen. The spore is still slightly polygonal in outline, and is about 12μ in diameter. The nucleus stains quite homogeneously, and the nucleolus has not yet been reconstructed after the homotype division. The nucleus of the young spore soon forms a distinct nucleolus (FIG. 80) and the spore now enters upon the series of changes concerned in the formation of a wall.

The first indications of wall formation were found in smears made at 5·30 p.m. Many of the spores present were at the stage shown in FIG. 81. A distinct vacuole has appeared round the nucleus, and the protoplasm exhibits a slightly different structure, staining somewhat more homogeneously. This renders it rather muddy in appearance as described in Reticularia (71, p.583). There is a distinct layer round the spore even at this early stage, and it soon increases in width until it has attained that found in the mature wall. [illegible] (PL. XVII FIG. 82).

Meantime vacuolation has increased until four or five large vacuoles are present. The protoplasm now stains very deeply and homogeneously, and has completely lost its alveolar structure. This stage/

stage is seen in the smears made at 6 p.m. A little later about 6·30 p.m. the stage figured in FIG. 83 is found. The protoplasm has contracted somewhat and the diameter of the young spore has decreased from 12μ the diameter at the stage shown in FIG. 80, to 10μ. In consequence the wall has fallen into ridges and this forms the spines which are such an obvious feature of the ripe spore. The vacuoles have decreased in size and number; two or three are present at this time which are filled with brownish contents, somewhat crystalline in appearance. The protoplasm still stains very deeply and homogeneously, and the nucleus is often very inconspicuous and much distorted in shape. In the smears made at 7.30 p.m. many of the spores had reached the stage shown in FIG. 84. A single vacuole is present filled with a yellow body, somewhat lighter in colour than those seen in FIG.83. The protoplasm does not stain quite so deeply, but it is still homogeneous in structure and no sign of a network can be distinguished. Contraction of the protoplasm continues after the wall has been fully formed and there is now a distinct space between the spore wall and the periphery of the protoplast. The nucleus has generally regained its normal rounded shape by this time, and has become much more conspicuous than it was in FIG. 83.

The final stages in the formation of the ripe spore were found in the smears made at 8·30 p.m. The single vacuole with its inclusions is still present in the spore drawn in FIG. 85, but the inclusion is not so definite in shape and it is paler yellow in colour. The protoplasm no longer stains homogeneously but has regained its original alveolar structure. In the spore shown in FIG. 86 the definite vacuole and inclusion have disappeared almost entirely, but their position is still indicated by a paler area. In FIG. 87 a drawing of a ripe spore, this slight indication has disappeared, and the mature condition is attained. The spore in FIG. 87 resembles in every detail that shown in PL.1 FIG. 1. but as the smear preparations, are stained with FLEMMING'S triple stain it is possible to indicate the nucleus and the alveolar structure of the protoplasm in the final drawing.

The formation of the spore wall in Didymium nigripes resembles closely in its essential details that found in the case of Reticularia Lycoperdon, but in the latter species no crystalline deposit was detected as being present in the vacuoles at any time during the process. The presence of this deposit is puzzling, and it cannot be accounted for satisfactorily, but there is very little doubt that it is closely connected with wall formation. The curious fact is that the material should become so much more conspicuous/

conspicuous after the building of the wall than it was before this took place. There are two explanations which might account for the presence of this deposit. Firstly, it may consist of waste products secreted during the formation of the wall. Secondly, it may consist of a surplus of the material used in the building of the wall. The first explanation does not appear to be satisfactory since it seems difficult to assume that waste material could be excreted from a body enclosed in a very definite membrane. It is more probable that the inclusions in the vacuole consist of a surplus of material used in the formation of the wall, and that this material is reabsorbed by the protoplasm as the spore ripens.

The complete life-history from the germination of the spore to the formation of the ripe spore is now concluded and in Pl. XXIV FIG. 100 is seen a photograph of the mature sporangium. A very massive stalk has been formed, which spreads out into the lower region of the columella in a funnel-shaped manner. The columella itself is loosely filled with very faintly staining material, and over its dome-shaped surface a distinct wall is present. The capillitium consists of a small number of dark threads, most of which run from the surface of the columella to the periphery of the sporangium. The wall of the sporangium/

sporangium is exactly similar in structure to that of the columella. The interior of the sporangium is filled with masses of ripe spores, each of which possesses a dark, spiny coat.

DISCUSSION.

DISCUSSION OF RESULTS.

(a) SPORE GERMINATION.

The investigation of spore germination in Didymium nigripes confirms the decision arrived at in Reticularia (71, p.562) that the rupture of the spore coat is brought about by the osmotic passage of water through the semi-permeable membrane formed by the coat. The fact that the spore contents swell and rupture the membrane before contracting to divide points emphatically to this conclusion, which is in agreement with the statements made by JAHN (25) and GILBERT. (19) Such theories as that of PINOY (43, 44 & 45) concerning the action of bacteria, and that of CONSTANTINEANU (12) in regard to the chemical action of the nutrient solution appear to be unnecessary to explain the simple rupture of the spore coat. Indeed the present writer's experience has been that the fewer the number of bacteria present the more satisfactory the germination, and JAHN has proved conclusively that germination is retarded and finally inhibited entirely as the osmotic pressure of the nutrient solution approximates to or exceeds that of the spore contents. Germination is usually most successful in weak solutions, and it was found that Didymium/

Didymium nigripes germinates well in tap water. The action of the nutrient solution in regard to germination is a physical one, therefore, and not chemical in nature.

A number of monospore cultures have been put up in tap water and in nutrient solutions, but, as in Reticularia, no successful results have been obtained. This may be due, possibly, to the reason discussed in connection with Reticularia (71, p.585) and previously suggested by ROBERTSON. (52, p.103) He is of the opinion that in single spore cultures "some essential substance" termed by him a nuclear autocatalyst, "which is contributed to the medium by the organisms themselves is too much diluted, or too completely extracted from the cells in large volumes of culture media to sustain nuclear synthesis or even nuclear equilibrium." Under such conditions, therefore, germination would fail to take place, or if it did so the resulting swarm-cell would not develop normally. In Reticularia germination often occurs when three spores are present, but the cell which has emerged generally rounds off before a flagellum is produced. In the case of Didymium nigripes apparently each spore possesses a larger proportion of the autocatalyst than does that of Reticularia, for in the former, if only a very small number/

number of spores, five or six are present, germination takes place readily, and the swarm-cells produce flagella and divide several times before they round off and development ceases. In one culture in which ten spores were present small plasmodia, actually, were formed. In Didymium nigripes, however, as in Reticularia, the most satisfactory development is obtained in cultures which contain a comparatively large number of spores.

This may be due to the theory put forward by ROBERTSON but it may also be due, in part, to the presence of bacteria. The swarm-cells of Didymium nigripes spend a great deal of their time in the amoeboid condition, in which they probably engulf and digest bacteria very readily.

The greater the number of swarm-cells present, obviously the more efficiently will the bacteria be kept under control, and the culture be permitted to arrive at fruition. Nearly all unsatisfactory cultures are swamped by bacteria.

(b)/

(b) THE BLEPHAROPLAST AND ASSOCIATED STRUCTURES.

The origin of the blepharoplast has been discussed fully in Reticularia (71,p.585) and it is quite comparable in the case of Didymium nigripes. Here, again, the blepharoplast obviously arises in connection with the nucleus, either as a fragment of the nucleolus, or de novo on the nuclear membrane. Unfortunately, as stated previously, the stage showing the passage of the fragment through the nuclear membrane, so clearly seen in Reticularia (71,Pl.1.fig. 10) was not obtained in Didymium, so that the evidence for the intra nuclear origin of the blepharoplast is not so strong, but the method of origin is probably identical in the two species. It should be made quite clear that blepharoplast and centrosome are regarded as practically interchangeable terms, in fact, as was stated in connection with Reticularia (71,p.585) the granule originating in the nucleus can function as either and should perhaps be referred to more correctly as a centroblepharoplast, a term introduced by KOFOID and SWEZY (68) in connection with a similar granule which they described in Trichomonas. The course of events is not quite the same in Didymium as it is in Reticularia, for in the former/

former the centroblepharoplast, after its origin, first functions as a centrosome in the division within the spore coat, whilst in the latter it forms the blepharoplast of the single swarm-cell which emerges from the spore. As the same granule may act as either blepharoplast or centrosome it is not regarded as a matter of great importance which role it assumes in the first place. Indeed there is evidence that the order is not always identical, even in the same species. Many investigators have stated that the spore of Didymium nigripes may germinate giving rise to a single swarm-cell, though they also observe that division may take place within the spore coat. The present author has found that, as a general rule division always precedes germination in Didymium nigripes, but the possibility that events may occasionally take place in the reverse order cannot be excluded. On the other hand, the present author has observed a single case of division within the spore coat in Reticularia, so that in this species also the order of events is not invariably the same.

The only other investigator, TERBY (69) who has described the origin of the blepharoplast in the Mycetozoa, states that before the first sporogenic division in Plasmodiophora Brassicae the centrosomes arise independently, apparently de novo. After the division they pass to the daughter cells, and/

and in each divide in contact with the nuclear membrane, the resulting bodies passing to the poles and becoming the centrosomes of the second division. After this division they pass to the young spores, where they lie in contact with the nuclear membrane. On germination they elongate, pass to the periphery of the cell, and become the blepharoplasts. The origin of the blepharoplast in Plasmodiophora, therefore, differs markedly from that in Reticularia and Didymium, in both of which it arises in connection with germination and is never present in the young spore.

The significance of the disappearance of the blepharoplast has been discussed in Reticularia (71,p.587) but it is interesting to note that in Didymium nigripes it takes place at a different point in the life-history.

In Reticularia the blepharoplasts are withdrawn into the nucleus just after the commencement of the cell fusion of the two gametes and before the nuclei approach each other. In Didymium nigripes, on the other hand, withdrawal of the blepharoplast is connected with the very definite transformation of the swarm-cell into a myxamoeba.

The beaking of the nucleus towards the blepharoplast just before its withdrawal, which was clearly/

clearly seen in Reticularia, (71, Pl. II, fig. 41 & 42) is also quite marked in Didymium nigripes (Pl, VI. figs. 34 & 35) so that an additional proof is furnished that the ultimate fate of the blepharoplast is absorption in the nucleus from which it originated. The beaking may also indicate increase in nuclear activity, as suggested in Reticularia and it is probably present during the growth of the flagellum in the young swarm-cell, as it was in Reticularia, (71, Pl, I. fig. 15) but no stages showing this have been found in the case of Didymium.

The bell-shaped structure, the "Verbindungstück" of JAHN, which connects nucleus and blepharoplast, is very clearly seen in Didymium. It was stated that in Reticularia (71,p.587) this structure always arises in connection with the blepharoplast, being formed as the latter passes out towards the periphery of the cell, that it disappears before the division of the swarm-cell, and is formed again in the daughter cells by the terminal portions of the mitotic spindles. In Didymium the "Verbindungstück" arises in exactly the same way, being formed as the centroblepharoplast passes out from the nucleus previous to the first division within the spore coat, but there is some evidence that it does not disappear until, with the blepharoplast, it is withdrawn into the/

the nucleus when the swarm-cell is transformed into a myxamoeba. In Pl.II FIG.8 which shows the division of the centroblepharoplast previous to the first division, and in Pl.V FIG.26 which shows it just before the second division, the Verbindungstück also has divided, and a portion of it will probably pass with each centrosome to opposite poles of the nucleus, and there assist in building up the terminal portions of the spindle. This leads one to the conclusion that the terminal portion of the spindle and the 'Verbindungstück' are homologous structures just as are the centrosome and the blepharoplast.

(c) DIVISION OF THE SWARM-CELL.

The division of the swarm-cell in Didymium nigripes is exactly similar to that in Reticularia (71.p.588) with the exception, perhaps, of the division in the spore coat, which, of course, cannot readily be compared with a similar one in Reticularia. In the division within the spore coat the daughter nuclei are completely reconstructed before cell division commences, whilst, in succeeding divisions the chromosomes, usually, have not completely fused together before cell-division is accomplished.

The very late separation of the daughter cells, and the curved shape of cell and spindle often found in the first division, may be correlated with the/

the fact that the space within which the division is taking place is much restricted.

The plane of division is obviously transverse in both types of division connected with the swarm-cell of Didymium nigripes, and therefore, this species agrees with Reticularia in this particular, and SKUPIENSKI'S account of a longitudinal fission (57, p.60) is incorrect. Neither his description nor his figures (57.) FIGS. 8-12 are convincing, and his drawings do not show the typical spindle figured in Pl. V FIG. 27. His FIG. 10. probably depicts one of the abnormal swarm-cells possessing flagella which are known to occur in several species of the Mycetozoa.

(d) THE PRESENCE OF A DEFINITE MYXAMOEBOID STAGE.

Didymium nigripes differs very markedly from Reticularia in possessing a definite myxamoeboid stage. All investigators who have examined the former species agree in stating that amoeboid structures are present in the life-history, and are derived from the swarm-cells by the withdrawal of the flagellum, but some confusion has arisen concerning the appropriate term to apply to these structures.

The/

The terms 'amoeba', 'swarm-cell' and 'myxamoeba' need very careful definition, as they have been used somewhat loosely, and have even been regarded as interchangeable. 'Myxamoeba' first introduced by CIENKOWSKI who reserved it exclusively for the plasmodium, is the term usually applied to any stage in the life of the swarm-cell at which the flagellum is not present, and it is in this sense that SKUPIENSKI makes use of the word in connection with Didymium nigripes. When an attempt was made to employ the term during the life-history of Reticularia it was found that it could only be used in connection with two very short periods, the condition immediately after germination and that just preceding the division of the swarm-cell. Therefore it was considered unnecessary to employ two terms, and 'swarm-cell' was applied to the structure during the entire period between spore germination and the commencement of sexual fusion. The word 'Amoeba' has often been used as a synonym for 'myxamoeba' particularly by JAHN, though he also applied the term to the young zygote immediately after sexual fusion. By the earlier authors 'swarm-cell' was regarded as synonymous with 'zoospore', indeed DE BARY, in his earlier writings, used the latter term, 'swarm-cell' being introduced/

introduced later by CIENKOWSKI. SKUPIENSKI, in his papers, always employs the term 'zoospore'. As it has been definitely established that, at least in a number of species, the swarm-cell is not always flagellate but may become amoeboid, it can no longer be considered as entirely homologous with the actively motile zoospore. In the case of Didymium nigripes in which two very definite stages are present during the period between spore germination and sexual fusion, it is considered advisable by the present author to make use of two terms, 'swarm-cell' being applied to the structure so long as it possesses a blepharoplast and is capable of developing a flagellum again should the latter have been withdrawn, whilst 'myxamoeba' is reserved for the condition in which both flagellum and blepharoplast are absent. 'Myxamoeba' is regarded as being preferable to 'Amoeba' since the latter term is already applied to a well known genus of the Protozoa.

No previous investigator has described the withdrawal of the blepharoplast, a very important point since it must result in the division of a myxamoeba being very different from that of the swarm-cell. SKUPIENSKI (61.) observed a definite period/

period of encystment, lasting twelve hours, which marked the transformation of swarm-cell into the myxamoeba in the case of Didymium difforme, but he does not give any information regarding the fate of the blepharoplast, and he does not describe such encystment in Didymium nigripes. The present author has certainly found that the transition from swarm-cell into myxamoeba is not marked by encystment, as definite cysts have never been found in satisfactory cultures. The withdrawal and disappearance of the blepharoplast entails that, in any division of the myxamoeba, the centrosome would have to be formed de novo, or it might be absent altogether, the division, as a result, resembling that in the plasmodium. No evidence has been obtained in support of either of these alternatives, and the present author is of the opinion that division of the myxamoeba does not take place. She is supported in this conviction by the experiment of SKUPIENSKI, mentioned previously in the description of the living cultures. Investigation of many cultures has led to the conclusion that increase in the number of amoeboid structures present is only marked during the first twenty-four hours after germination, just at the very time that active swarm-cells are most abundant/

abundant. It has also been found, from an examination of cultures containing a limited number of spores, that each swarm-cell may divide four or five times before entering the myxamoeboid stage, so that it seems quite unlikely that the myxamoebae continue to increase in numbers by division. All previous investigators have stated that the myxamoebae are capable of division, but in living cultures it is often exceedingly difficult to distinguish an amoeboid swarm-cell from a myxamoeba, and this may very likely have led to confusion.

From the present investigations it is quite clear that the myxamoeboid stage is much more definite and differs more markedly from the swarm-cell than previous workers, with the possible exception of SKUPIENSKI, have realised. The presence of this additional stage in Didymium nigripes renders its life history very different from that of Reticularia, as has been stated already, and it must be regarded as pointing to the fact that the former species is more highly developedthan the latter, for it is generally admitted (71,p. 589) that the flagellate condition is more primitive than the amoeboid state, and a species in which the latter is absent/

absent must be more primitive than one which possesses it.

(e) THE ASSUMPTION OF SEXUALITY BY THE GAMETES AND SEXUAL FUSION.

The fusion of amoeboid gametes in the Mycetozoa has been described by JAHN (28) and SKUPIENSKI (54,55,57,59,and 60). Indeed the only species in which the fusion of flagellate gametes has been found and described fully is Reticularia Lycoperdon. CAYLEY (9) has recently described and figured fusion of flagellates in Didymium difforme, but all her observations rely entirely on living cultures and are not supported by evidence obtained from stained and mounted preparations. It would be very surprising if a closely allied species like Didymium difforme should differ from Didymium nigripes in this most important point in the life-history. Most of the work in regard to sexual fusion in the Mycetozoa has been carried out with a few species of the Didymium type and the present investigation is in agreement with the results already obtained in regard to Didymium nigripes. It might be noted here, that the present author, before commencing to investigate Didymium nigripes, had worked/

worked for a considerable time with Reticularia and thus was much biassed in favour of finding a fusion of flagellate swarm-cells in the former species. Living cultures were most carefully examined for fused pairs of flagellate swarm-cells whose peculiar wriggling motion rendered them so conspicuous in Reticularia (71,p. 568). None of these were obtained, even in cultures in which actively swimming swarm-cells were very numerous. As the actively motile condition of the swarm-cell normally continues for only twenty-four hours at the longest, and fusions are most commonly found three to four days after germination, it is considered most unlikely that fusion between flagellate gametes takes place in Didymium nigripes. It might be argued that the figures shown in (Pl.VIIFIG.42 and Pl.VIIIFIGS 43 &44) could equally well portray the fusion of uninucleate plasmodia, but this is considered unlikely. The evidence obtained goes to prove that uninucleate plasmodia usually coalesce with larger, multinucleate plasmodia and not with each other.

The assumption of sexuality by the gametes is a point of great interest, and was fully discussed in connection with Reticularia (71,p.589.)

The/

The theory put forward by DANGEARD (14, p. 263.) that the development of sexuality is associated with lack of food material and that the gametes are therefore ordinary swarm-cells 'weakened and starved by continued cell division without intermediate periods of nutrition,' (71,p. 589) was accepted as satisfactorily explaining the facts in Reticularia Lycoperdon. In this species it was found that the swarm-cells pass through three or four divisions before they are able to fuse in pairs,and it was considered probable that, as these divisions follow each other very rapidly, usually taking place within the first twenty-four hours, after germination they result in a starvation and weakening of the swarm-cells which renders them capable of functioning as gametes. The condition of affairs is rather different in Didymium nigripes. Here again the swarm-cell probably undergoes several divisions, but before fusion can take place a further change is essential; the blepharoplast and 'Verbindungstück' must disappear, and the swarm-cell become transformed into a myxamoeba. The fact that the addition of fresh nutrient solution at regular intervals appears to retard the transformation of the swarm-cell into a myxamoeba, as stated in the description of the living cultures, rather/

rather supports the view that the assumption of sexuality is connected to some extent with the supply of food available.

Fusion of amoeboid gametes is not unknown amongst the more primitive species of the Protozoa, but in these lowly animals centrosomes are always present throughout the entire life-history. The withdrawal of the blepharoplast, and the subsequent absence of centrosomes during the sexual fusion in Didymium nigripes is interesting and is a marked difference from the state of affairs found in Reticularia. It would appear that the plant-like nature of the diploid portion of the life-history, which is emphasised by the absence of centrosomes at the nuclear division in the plasmodium, is actually transmitted to the final stages in the preparation of the gametes in such a species as Didymium nigripes.

The fact that some structures which may be either swarm-cells or myxamoebae, do remain to be engulfed by young plasmodia, indicates that all the potential gametes present do not fuse. Some remain which have not been able to secure a partner. The reason for this was discussed in Reticularia ((71, p. 592), and it was suggested there, that the gametes might/

might be of two kinds as SUPIENSKI had previously described in Didymium nigripes (57) and D.difforme, (59). Fusion in Reticularia, could, therefore, only take place between gametes of opposite sexes. This was not considered likely, as no obvious sexual attraction between the gametes could be detected. In Didymium nigripes, also, the impression is given that the gametes do not exert any kind of attraction, and that their meetings are purely accidental. The swarm cell must pass through several divisions and the flagellum, belpharoplast and 'Verbindungsstück' must disappear before it can function as a gamete. As germination can be spread over a comparatively lengthy period in Didymium, the chances of having gametes at very different stages in their development present in a culture at the same time are great, and one of them may pass through its entire life-history without encountering another with which it is able to fuse. It is not considered at all necessary, therefore, to assume the presence of male and female gametes in Didymium nigripes. The gametes are morphologically identical, and it is very probable that they do not differ physiologically.

The fusion of amoeboid gametes is correlated with the presence of a myxamoeboid stage in the life-history, and is considered as a further indication that Didymium nigripes is a more highly developed species than Reticularia Ecoperdon.

(f)/

(f) THE YOUNG PLASMODIUM AND THE DIVISION OF ITS NUCLEI.

The condition of the young plasmodium in Didymium nigripes differs very markedly from that found in Reticularia Lycoperdon. In the latter species the uninucleate plasmodium is usually in the throes of digesting the nuclei of the swarm-cells which coalesced with the fusing gametes during the later stages of sexual fusion (71, p. 573). RAYNER (51 a) has noted that the nuclei of host-cells engaged in the active digestion of fungal filaments are usually larger than the normal nuclei, and that the amount of chromatin present increases considerably. Active digestion, therefore, and the fact that the nucleus has just been reconstructed after sexual fusion account for its very large size in the uninucleate plasmodium of Reticularia, about 6μ in diameter as compared with 3μ, the diameter of the nucleus in the swarm-cell. No coalescence with unfused swarm-cells takes place in Didymium nigripes and so the nucleus of the young zygote is comparatively little bigger than that of the myxamoeba, the diameter in the former being, as stated, about 3 to 3·5μ and in the latter 2·5μ.

Nuclear/

Nuclear divisions were found quite frequently in the young uninucleate and binucleate plasmodia of Reticularia Lycoperdon whilst they have never been seen in such young plasmodia of Didymium nigripes. This is probably due to the fact that in the former species no coalescence of plasmodia takes place, at least in the early stages, and the number of nuclei present is increased by mitosis alone. In Didymium nigripes, on the other hand, very active coalescence of young plasmodia occurs, and therefore increase in the number of nuclei is not dependent on nuclear division. The various stages in the first two divisions of the fusion nucleus in Reticularia Lycoperdon are very distinct, the spindle is broad and the chromosomes large and readily counted. This is probably due to the very large size of the fusion nucleus. In Didymium nigripes the division figures are not nearly so large and clear. The spindle is hardly broader than that in the dividing swarm-cell, and the chromosomes are small and somewhat difficult to count. This is not surprising because, as stated above, the fusion nucleus never becomes so big in comparison as that in Reticularia, and if the young plasmodium does not engulf myxamoebae its nucleus soon decreases in size. There is some indication that nuclear divisions are/

are found more abundantly in cultures which are not progressing as successfully as they might, and in which no marked coalescence of plasmodia has been observed. In one such culture, which was examined nine days after germination, a large number of comparatively small plasmodia were present. No coalescence of plasmodia had been seen and no stages in nuclear division were obtained, but the number of nuclei present in each case was very suggestive. Many plasmodia were found containing one, two, four or eight nuclei, indeed one which apparently possessed sixteen nuclei was observed. The above figures certainly give a very strong impression that the nuclei had increased in numbers by division and not by coalescence,

(g) COALESCENCE AND INGESTION.

The process of coalescence in Reticularia has been very fully discussed (71, p. 593) and the distinction between coalescence and ingestion was clearly drawn. The present author makes use of the terms in exactly the same way so the definition given there may be repeated at this point. 'In the case of coalescence there is an intimate fusion between masses of protoplasm of identical structure and/

and similar physiological nature, the one merges into the other, and there is no line of demarkation between the two at any stage; in this process there is no digestion of one by the other, and the bulk of the resultant mass should theoretically be the sum of the two. On the other hand, ingestion can only take place when the masses of protoplasm are of different physiological structure, one, the larger, surrounding the smaller and digesting it, i.e. bringing it into solution and absorbing the soluble products.'

The phenomenon of coalescence in Didymium nigripes differs markedly from that described in Reticularia. In the latter the process commences at a very early stage in the life-history, before the gametic nuclei have fused. One is dealing, therefore, with coalescence between haploid swarm-cells and the protoplasm of the fusing gametes. Since the gametic nuclei have not yet fused, the protoplasm is not dominated by a diploid nucleus, and is therefore, probably in the same condition as that of the coalescing swarm-cells, so that coalescence can readily take place. By the time that the fusion nucleus is constituted the swarm-cells have completely coalesced with/

with the young zygote and this structure now contains a number of haploid nuclei surrounded by protoplasm dominated by a diploid nucleus. The fate of the haploid nuclei is not surprising; they disintegrate and are digested by the developing plasmodium. In Didymium nigripes the position is quite different. Uninucleate plasmodia fuse with a larger, usually multinucleate plasmodium, the coalescing structures, therefore, all being diploid in nature. Consequently the nuclei of the coalesced plasmodia do not disintegrate, but increase somewhat in size and become normal nuclei of the developing plasmodium. The process of coalescence just described is probably of a higher and more satisfactory type than that found in Reticularia. The nuclei of the coalesced plasmodia are not destroyed, but remain and perform a useful function in the young plasmodium, and so, apparently, there is less waste of material. This may be taken as a further indication that Didymium nigripes is higher in the scale of evolution than is Reticularia.

The process of coalescence was really observed and figured by CIENKOWSKI (11), though he failed to realise its significance. As stated in the/

the paper on Reticularia (71.p. 594) his description of the formation of the plasmodium is remarkably accurate. There is no doubt that he observed the fusion of two non-flagellate gametes, which he describes in the following words, (11,p. 419.) "Nach langem, erfolglosen Suchen gelingt es, zwei sich anlegende cilienlose Schwärmer in einen Korper verschmelzen zu sehen." He found that two or three non-flagellate swarm-cells may fuse, and that the resulting myxamoeba afterwards fused with non-motile swarm-cells with which it may come into contact. The young plasmodium then rounds itself off and becomes covered with a number of swarm-cells which may or may not have flagella. The multiple coalescence which follows the fusion of the two gametes is so much more conspicuous that it led CIENKOWSKI to discount the importance of the latter and he finally concluded that the plasmodium is formed by the fusion of a large number of non-flagellate swarm-cells. He was unable, at that early date, to make use of accurate methods of staining, and so could not observe the nuclear fusion which follows the cell fusion of the gametes. It was not till about fifty years later when JAHN'S work appeared that nuclear fusion/

fusion was discovered and figured. Unfortunately this discovery led JAHN and subsequent investigators, with the exception of WILSON and CADMAN, to disregard entirely CIENKOWSKI'S perfectly accurate description and figures of a multiple fusion. It is quite obvious that the process he saw and termed 'multiple fusion' is really the coalescence of uninucleate plasmodia with a large plasmodium. His account follows the course of events in Didymium nigripes even more accurately than it does in the case of Reticularia Lycoperdon, for in the former species one is dealing with the fusion of non-flagellate gametes, as described by CIENKOWSKI, in the latter species the fusing gametes are flagellate. CIENKOWSKI merely failed to interpret his results correctly because he had not the advantage of the modern methods of fixation and staining.

LISTER, in the second edition of his monograph, (37a, p. 8) gives a very accurate drawing of a young plasmodium of Didymium difforme which, according to his interpretation, is in process of formation by the fusion of a large number of non-flagellate swarm-cells. This drawing compares in every detail with the photograph (Pl.XVIII FIG. 89) and the/

the figure (Pl. X FIG.55) One can even distinguish the two types of nuclei observed in a young plasmodium of Didymium nigripes at a similar stage, those with large nucleoli which have been present in the plasmodium for some time, and those, smaller in size and with small nucleoli, which have just entered from the recently coalesced, uninucleate plasmodia. It is practically certain that LISTER'S drawing does not represent the formation of a plasmodium by the fusion of non-flagellate swarm-cells, but a multinucleate plasmodium in process of coalescence with a number of uninucleate ones. So imbued was MISS LISTER with the importance of JAHN'S great discovery of sexual fusion that, when she edited the third edition of her father's monograph, she omitted this very fine drawing, since multiple fusion did not find a place in the new account of the life-history of the Mycetozoa.

The presence of large groups of uninucleate plasmodia clustering round a multinucleate one indicates that the latter exerts some kind of attraction over the former. No reason can be suggested for this attraction, but it is a definite fact that the larger the plasmodium the greater the attractive force/

force it possesses, even though the smaller plasmodia in its neighbourhood should be multinucleate also. This accounts for the formation of the single large plasmodium in a successful drop culture. It is suggested, therefore, that a uninucleate plasmodium which has engulfed and is in process of digesting several myxamoebae or swarm-cells has a greater power of attraction than one which has not done so. Coalescence probably commences by the fusion of a vigorous, uninucleate plasmodium possessing a number of inclusions with several less flourishing, uninucleate plasmodia in which no inclusions are present. The first formed plasmodia in a culture will naturally be in a condition to engulf and digest myxamoebae and swarm-cells before those arising later, and this will probably give the older ones a better start in life. On the other hand, it is possible that the centre of attraction in the early stages of coalescence may be a binucleate plasmodium, the two nuclei having arisen by the division of the fusion nucleus. The latter alternative is considered the more unlikely as divisions in uninucleate plasmodia have never been found.

The process of ingestion probably precedes that of coalescence in Didymium nigripes, and is of considerable/

considerable importance in this species. It has been stated previously that four or five days after germination small plasmodia appear which, in many cases, have engulfed five or six myxamoebae or swarm-cells. These plasmodia, very soon afterwards, form the centre of attraction round which the uninucleate plasmodia without inclusions cluster. Coalescence and ingestion are, therefore, proceeding simultaneously, and it is obvious that there must be some essential difference between the uninucleate structures which coalesce and those which are ingested, though ~~morphologically~~ apparently they are identical. The most obvious explanation, as was indicated previously is that it is the diploid structures which coalesce, and the haploid structures which are ingested. Only one example has been observed in which a large plasmodium has engulfed a small one,(Pl. X FIG.55) To the left of the figure is a large vacuole containing a uninucleate plasmodium which itself has engulfed and is digesting a swarm-cell or myxamoeba. Though the small plasmodium has been engulfed by the larger one, it is not at all certain that it would have been digested by the latter. LAPAGE (31a) has described a similar case in Amoeba vespertilio in which one individual/

individual may be enclosed by a second, the second by a third, and the third by a fourth. He states that the individual enclosed is never digested but is finally ejected, sometimes in a living condition, when it may continue to develop. He observed that the amoebae engulfed were always less vigorous than those enclosing them, and were usually in a rounded, quiescent condition. A similar explanation might be suggested in the case of the example figured in Didymium nigripes. The uninucleate plasmodium engulfed was obviously very actively engaged in digestion when it encountered the large plasmodium. This may have rendered it quiescent and incapable of coalescing with the latter, which consequently flowed round it. It should be noted that the nucleus of the small plasmodium has not disintegrated, and there are no signs that digestion by the large plasmodium has commenced.

It should perhaps be stated here that most of the various stages in ingestion were found in preparations obtained from a culture nine days old, in which coalescence had not progressed favourably. The nucleoli are not so large as in those cases in which coalescence has taken place , and inclusions are not so numerous. As stated before RAYNER has observed/

observed that the amount of chromatin increases when active digestion is proceeding, so this probably accounts for the large size of the nucleoli in the flourishing plasmodia present in cultures in which coalescence and ingestion are progressing favourably. Probably the stages in ingestion were obtained more readily in unfavourable cultures because the process itself was proceeding more slowly.

It is exceedingly interesting that in the case of both Reticularia Lycoperdon and Didymium nigripes coalescence should apparently be an essential factor in the successful development of the young plasmodium. Certainly it is a means by which a rapid increase in size can be readily effected, as the material is at hand, and easily made use of. This applies, also, to the process of ingestion. The importance of coalescence in the life-history, strengthens the opinion that single spore cultures are not destined to be very successful. Since a single swarm-cell only divides three or four times, it is difficult to see how the number requisite to supply the requirements of more than two or three comparatively small plasmodia, at the very most, can arise, and it is almost certain that a much greater number of small plasmodia must unite to form a single, successful, large one.

(h) THE DEVELOPMENT of the STALK, CAPILLITIUM and WALL of the SPORANGIUM.

The presence of an exceedingly large amount of degenerating protoplasm, during the earliest stages of sporangium formation has not previously been observed, except in the case of Reticularia Lycoperdon (71). In this species sporangium wall and pseudo-capillitium are formed entirely by the degeneration of large masses of protoplasm. In Didymium nigripes the stalk alone is built up as the result of degeneration, and sporangium wall and capillitium are formed in an entirely different way. Very few previous investigators, with the exception of KRANZLIN (31), have described the formation and structure of the stalk in any of the Mycetozoa; in fact, it has apparently been regarded by later workers, as a matter of no great importance. KRANZLIN found that the stalk in Trichia and Arcyria was formed by the degeneration of masses of protoplasm, and Didymium nigripes, therefore, agrees with these two species in this particular. HARPER (23), states, that the stalk in Didymium melanospermum is filled with coarse concretions, but he does not give any further details concerning its structure. The failure by many/

many of the previous investigators, to realise the presence of degenerating protoplasm, has led to much confusion in regard to the different types of nuclei present. Both DANGEARD (14) and ROSEN (53), describe two types of nuclei in the young sporangium. Nuclei of the first type possess a nucleolus and stain comparatively normally, whilst those of the second type stain homogeneously and deeply. DANGEARD explained the presence of the two types of nuclei, by stating that the nuclei of the second type were ridding themselves of a superabundance of chromatin before entering upon the reduction division. This explanation is, obviously, incorrect in the case of Didymium nigripes. The nuclei of the second type are, most probably nuclei in process of degeneration, similar to those found in the developing stalk of Didymium nigripes. HARPER found three types of nuclei present in the young sporangium of D.melanospermum. Two of his types resemble those described previously, and therefore, are probably normal and degenerating nuclei. His third type almost certainly consists of nuclei which have just commenced to degenerate, and in which the nucleolus is still visible.

JAHN (51) found that in Comatricha nigra the stalk is formed within the developing sporangium and the/

the active protoplasm creeps up over the surface of the stalk as the latter is built up. In Dictydium and Cribraria the protoplasm creeps through the interior of the stalk to the top. Didymium nigripes resembles Comatricha nigra more closely than Dictydium and Cribraria, but the impression is given that the stalk in Didymium nigripes is built up from the degenerating protoplasm present in the interior of the sporangium and raises up the active protoplasm gradually, rather than that the active protoplasm creeps up over the surface of the developing stalk.

The formation of the capillitium has received a considerable amount of attention, the chief investigators in this field being STRASBURGER (63), KRANZLIN (31), HARPER (22), and BISBY (4). As stated in the review of literature STRASBURGER, KRANZLIN and HARPER, were dealing with sporangia of the advanced Arcyria and Trichia type, and they agree that the capillitial threads are laid down in the walls of vacuoles. BISBY is of the opinion that it is more accurate to regard the furrows formed as invaginations into the protoplasm from the surface of both columella and sporangium, rather than as true vacuoles, and this certainly agrees more closely with the condition found in Didymium nigripes. The formation of the wall/

wall of the thread by secretion from the protoplasm is generally accepted by the investigators, who, as a rule, follow the lead of STRASBURGER, though they do not appear to confirm his statement that the secretions first appear in the form of microsomes. Both HARPER and KRANZLIN, consider that the nuclei play an important part in providing material for the formation of the wall of the thread. KRANZLIN found that the groundwork of the elaters in Trichia and Arcyria is cut out by astral rays emanating from the centrosomes of the nuclei, but HARPER considered that KRANZLIN'S astral rays are really lines of flow between the nuclei and the wall of the capillitium along which material passes to build up the latter. The present author is of the opinion that the nuclei are not directly concerned in the formation of the capillitial threads in Didymium nigripes. She has never found that the nuclei group themselves along the furrows which initiate the capillitium, as was described by HARPER, and believes that the formation of the wall and capillitium in the case of D.nigripes can be explained in the same way as the laying down of the wall in the young cell of a green plant. STRASBURGER (64), states that the cell-wall is laid down by/

by secretion, in the form of microsomes, and gradually increases in thickness. The theory held by the new school of thought, the chief exponent of which is GLEISBERG (20b), is that a wall consisting of cellulose is the result of a chemico-physical action taking place on the surface of living protoplasm in contact with air, and controlled by the protoplasm. This action results in the transformation of the protoplasm into simpler substances, such as cellulose.

It is certain that the wall of the sporangium, the wall over the columella and the capillitium are all formed in exactly the same way. The same type of structureless material which forms such a wide layer in the earliest stages of wall formation is also present in the invaginationsinto the sporogenous tract which initiate the formation of the capillitial threads. The threads themselves are formed by the condensation of the material almost certainly through loss of water, just as were the walls of sporangium and columella. The structureless material resembles agar-agar very closely in appearance and in staining properties and it is, therefore, very probably gelatinous in the first place, but becomes membranous as condensation proceeds. It is interesting to note that LISTER (p.23.38), states that the sporangium wall of Physarum nutans is gelatinous/

gelatinous at first but later becomes membranous. HARPER (23), found that the columella of Didymium melanospermum was full of extruded gelatinous material, but he does not associate this with the wall formation, and he did not observe it on the surface of the sporangium. This is probably due to the fact that he did not obtain sections showing the sporangium wall in its gelatinous condition, a very evanescent stage.

The formation of sporangium wall and capillitium, in Didymium nigripes differs very markedly from that of Reticularia Lycoperdon, in which species they are built up by masses of degenerating protoplasm. Didymium nigripes, therefore, possesses a true capillitium as compared with the pseudo-capillitium of Reticularia, and since the former is produced in a manner similar to that found in the wall formation of higher plants, this may be regarded as further proof that Didymium nigripes is a more highly developed member of the Mycetozoa than is Reticularia Lycoperdon.

The presence of degenerating protoplasm within the sporogenous tract is surprising as, apparently, it is cut off from taking a part in the formation of the stalk by the wall covering the surface of the columella. Two suggestions may be put forward/

forward as to the subsequent fate of this degenerating material.

FIRSTLY. It may possibly serve as a food supply for the sporogenous tract, like the tapetum in the anther of flowering plants, and disappear early in the formation of the young spores, probably during the first stages in cleavage.

SECONDLY. The degenerating protoplasm may eventually take part in the formation of the stalk. In some sections obtained just before cleavage commenced, the impression was given that the gelatinous layer over the columella could form again behind the distintegrating material and so cut it out from the sporogenous protoplasm, in which case degeneration would continue and the mass subsequently form part of the stalk.

(i) THE MEIOTIC DIVISIONS.

The discovery of two divisions immediately preceding spore formation, constituting typical meiosis, brings Didymium nigripes into line with Reticularia Lycoperdon, Ceratiomyxa, Plasmodiophora Brassicae and Spongospora subterranea, the only members of the Mycetozoa in which two divisions have been described previously. There is little doubt that the second division preceding spore formation is present in the Plasmodiophoraceae and also in Ceratiamyxa, but Didymium nigripes in only the second species belonging to the Endosporeae, in which it

has/

has been discovered. Cleavage and meiosis occur simultaneously in Didymium nigripes, and this has probably rendered it difficult to follow the two divisions successfully. In a large number of cases the cleavage furrows separate the daughter nuclei resulting from the first division at an early stage, and it is somewhat difficult to find undoubted pairs, particularly as the nuclei in this condition stain very faintly. It is only after a careful consideration of the shape of the chromosomes, U-shaped in the heterotype division and spherical in the homotype division, and the difference in the width of spindle, that one can determine whether a nucleus is dividing for the first or second time in meiosis. Reticularia Lycoperdon is a much more favourable species for the investigation of meiosis than Didymium nigripes, since in the former both divisions are completed before cleavage commences. It is probably because all previous investigations had been carried out on species of the Didymium type that typical meiosis in the Endosporeae was not discovered until it was described in Reticularia Lycoperdon in 1928.

It is not surprising that heterotype and homotype divisions should precede spore formation in Didymium nigripes as this brings the species into line with practically all other organisms, both plant and animal, and it is almost certain that further investigation/

investigation will prove that this is the rule and not the exception in the Endosporeae. JAHN (26), describes only one division before spore-formation, but states that the homotype division is the first division immediately occurring after germination or possibly within the spore coat in some species. SKUPIENSKI (57), referring to Didymium nigripes, considers that the second meiotic division takes place at some stage in the series of divisions through which the swarm-cell passes before it can function as a gamete. He is not sure whether it is at the first division of the swarm-cell or not, and with it he associates sex-separation, since the spores are potentially bisexual. CAYLEY (9), agrees with SKUPIENSKI that sex-separation must take place after spore germination in Didymium nigripes, but is certain from her own investigations that it is at the first division of the swarm-cell. She refers to JAHN'S observation that he saw indications of reduction in chromosome numbers at the first division of the swarm-cell in Amaurochaete, Reticularia, Trichia, Badhamia, Stemonitis and Didymium, and on this rather indifferently founded statement, she bases her conclusion that meiosis and sex-separation are closely connected. Two contentions might be advanced which throw considerable doubt on CAYLEY'S conclusion. In the first place the/

the investigations carried out by the present author in connection with Reticularia Lycoperdon and Didymium nigripes have given her the impression that there is no essential difference, morphological or physiological, between the gametes in either of these species. If, therefore, there is no differentiation in sex, there is obviously no need for sex-separation. Secondly, if sex-separation takes place, and the gametes differ physiologically, it is not essential that it should be connected with meiosis. In flowering plants the formation of stamen and pistil precedes the reduction division, and in many mosses and liverworts sex-separation , as indicated by the formation of antheridia and archegonia, occurs a considerable time after the meiotic divisions which resulted in the formation of haploid spores. If sex-separation does take place at meiosis then the spores of Didymium nigripes must be unisexual and SKUPIEN[illegible]SKI'S statement that they are bi-sexual would be incorrect. It is hoped to carry out further investigations to settle this important point.

(j) CONCLUSION.

In the above description of the life-history of Didymium nigripes, a comparison has been drawn between that species and Reticularia Lycoperdon as/

as a result of which the conclusion has been reached that the latter is a more primitive species than the former. The chief points of difference may be repeated very shortly here.

(1). In Didymium nigripes the first division takes place within the spore coat, in Reticularia Lycoperdon it occurs some time after germination.

(2). In D. nigripes there is a definite myxamoeboid stage, in R.Lycoperdon there is not.

(3). In D. nigripes fusion takes place between amoeboid gametes, in R.Lycoperdon the gametes are flagellate.

(4). In D. nigripes coalescence of uninucleate plasmodia occurs, in R.Lycoperdon there is a coalescence of flagellate swarm-cells.

(5). In D. nigripes meiosis and cleavage take place simultaneously, in R.Lycoperdon meiosis precedes cleavage.

(6) In D. nigripes a true capillitium is formed, in R.Lycoperdon a pseudo-capillitium is present.

It was stated in the paper on Reticularia that this species is a primitive member of the Mycetozoa. The conclusion arrived at is strengthened by the investigation of D.nigripes.

SUMMARY.

The life-history and cytology of Didymium nigripes have been worked out in detail. The organism grows well in carrot-agar and takes about a fortnight to six weeks to complete its life-history, the time required depending very largely on external conditions.

The spore coat is ruptured by osmotic pressure and the single nucleus then divides karyokinetically, four chromosomes being present. The centrosome arises in the nucleus and passes outwards, leaving behind it the 'Verbindungstück'. Division of centrosome and Verbindungstück precede the nuclear division. Nuclear division is followed by division of the protoplast, and two swarm-cells emerge from the spore coat. The centrosome subsequently functions as the blepharoplast.

After three or four divisions the swarm-cell withdraws the flagellum, 'Verbindungstück' and blepharoplast and becomes a myxamoeba. The myxamoebae fuse in pairs to form the zygote or young plasmodium.

The young plasmodium can ingest swarm-cells and unfused myxamoebae, and can also coalesce with smaller plasmodia. About five or six days after germination, the larger plasmodia exert a distinct attraction/

attraction on the uninucleate plasmodia in their neighbourhood and these coalesce with them in large numbers.

Nuclear divisions are comparatively rare in the young plasmodium. The division is karyokinetic, intracuclear, and no centrosomes are present. Eight chromosomes are formed. The plasmodium increases in size by coalescing with any small plasmodia it encounters.

The stalk of the sporangium is formed by the degeneration of large masses of protoplasm. The wall and capillitium probably arise as a result of a chemico-physical action taking place on the surface of protoplasm exposed to air and controlled by the protoplasm itself.

Cleavage of the sporogenous protoplasm and meiosis proceed simultaneously. The meiosis is typical and consists of the usual heterotype and homotype divisions. Both divisions are intra-nuclear, without centrosomes, and with four chromosomes. In the heterotype division the chromosomes are U-shaped, in the homotype one they are spherical. The final cleavage into singel spores takes place during the concluding stages of the homotype division.

Didymium nigripes is considered to be a more advanced type than Reticularia Lycoperdon, because of the presence of myxamoebae, the fusion of amoeboid gametes, and the formation of a true capillitium.

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P L A T E S.

All the drawings were made with a Zeiss Apochromatic lens, aperture 1.3, and an 18 compensating ocular, except in the case of the plasmodium shown in PL. X. , FIG. 55 for which an 8 compensating ocular was used.

The preparations, with the exception of those illustrating the developement of the sporangium, were stained with iron haemotoxylin and Orange G. The sections through the sporangia were stained with Flemming's triple stain.

A camera lucida was used in making the drawings.

PLATE I.

FIG. I. Ripe spore mounted in glycerine jelly. A distinct space is present between the protoplast and the spore coat.

FIG. II. Spore, after immersion in nutrient solution. The protoplast has swelled until it has reached the spore wall.

FIG. III. The spore coat has been ruptured, the protoplast has commenced to contract, and the nucleolus has divided into two.

FIG. IV. The nucleolus has divided into five fragments, one of which is approaching the nuclear membrane.

FIG. V. The centroblepharoplast is present on the exterior of the nuclear membrane.

FIG. VI. The centroblepharoplast has moved some distance from the nuclear membrane, leaving the 'Verbindungstück' behind it.

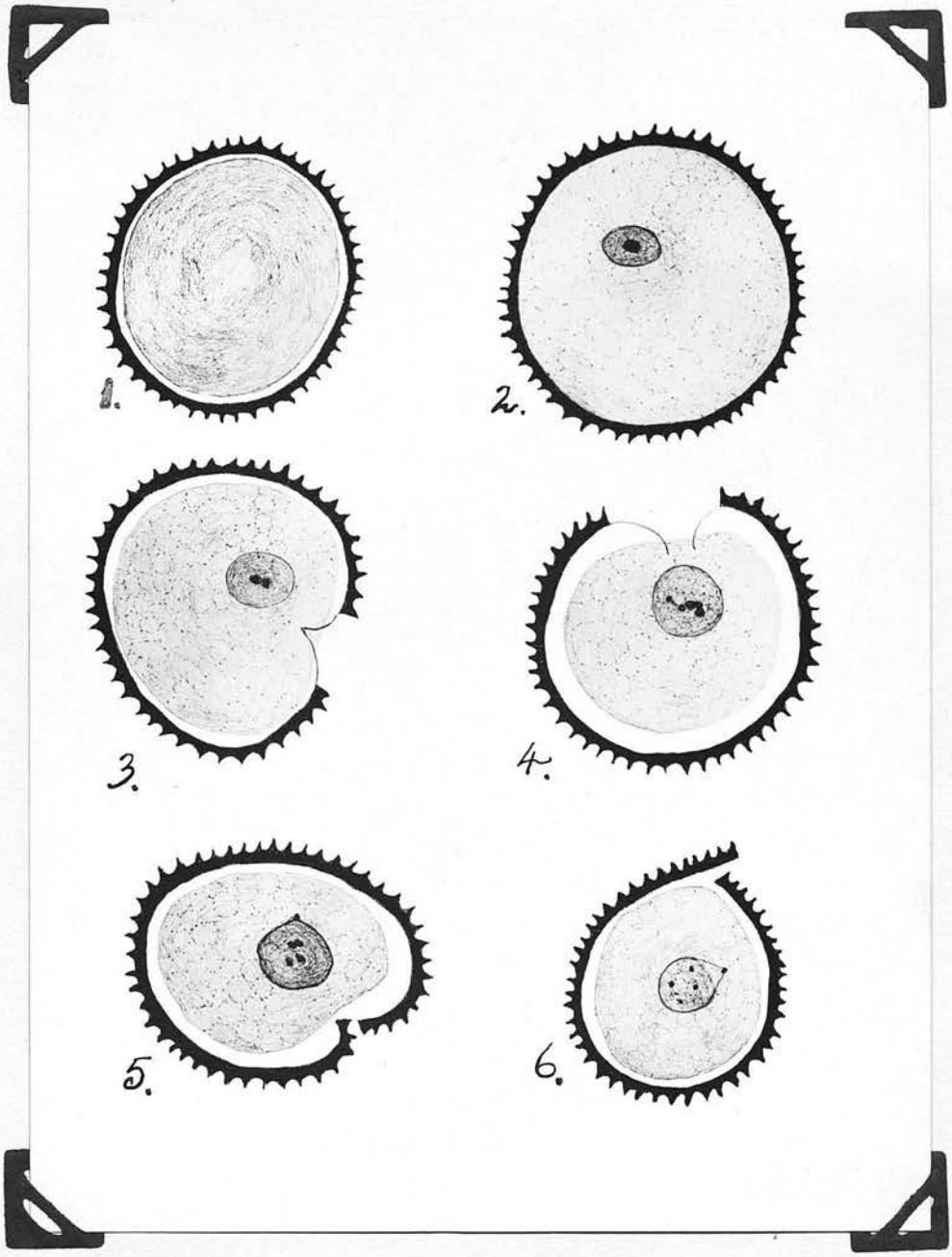


PLATE I.

PLATE II.

FIG. VII. The centroblepharoplast has divided to form two daughter centrosomes, which are connected by the centrodesmose. The 'Verbindungstück' has widened considerably.

FIG. VIII. Prophase of the first division. The spindle is commencing to form, and fou chromosomes are present.

FIG. IX. Anaphase of the first division.

FIG. X. Early telophase of the first division.

FIG. XI. Late telophase of the first division.

FIG. XII. The reconstruction of the daughter nucle

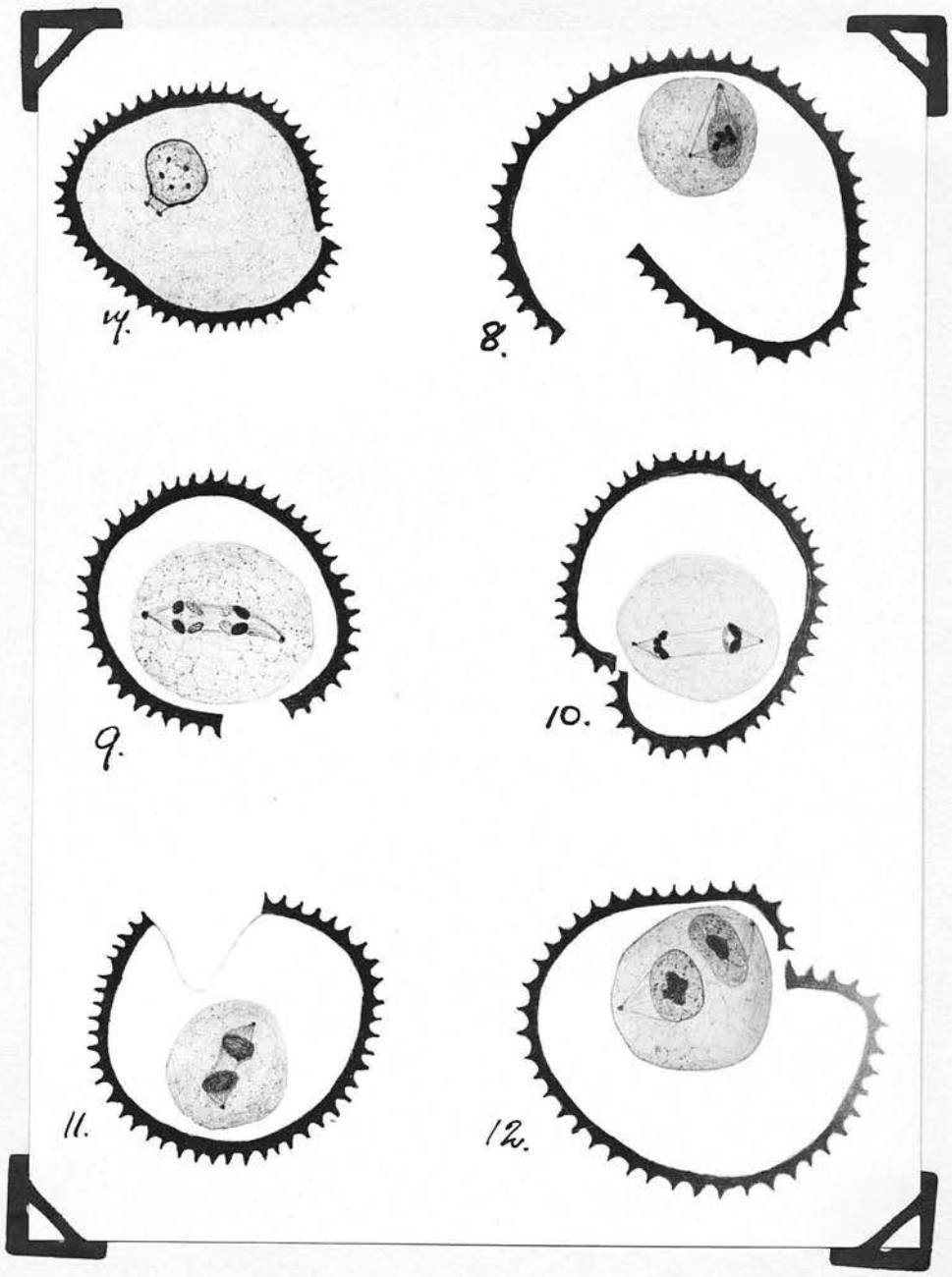


PLATE II.

PLATE III.

FIG. XIII. The reconstruction of the daughter nuclei has been completed and the protoplast is bent in a C. shaped manner.

FIGS. XIV. & XV. Stages in the cell division of the protoplast.

FIG. XVI. The cell division of the protoplast has been completed, and two daughter swarm-cells have been formed.

FIG. XVII. Emergence of the swarm-cells.

FIG. XVIII. An unusual case in which the protoplast has emerged from the spore coat during the later stages of the first division.

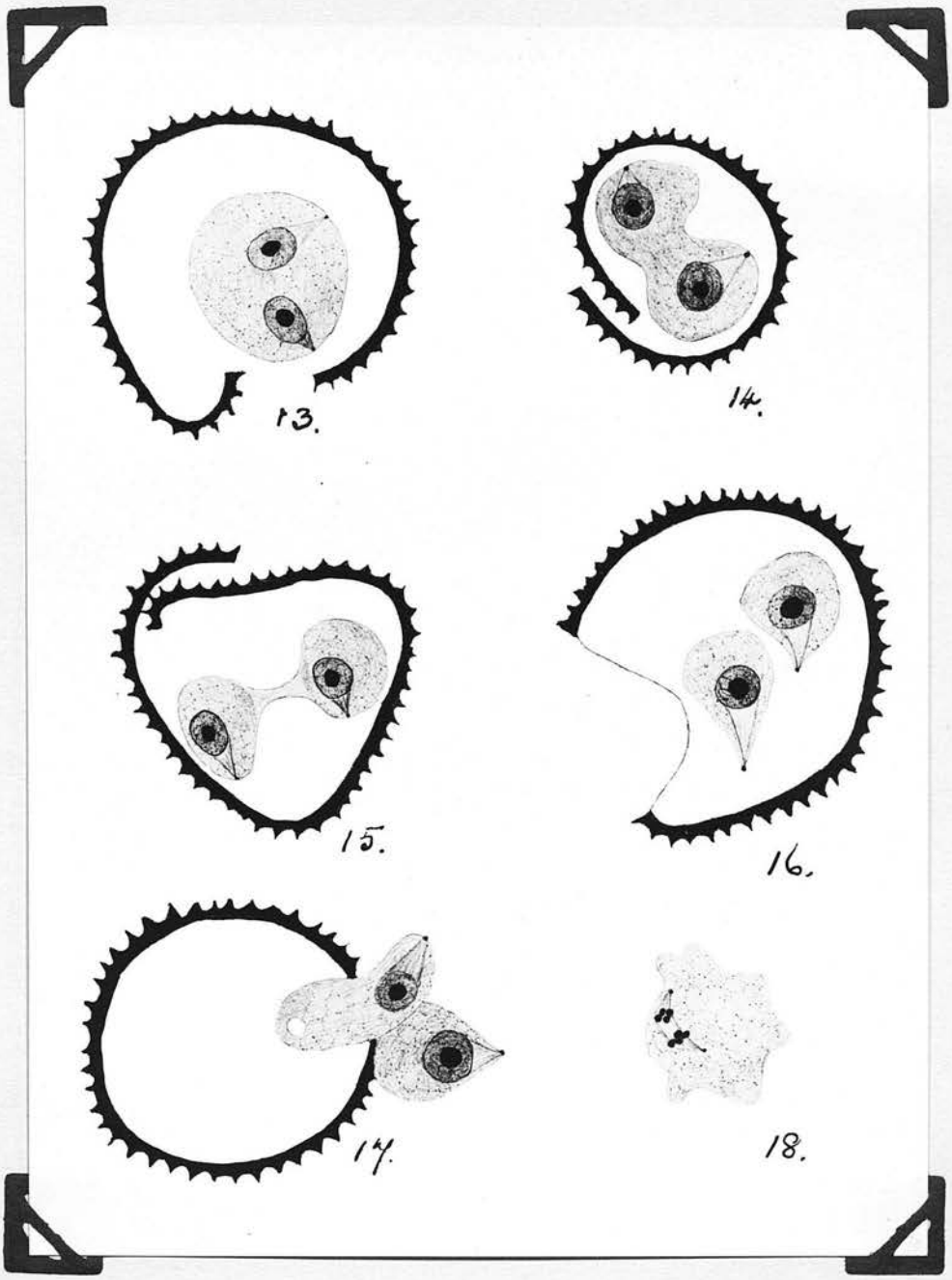


PLATE III.

PLATE IV.

FIG. XIX The protoplast has emerged before cell-division commenced.

FIG. XX The protoplast has emerged before the reconstruction of the daughter nuclei was completed.

FIG. XXI Cell-division has commenced and the flagella are growing out from the blepharoplasts.

FIG. XXII Mature swarm-cell.

FIG. XXIII Mature swarm-cell showing lipoid granules.

FIG. XXIV Amœboid swarm-cell.

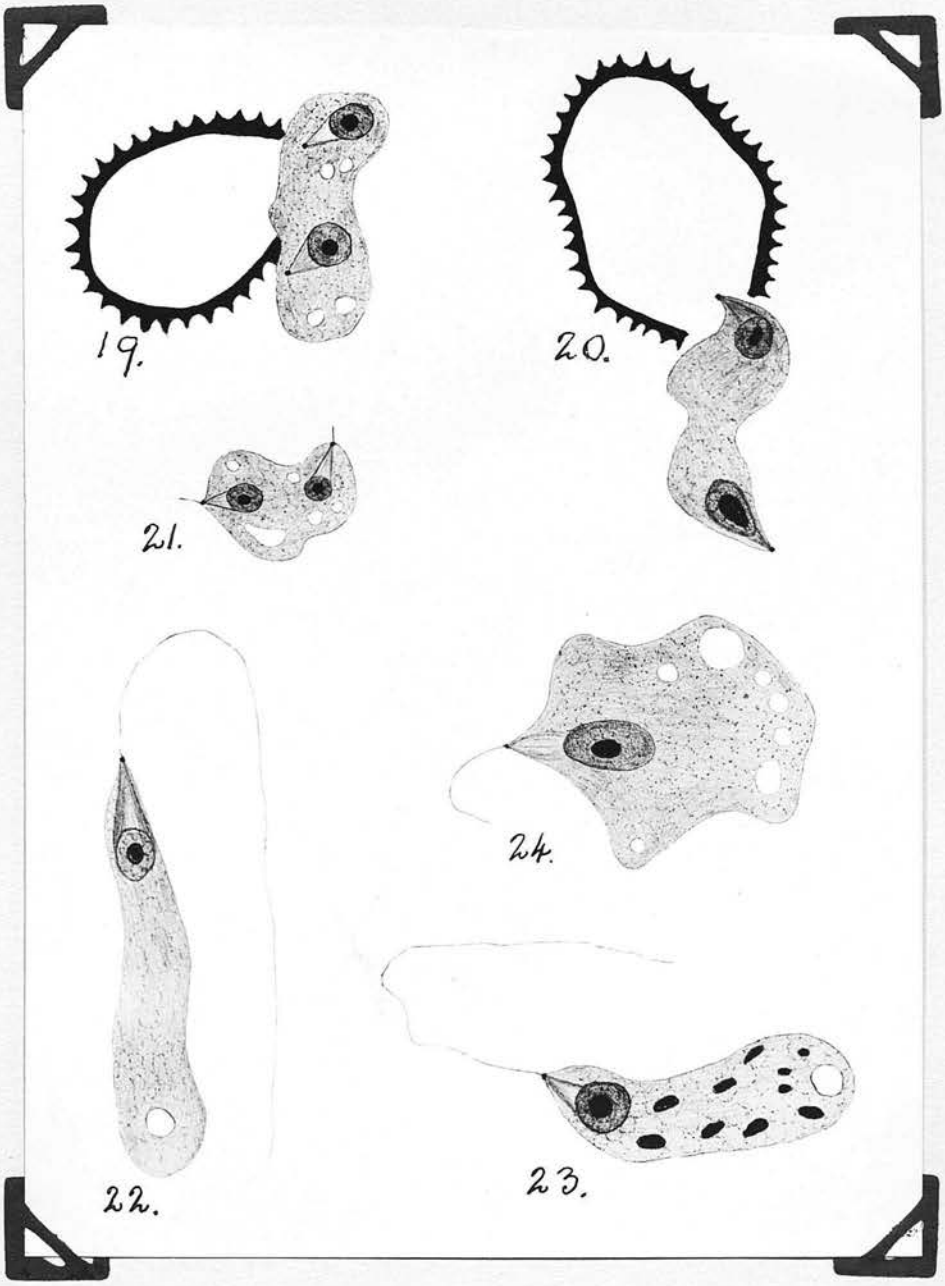


PLATE IV.

PLATE V.

FIG. XXV Unusual case in which two divisions have taken place within the spore-coat giving rise to four swarm-cells.

FIG. XXVI An early stage in the second division. The blepharoplast and 'Verbindungsstück' have divided.

FIG. XXVII Late prophase of the second division.

FIG. XXVIII Metaphase of the second division.

FIG. XXIX Anaphase of the second division.

FIG. XXX Telophase of the second division; cell division has commenced.

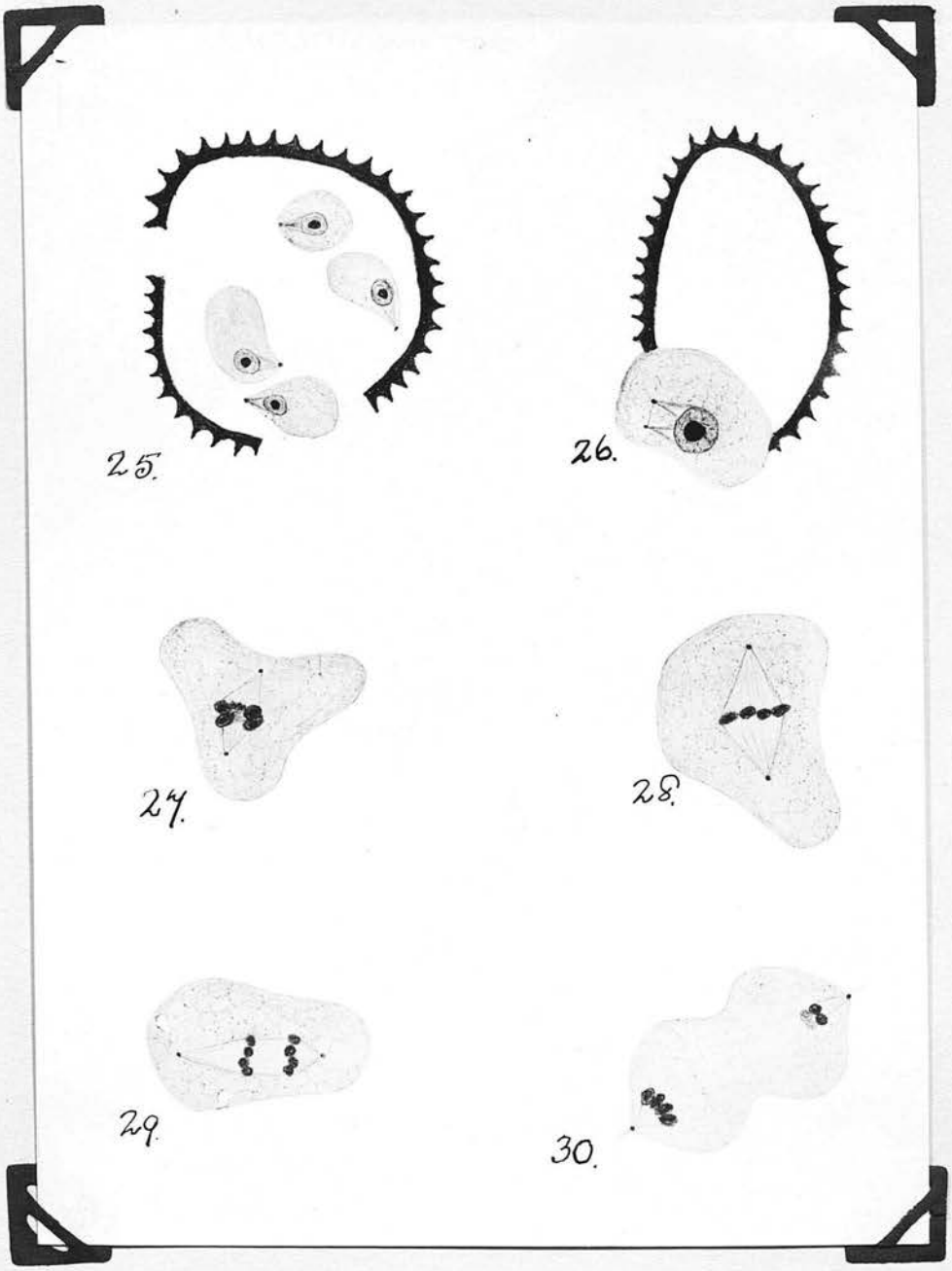


PLATE V.

PLATE VI.

FIG. XXXI Amoeboid swarm-cell in which the flagellum has been withdrawn.

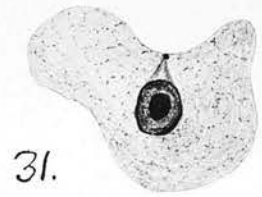
FIG. XXXII Stage in withdrawal of the flagellum. The flagellum is quite short and the swarm-cell is becoming rounded.

FIG. XXXIII A somewhat later stage. The swarm-cell has become quite rounded.

FIG. XXXIV The flagellum has been withdrawn, but the blepharoplast is still present on the periphery of the cell.

FIG. XXXV The blepharoplast has left the periphery and is seen just within the cell.

FIG. XXXVI The blepharoplast is halfway between the periphery of the cell and the nuclear membrane.



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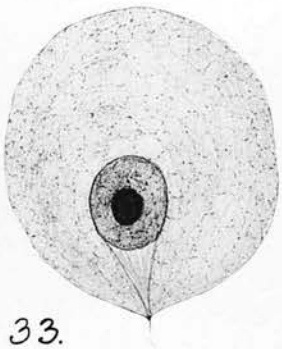

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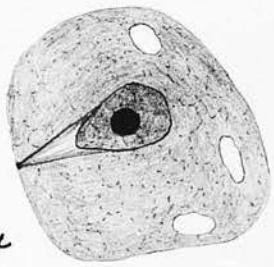

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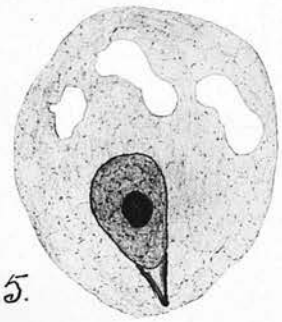

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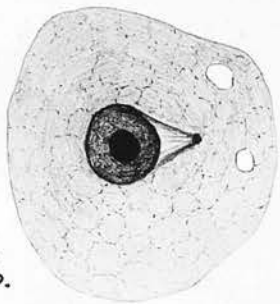

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PLATE VI.

PLATE VII.

FIG. XXXVII The blepharoplast has reached the nuclear membrane.

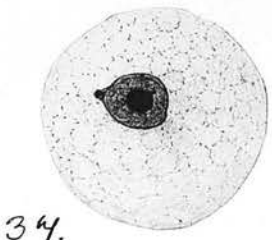
FIG. XXXVIII The blepharoplast has disappeared, but a slight thickening is present at one point on the nuclear membrane.

FIG. XXXIX Myxamoeba which is rather rounded in shape.

FIG. XL Stage comparable with PL. VI, FIG. XXXVI, but the cell is distinctly amoeboid.

FIG. XLI Amoeboid myxamoeba with three vacuoles, one of which contains a flagellate.

FIG. XLII Early stage in the fusion of two myxamoebae. The gametes are connected by a narrow bridge of protoplasm.



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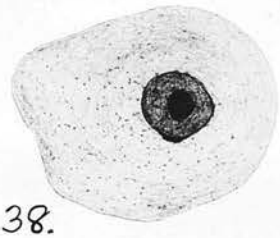

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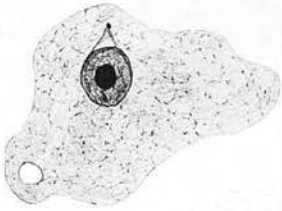

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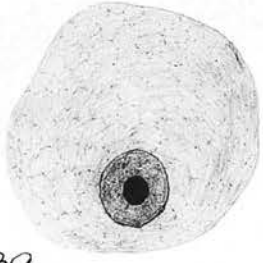

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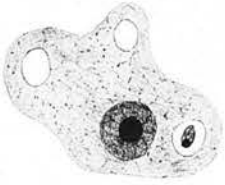

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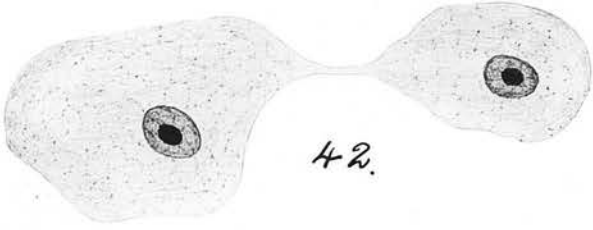


42.

PLATE VII.

P L A T E VIII.

FIGS. XLIII & XLIV Stages in the contraction of the bridge between the fusing gametes.

FIG. XLV The bridge between the gametes has almost disappeared. In the cell to the left a vacuole is present containing a bacillus.

FIG. XLVI Only a slight constriction is present between the fusing gametes. One large vacuole is present.

FIG. XLVII The nuclei of the gametes are commencing to approach. One vacuole is present between the gametes.

FIG. XLVIII The gametic nuclei are quite close together. Four vacuoles are present.

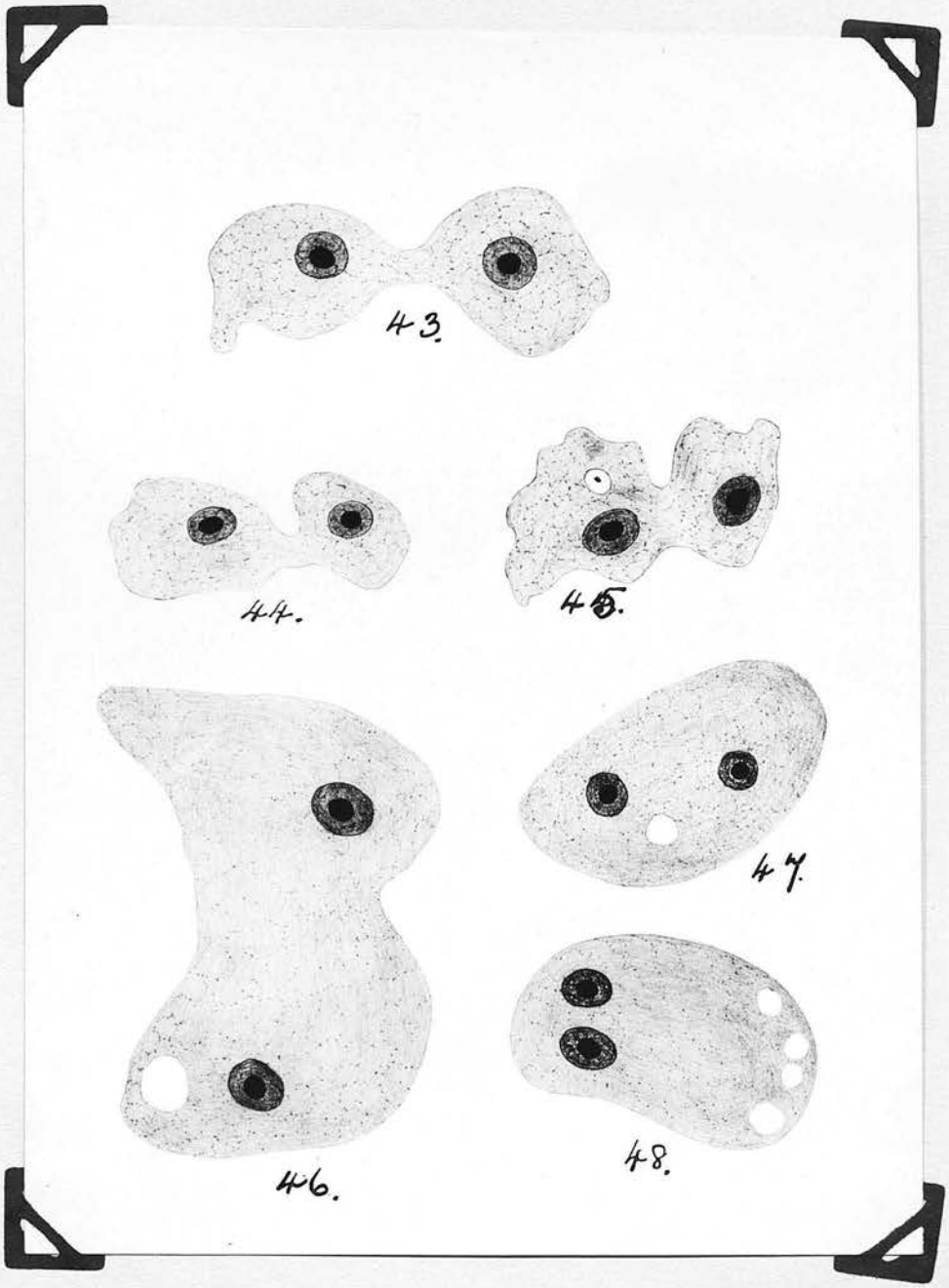


PLATE VIII.

P L A T E IX.

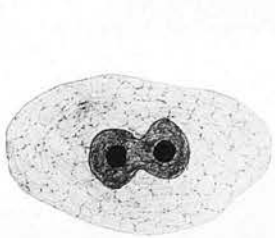
FIG. XLIX — The membranes between the gametic nuclei have disappeared and the nuclei have fused together.

FIG. L — The nuclei are in process of fusion.

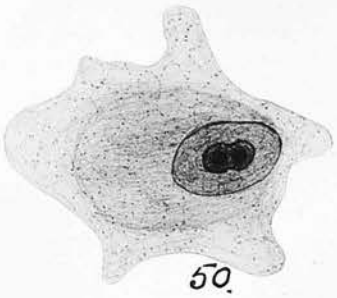
FIG. LI — Uninucleate plasmodium immediately after fusion.

FIG. LII — Plasmodium with three nuclei, one of which is in the resting condition, and two are at metaphase.

FIG. LIII — Seven nucleate plasmodium. Two of the nuclei are in the resting condition, three are at metaphase and two are at anaphase. One of the nuclei at anaphase is not shown because it is placed over the large inclusion, probably a myxamoeba.



49



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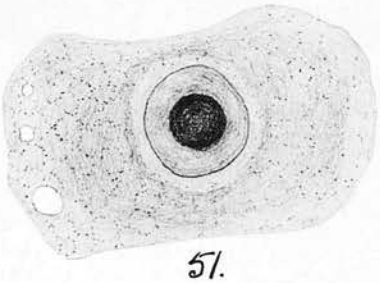

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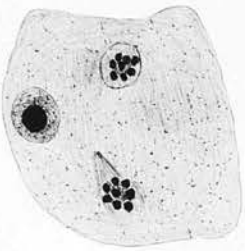
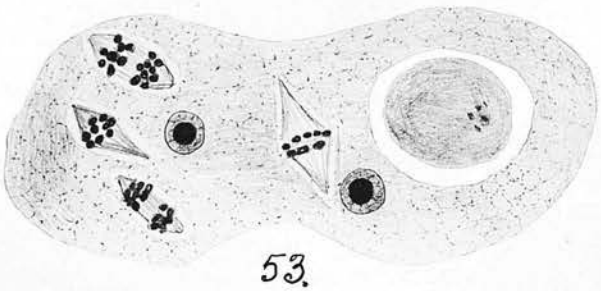


52.



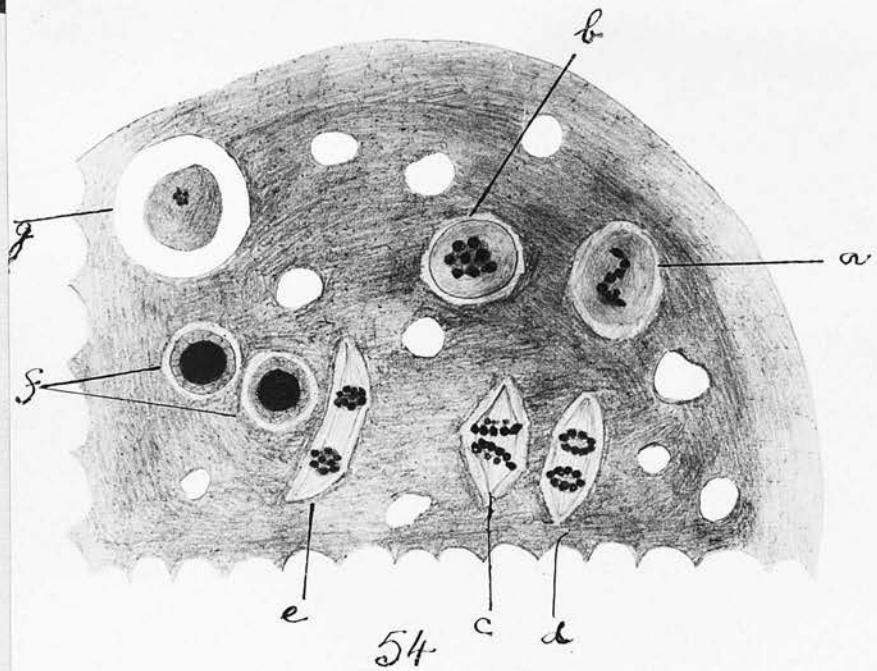
53.

PLATE IX.

PLATE X.

FIG. LIV Portion of a plasmodium showing various stages in division. (a) The chromatin is arranged in a spiral thread and is separating out to form the chromosomes. (b) Equatorial plate view of metaphase. (c) Early anaphase. (d) Later anaphase. (e) Early telophase. (f) Nuclei in the resting condition. (g) A vacuole containing a myxamoeba.

FIG. LV Plasmodium which has just coalesced with three uninucleate plasmodia. Five of the six large nuclei are shown. The three small nuclei belong to the uninucleate plasmodia which have coalesced. At (a) a uninucleate plasmodium has been engulfed which has itself engulfed a myxamoeba.



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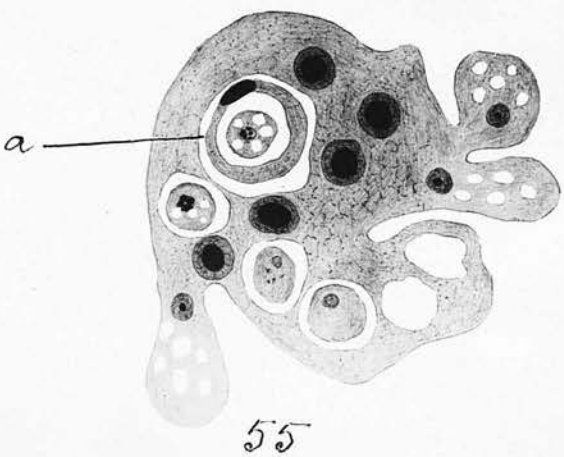

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PLATE X.

P L A T E XI.

FIG. LVI. Portion of plasmodium photographed in PL. XVIII, FIG. LXXXIX. (a) Plasmodia which have coalesced with the large plasmodium and contain nuclei of the small type. (b) A somewhat larger nucleus the nucleolus of which has not yet commenced to increase in size. (c) Large nucleus with nucleolus one-third the diameter of the nucleus. (d) Large nucleus with nucleolus one-half the diameter of the nucleus. (e) Normal large nuclei with nucleoli three-quarters the diameter of the nucleus.

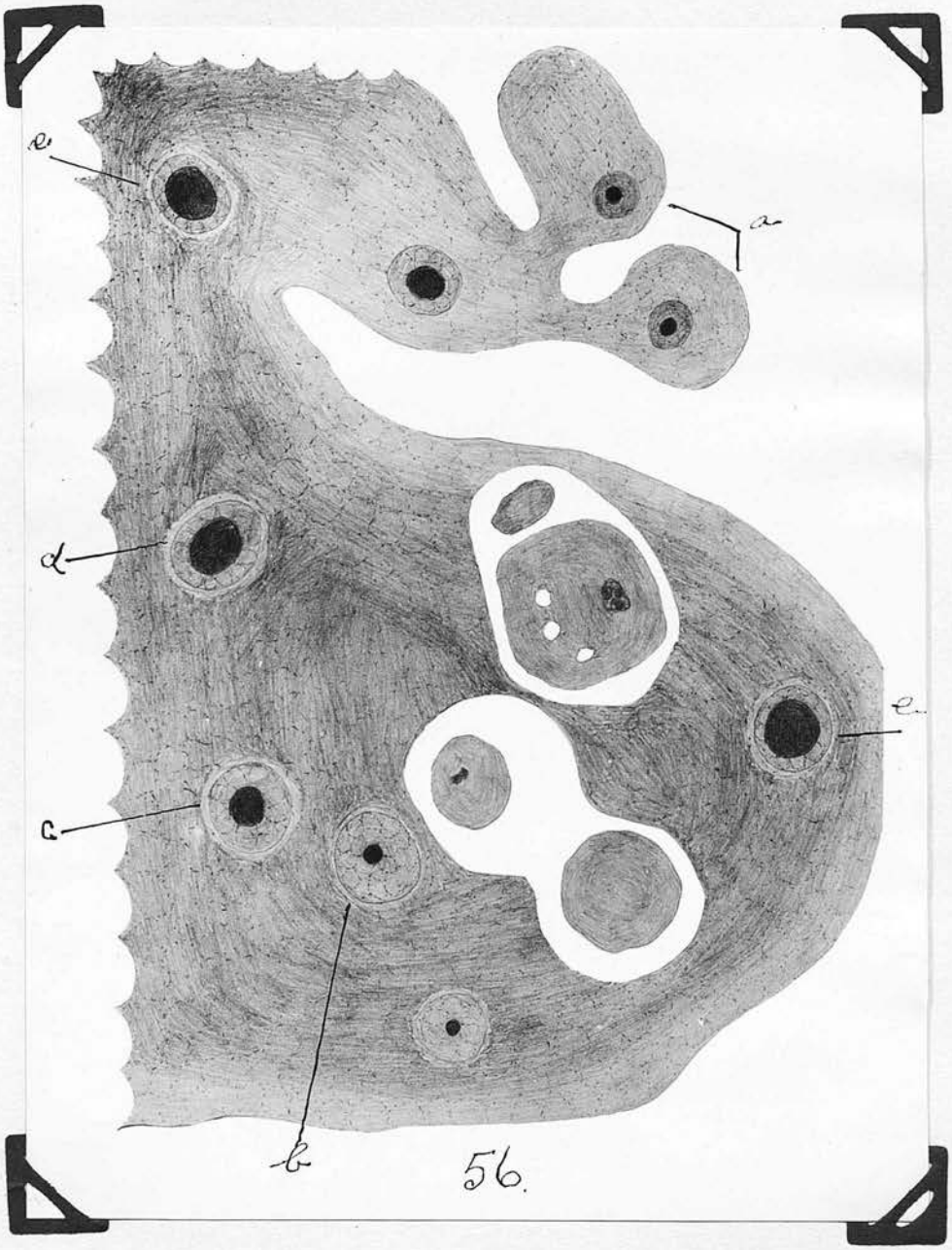


PLATE XI.

P L A T E XII.

FIG. LVII An 8-nucleate plasmodium about to ingest a myxamoeba. In the bottom right hand corner a myxamoeba is present in an advanced stage of digestion.

FIG. LVIII A 14-nucleate plasmodium, only 10 of the nuclei being shown. A pseudopodium has grown out and almost enclosed a myxamoeba. Two inclusions are present which are at an advanced stage of digestion.

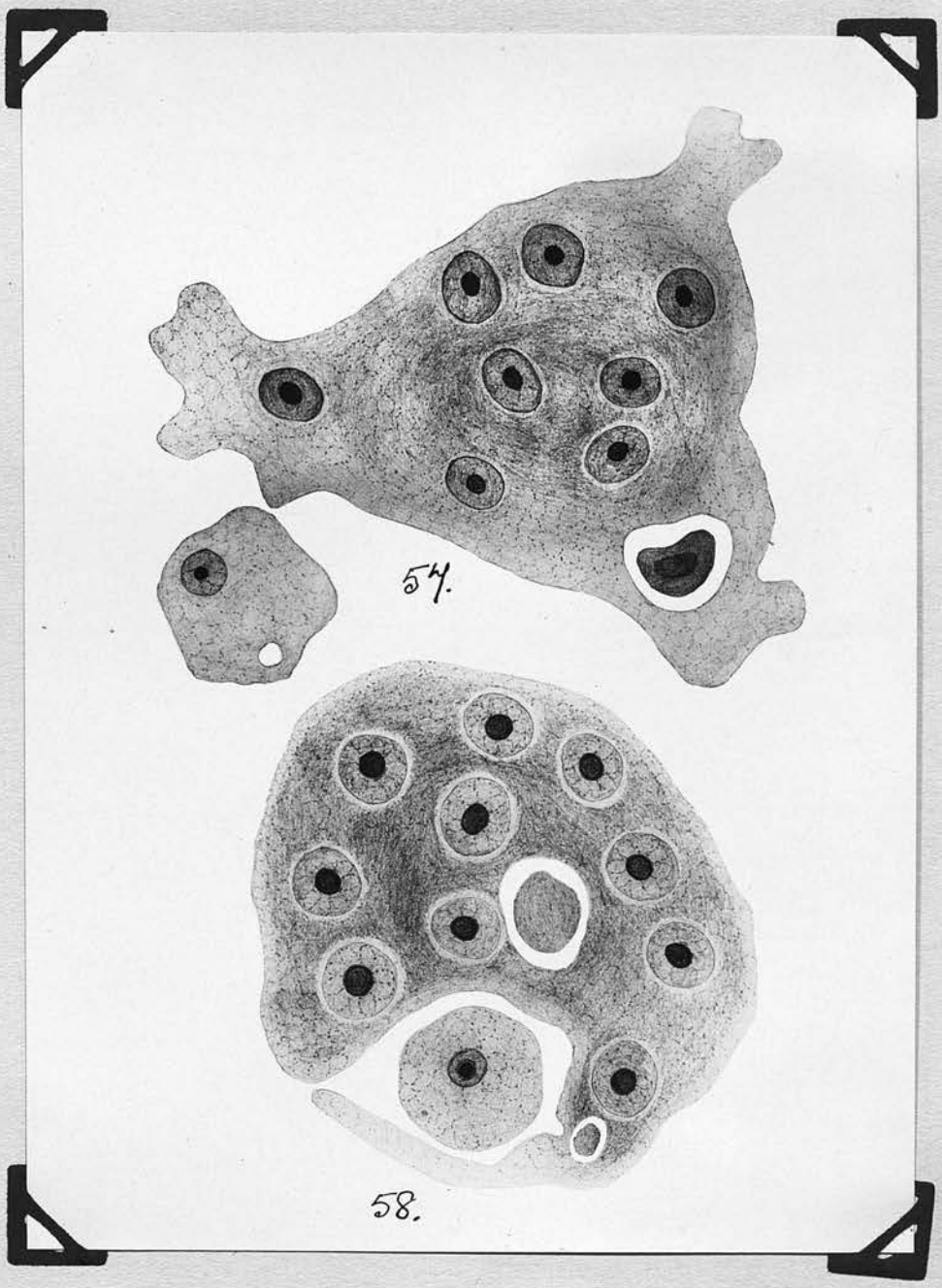


PLATE XII.

PLATE XIII

FIG. LIX A 4-nucleate plasmodium with three vacuoles containing myxamoebae at various stages of digestion. In the two upper vacuoles the myxamoebae are at a very early stage; in the lower one the myxamoeba is at a very advanced stage.

FIG. LX 1-nucleate plasmodium the nucleus of which is not shown. Two myxamoebae at an advanced stage of digestion are present.

FIG. LXI Portion of mature plasmodium just before spore formation. (a) Three nuclei (b) Two empty spore coats.

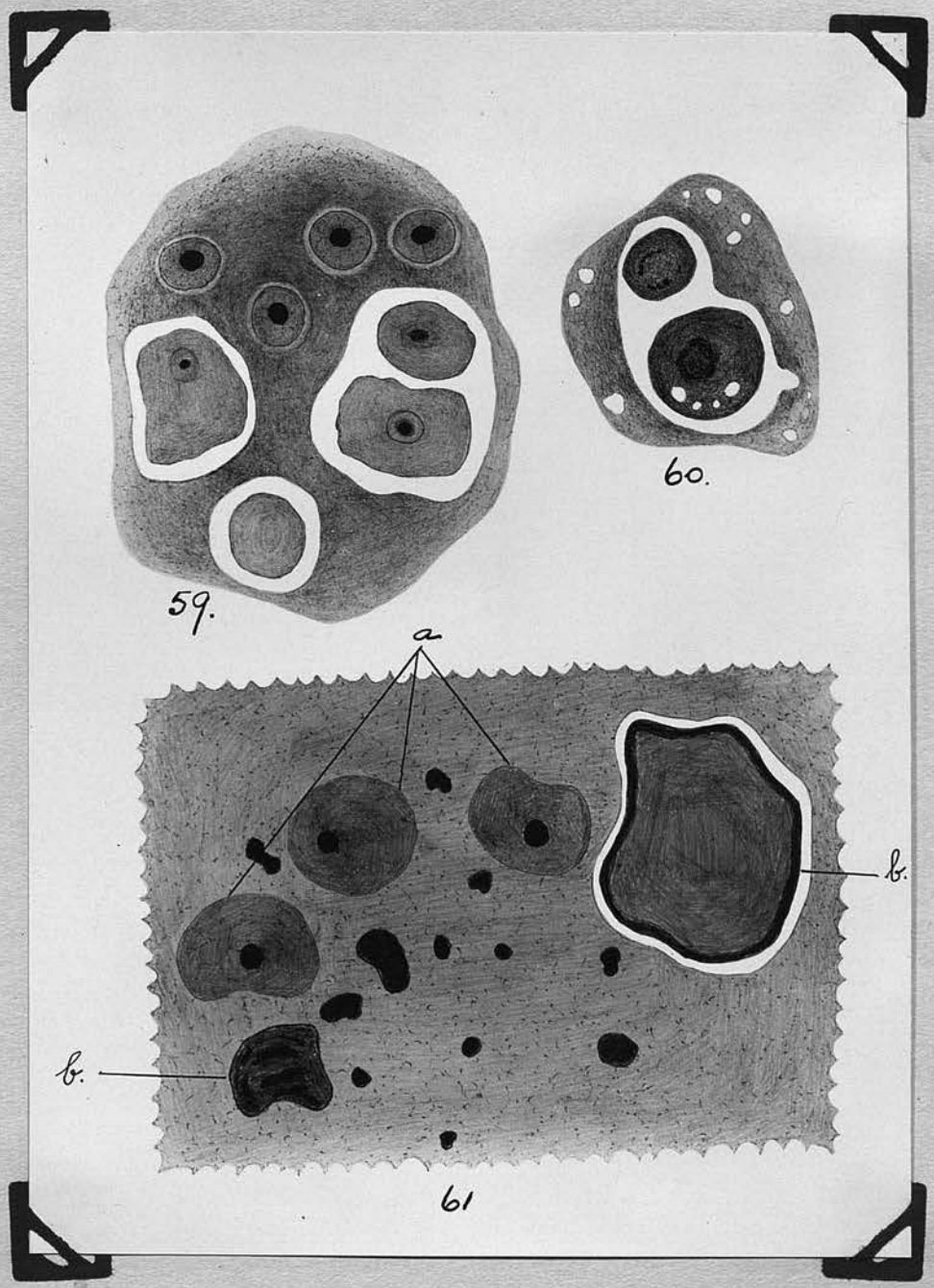


PLATE XIII

PLATE XIV.

FIG. LXII. Portion of the hypothallus, showing the large number of all kinds of inclusions present.

FIG. LXIII. Portion of sporogenous layer showing the numerous nuclei. Condition at 9.a.m.

FIG. LXIV. Portion of the degenerating protoplasm present in the centre of the sporangium at 9.a.m. (a) degenerating nuclei. (b) Normal nucleus in a vacuole.

FIG. LXV. A nucleus which has divided by direct division previous to degeneration.

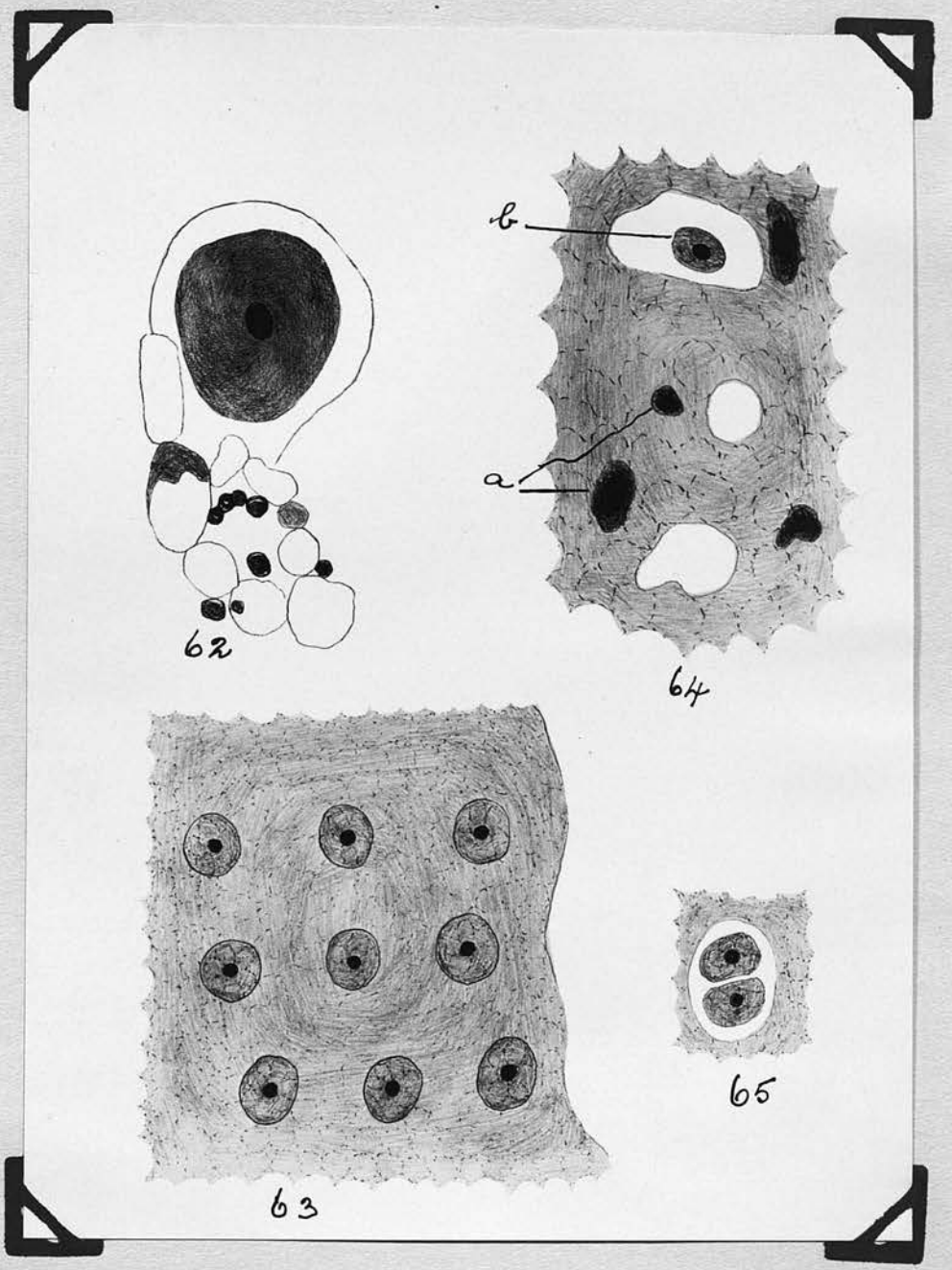


PLATE XIV.

P L A T E XV.

FIGS. LXVI & LXVII — Stages in synapsis of the heterotype division.

FIG. LXVIII — Stage just preceding metaphase. The nuclear membrane is present and 4 U-shaped chromosomes can be seen.

FIG. LXIX — Metaphase.

FIG. LXX — Anaphase.

FIG. LXXI — Early telophase.

FIG. LXXII — First stage in the homotype division. The spindle of the heterotype division is still visible and the spindles for the homotype division have commenced to form. The chromosomes have not yet been formed.

PLATE XV.

P L A T E XVI.

FIG. LXXIII Metaphase of the homotype division. Four spherical chromosomes are present.

FIGS. LXXIV & LXXV Anaphase. In FIG. LXXV the daughter nuclei are being separated by a cleavage furrow.

FIG. LXXVI Anaphase in a piece of protoplasm of two-spore capacity.

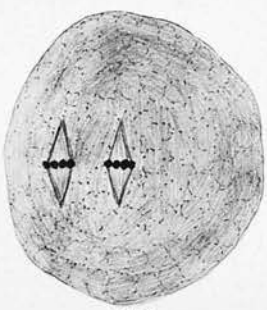
FIG. LXXVII Telophase.

FIG. LXXVIII Late telophase. A deep constriction of the protoplasm is seen between the daughter nuclei.

FIG. LXXIX Young uninucleate spore. The nucleus stains homogeneously.

FIG. LXXX Young spore in which the nucleus has been reconstructed and a distinct nucleolus is present.

FIG. LXXXI. First stage in wall formation. A vacuole has appeared round the nucleus and the protoplasm has become slightly muddy in appearance.



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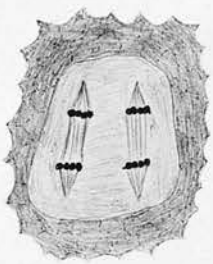
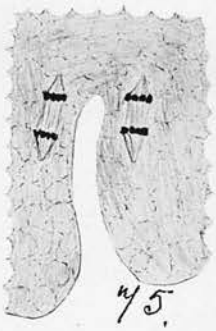
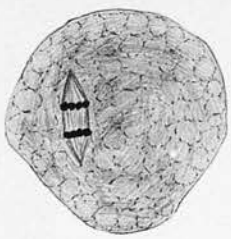


44.



45.



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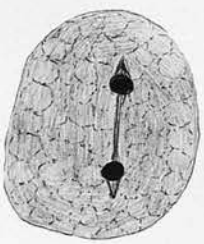
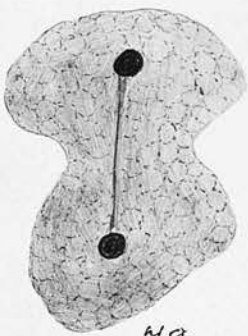


47.



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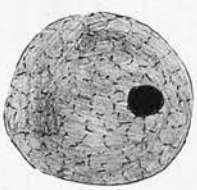

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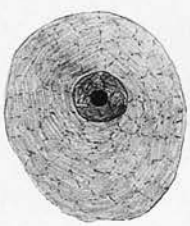
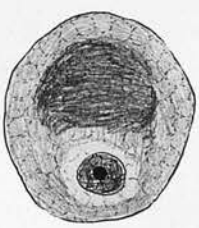


80.



81.

PLATE XVI.

P L A T E XVII.

FIG. LXXXII Somewhat later stage. There is a thin layer present round the periphery of the cell. Five large vacuoles are present and the protoplasm stains homogeneously and deeply.

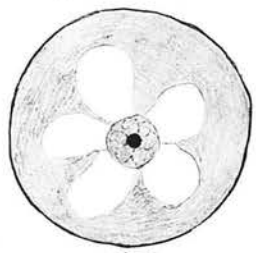
FIG. LXXXIII The diameter of the spore has decreased and the spore coat has fallen into ridges. Two vacuoles are present filled with brownish contents. The nucleus is inconspicuous and distorted in shape.

FIG. LXXXIV A single vacuole is present containing a yellowish body. There is a distinct space between the protoplast and the spore wall. The nucleus is normal and the protoplasm stains less deeply.

FIG. LXXXV Vacuole and inclusion still present. The protoplasm has regained its alveolar structure.

FIG. LXXXVI Vacuole and inclusion have almost disappeared but a paler area indicates their position.

FIG. LXXXVII The ripe spore. No signs of the vacuole and its contents remain.



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87.

PLATE XVII.

P L A T E XVIII.

FIG. LXXXVIII Photograph of a plasmodium growing on carrot agar in a petri-dish. Magnified about 3 times.

FIG. LXXXIX Microphotograph of a young plasmodium in process of coalescence with a number of uninucleate plasmodia magnified about 550 times.

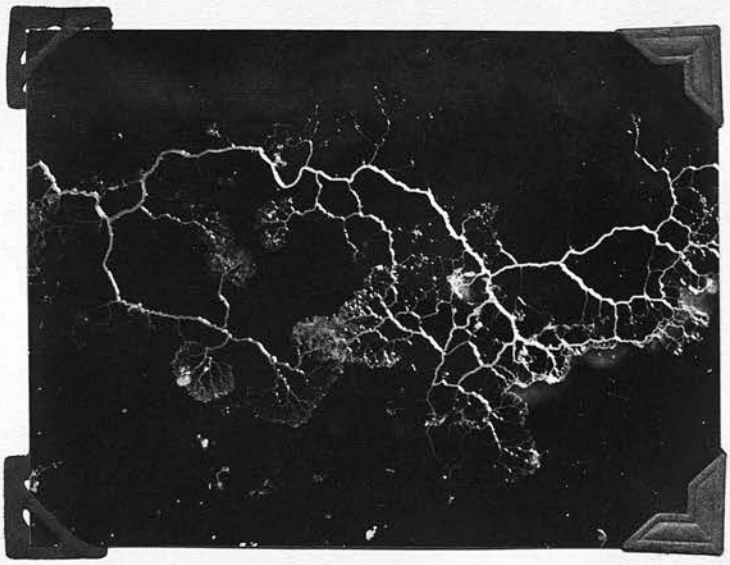

FIG. LXXXVIII

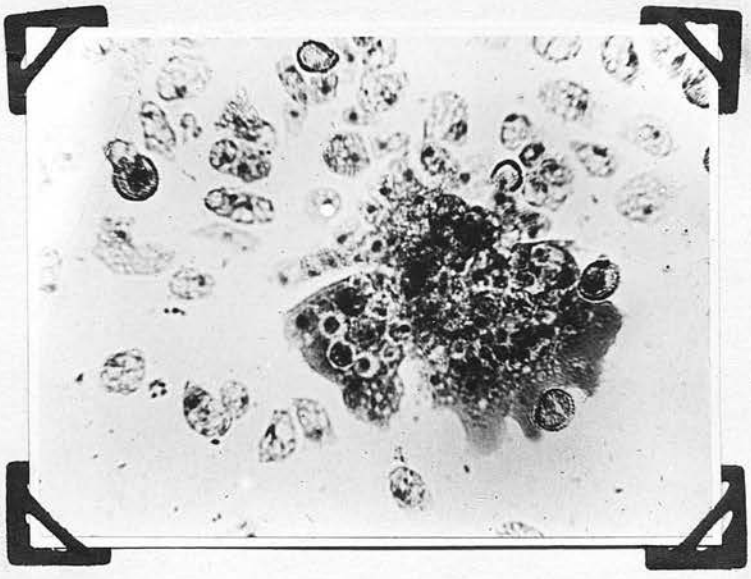

FIG. LXXXIX.

PLATE XVIII.

PLATE XIX.

FIG. XC Photograph of two young sporangia about the time at which meiosis is taking place. Magnified 10 times.

FIG. XCI Photograph of the same two sporangia after the spores have ripened. Note the decrease in size. Magnified 10 times.

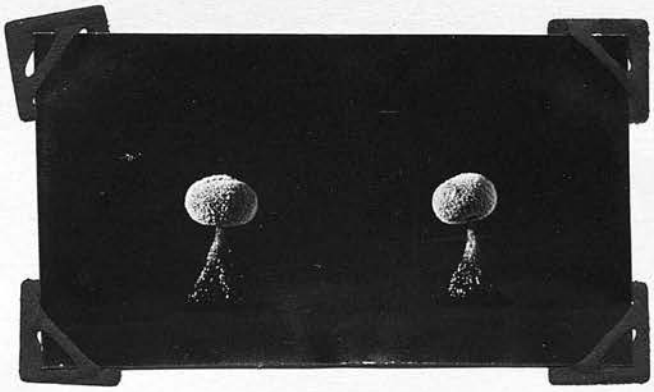

FIG. XC.

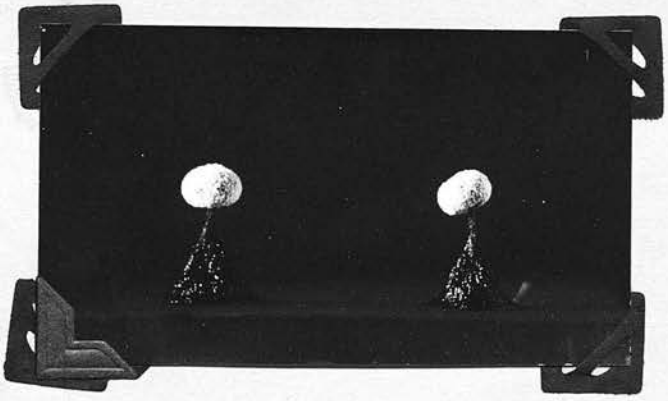

FIG. XCI.

PLATE XIX.

The following figures are microphotographs of sections through young sporangia at successive stages in development. All the preparations were stained with Flemming's triple stain.

P L A T E XX.

FIG. XCII Young sporangium which has just risen from the surface of the nutrientmedium magnified 65 times.

FIG. XCIII Slightly older sporangium. (a) hypothallus (b) stalk (c) Sporogenous tract (d) degenerating protoplasm. Magnified 65 times.

Condition at 9 a.m.

FIG. XCII.

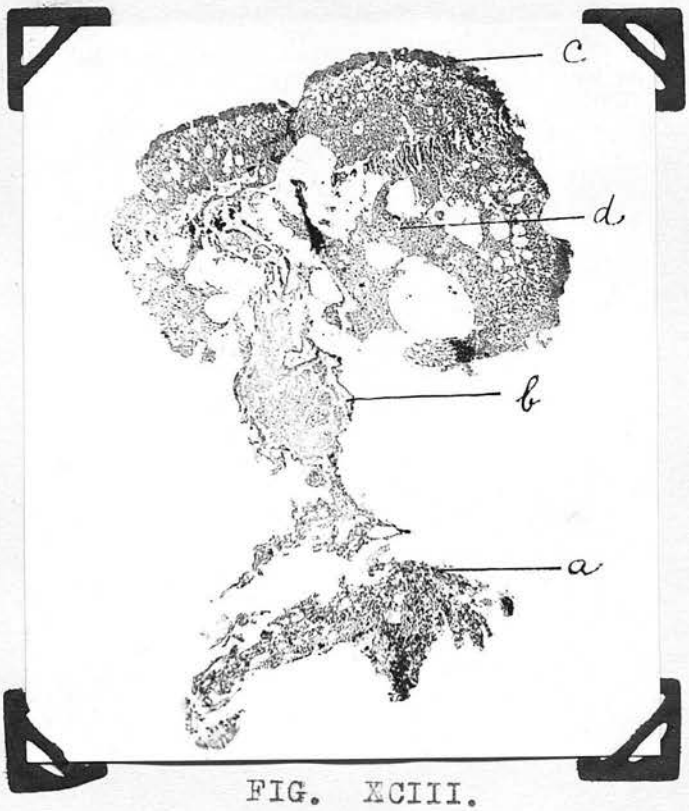

FIG. XCIII.

PLATE XX.

P L A T E XXI.

FIG. XCIV Section through young sporangium at 10-45 a.m. (a) Broad, structureless layer which will condense to form the wall. (b) Furrows into sporogenous protoplasm. (c) Degenerating protoplasm at inner margin of sporogenous tract. Magnified 150 times.

FIG. XCV A slightly later stage in which the wall of the sporangium has been formed by the condensation of the broad, structureless layer. Magnified 125 times.

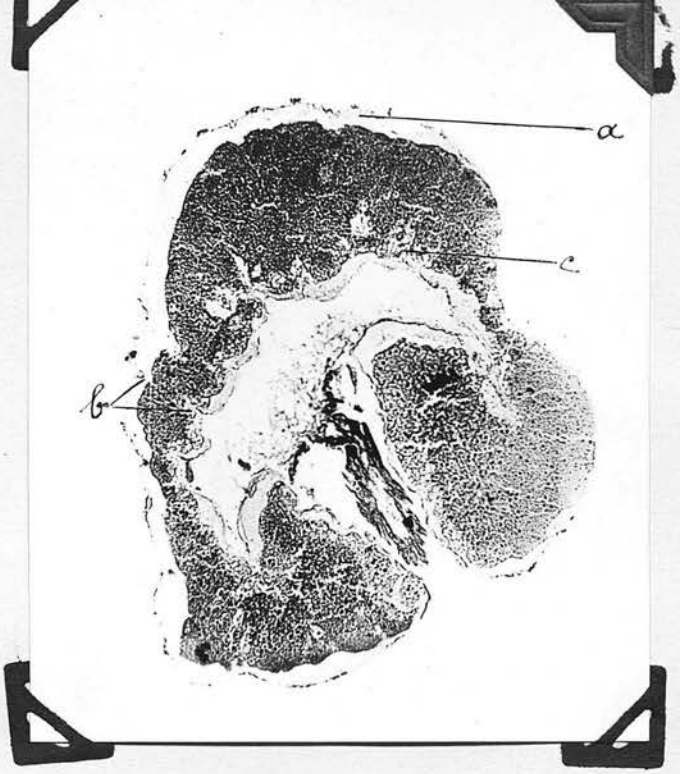

FIG. XCIV.

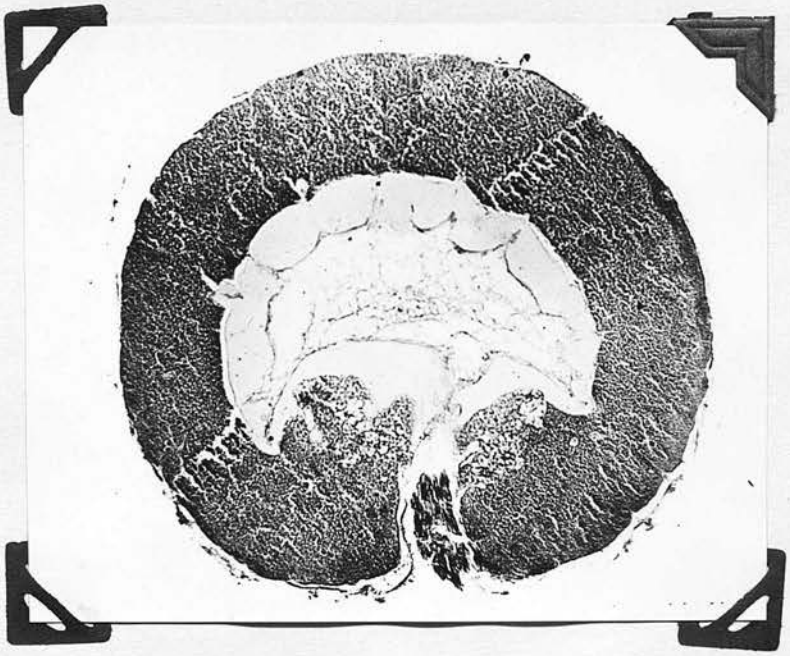

FIG. XCV.

PLATE XXI.

P L A T E XXII.

FIG. XCVI Section through young sporangium at noon.

(a) Furrow containing capillitial thread. The furrows have increased in number. Magnified 100 times.

FIG. XCVII Section through sporangium at 2 p.m.

(a) Capillitial thread. Cleavage of the sporogenous tract has taken place. Magnified 150 times.

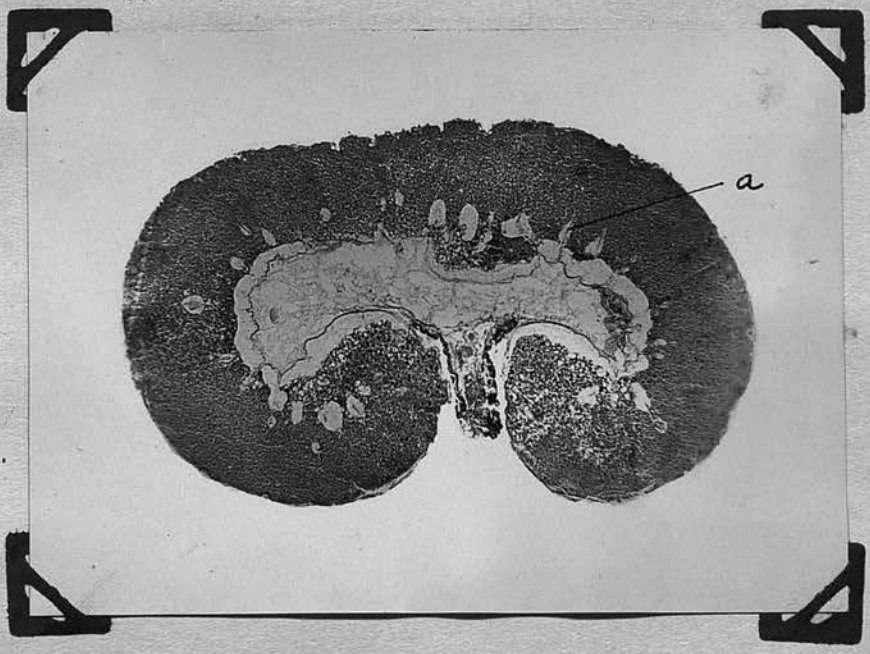

FIG. XCVI.

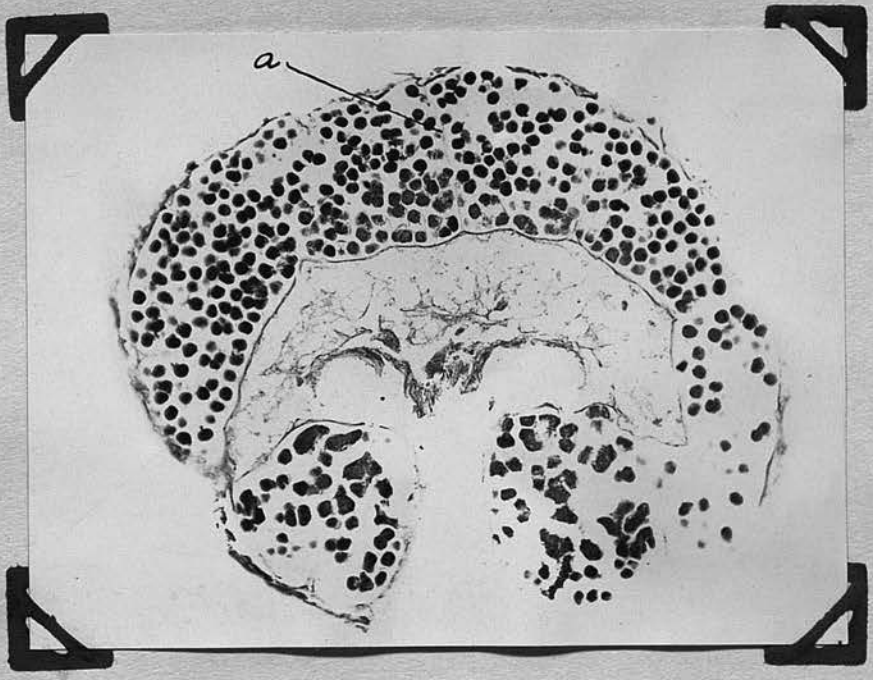

FIG. XCVII.

PLATE XXII.

P L A T E XXIII.

FIG. XCVIII Abnormal case in which a large piece of protoplasm in the right lower corner has been cut off from the general course of development and is degenerating. Magnified 150 times.

FIG. XCIX Somewhat earlier stage in cleavage than that shown in FIG. XCVII. Magnified 90 times.

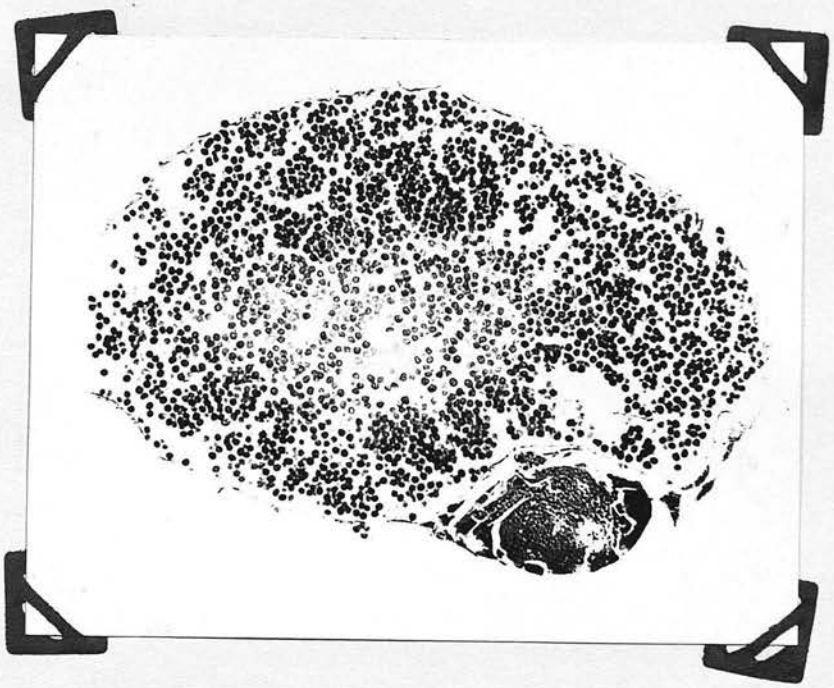

FIG. XCVIII.

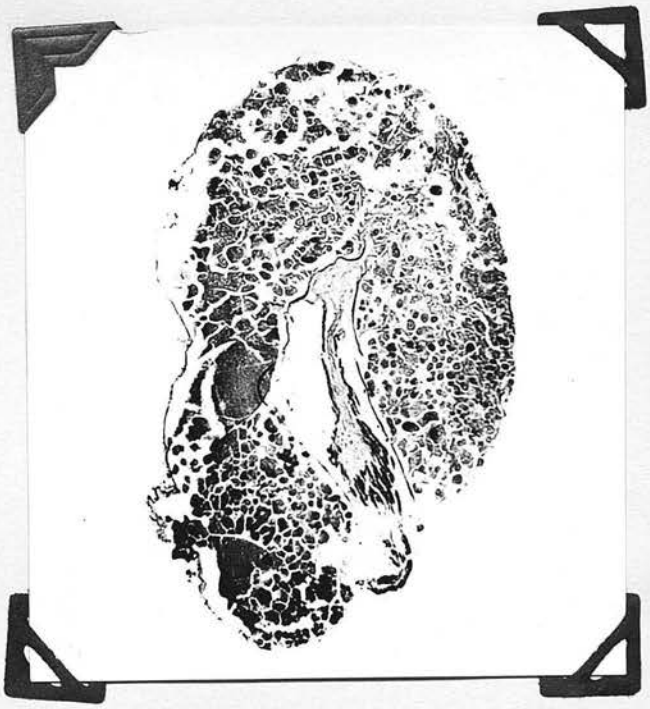

FIG. XCIX.

PLATE XXIII.

PLATE XXIV.

FIG. C Section through ripe sporangium at 4-45 p.m. Magnified 115 times.

FIG. C.

PLATE XXIV.