Cytology of the Ascomycetes. Pustularia bolarioides Ramsb.

I. Spore Development.

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With Plates V–VIII and three Figures in the Text.

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I. Introduction.[1]

OUR fundamental knowledge of the cytology of the Ascomycetes has been rendered almost complete by the researches of Dangeard (20, 21, 22), Harper (52, 53, 54, 55, 56, 57, 58), Maire (62, 63), Guillermond (49, 50, 51), Blackman and Fraser (7, 8), Claussen (15, 16), Fraser (36, 37), Fraser and Brooks (41), and Fraser and Welsford (43). It is now admitted that the Ascomycetes have a sexual process, however modified and reduced, in their life-cycle. It is also admitted that there is a process of reduction of chromosomes which in all essential details follows the scheme of reduction of chromosomes of the meiotic phase of animals and plants.

The Ascomycetes in general exhibit an alternation of generations which can be compared in all important details with that of the higher plants. The two generations are not very sharply separated from one another, and the separation line may be shifted in the life-cycle of the members of different families.

Researches on the life-history of the Green Algae (2), Brown Algae (32, 86), Red Algae (85), Rusts (6, 9, 14), Mosses (35, 64), and Ferns (30) have thrown sufficient light on the question of alternation of generations in general. A comparative study of the life-history of these organisms shows that the cytological alternation of generations is not always restricted to morphologically differentiated structures. In the lower groups of plants one is struck with the great diversity of the organs where reduction takes place, while in the other groups the representative units of the two generations may be included in an organ which is morphologically and physiologically identical. The Ascomycetes, with certain modifications, present a type that falls into the last category.

The doubling of the number of chromosomes takes place during the process of fusion of the sexual nuclei. This fusion is completed in the ascogenous hyphae, and the ascogenous hyphae containing the paired nuclei corresponds to the sporophyte, and the young ascus with the definitive nucleus to the spore mother-cell. The sporophytic generation is terminated by the divisions in the ascus, which precede the formation of ascospores when the numerical reduction of chromosomes takes place. The spore on germination produces mycelium, which corresponds to the gametophyte, and in the case of highly sexual Ascomycetes the sexual organs, the homologues of antheridia and the archegonia, are borne on it and normal fertilization is effected, while in the absence of these sexual organs or their homologues

[1] In naming this fungus I am indebted to Mr. J. Ramsbottom, M.A., F.L.S., President of the British Mycological Society, for his kindness in supplying me with the names of the nearest generic and specific allies, as well as the authorities on which this identification is based. The fungus will be fully described in a later publication.

there is, naturally, a wide range of modification of the process by which the two sexual units are brought together. The cycle of alternation of generations is thus complete when we reach the ascogenous hyphae with paired nuclei, the sporophyte.

The complete scheme of alternation of generations in the life-history of the Ascomycetes was first advanced by Harper (58); later on it has been supported by Overton (70) and Strasburger (82, 83), and more recently by Claussen (16). There are, however, several important points of controversy in the detailed account of the nuclear history.

Harper (57, 58), Blackman and Fraser (7, 8), Blackman and Welsford (10), Fraser (36, 37, 39), Fraser and Welsford (43), Fraser and Brooks (41), Cutting (17), Carruthers (13), and Claussen (15) hold that there are two nuclear fusions in the life-history of the Ascomycetes. The first of these two fusions has a true sexual significance. It may take place between the normal gametophytic nuclei of the antheridium and ascogonium. Such processes of normal fertilization in the Ascomycetes have been reported by Harper in *Sphaerotheca* (53, 54), *Erysiphe* (54), *Pyronema* (57), and *Phyllactinia* (58), by Barker in *Monascus* (3), and by Blackman and Fraser in *Sphaerotheca* (7). The absence of such functional male organ involves a reduced sexual process. Fusion takes place between the nuclei of the same female organs. This may again take place in the presence of an abortive male organ, as is recorded in *Ascobolus furfuraceus* by Miss Welsford (84), in *Lachnea stercorea* by Fraser (36), in *Aspergillus repens* by Dale (19); or the male organ may be entirely absent, as in the case of *Humaria granulata* recorded by Blackman and Fraser (8), and in *Lachnea cretea* by Fraser (39).

Similar abnormal sexual process, 'where the sexual fusion of gametes is replaced by a fusion of ordinary gametophytic nuclei which morphologically are not sexually differentiated', has been previously described by Farmer and Digby (30), who worked on Ferns, as *Pseudo-aspogamy*. Certain cases of extreme modification of this process have been discovered by Fraser (37) in *Humaria rutilans*, and by Carruthers in *Helvella crispa* (13), where, in the absence of the ascogonium, the gametophytic nuclei are supplied by the vegetative hyphae of the hypothecium. Such phenomena, as exhibited by *Humaria rutilans* and *Helvella crispa*, where a hypothecial nucleus migrates into an adjoining cell to effect an apogamous fusion, have been defined by Fraser and Chambers (42) as *Pseudogamy*; and this has been compared to the reduced sexual process observed by Farmer and Digby (30) in the prothallium of *Lastrea pseudomas*, var. *polydactyla*.

The nature of the subsequent fusion of the nuclei is 'asexual' (Harper (58), Blackman and Fraser (7), and Fraser (37, &c.)), and its significance is to bring about the 'nucleo-cytoplasmic equilibrium' (Harper (58)) in the ascus.

Thus it may be seen that, according to this doctrine, the fusion in the ascus is always preceded by a fusion in the ascogonium or in an organ homologous to it. As a consequence of the two nuclear fusions, it is claimed that the chromosomes are subjected to two reduction processes (Fraser (37, &c.)); the first of which is *meiotic*, when a true numerical reduction takes place as laid down by Farmer and Moore (31) for the reduction of chromosomes during the meiotic phase of animals and plants. The succeeding division is *brachymeiotic*, which, 'as it lacks a second contraction, admits of less variation in its products than meiosis, and implies either the separation of entire nuclei which fused or at any rate sorting of unaltered chromosomes' (Fraser and Welsford (43)).

Dangeard (22, 23) and Maire (63), however, are in agreement on the question of sexual fusion at the origin of the definitive nucleus, but, contrary to the hypothesis of Harper, Blackman, and Fraser, they have maintained that there is only one nuclear fusion in the life-history of the Ascomycetes, which is followed by one reduction of chromosomes. Maire has explained that the association of the nuclei takes place in the ascogonium. The nuclei divide conjugately, as some sexual nuclei of animals. The fusion of the paired nuclei takes place in the ascogenous hyphae. He has designated this process by the name of *synkarion*. He has thus brought the sexuality of the Ascomycetes into line with the Basidiomycetes (62). The later discoveries of Blackman (6, 9) and Christman (14) on the formation of synkarion in the Uredineae have given support to the hypothesis of Maire.

Claussen (16) has reworked the life-history of *Pyronema confluens* in great detail, and has upheld Maire's observations on the synkarion formation of ascus nuclei. The paired nuclei travel along the ascogenous hyphae and ultimately migrate into the crosier of the young ascus, where they divide, and a *single* union between the descendants of the sexual pronuclei takes place in the young ascus. As regards the three divisions of the ascus nuclei, Claussen supports Guilliermond and others who disagree with two reductions, and confirms the view that the number of chromosomes remains the same in the metaphases as well as in the telophases of all the three divisions; according to his contention there is no sufficient basis for the hypothesis of *brachymeiosis*.

Faull (34) has strongly supported Claussen's view of nuclear migration from antheridium to oogonium where pairing of nuclei takes place, and is followed by a series of conjugate divisions and final fusion of the sexual nuclei in the young ascus; while on the ground of his own observations on the cytology of *Laboulbenia chaetophora* and *L. Gyrinidarum* he has called in question the phenomenon of second reduction.

Following Claussen's work, Shikorra has reworked the life-history of *Monascus* (78) and Ramlow that of *Ascobolus furfuraceus* and *Ascophanus*

carneus (72), and both have confirmed Claussen's observation with regard to the single nuclear fusion in the life-history of the Ascomycetes; and Strasburger (83) has criticized the theory of *brachymeiosis* of Fraser, and has given his support in favour of Claussen.

It seemed, therefore, that the problem was open to further investigation, and a study of the cytology of the Ascomycetes with improved knowledge of technique might help to reconcile the two different views. The following research explains to a certain extent some of the discordant views of the two accounts, and at the same time extends our rather limited knowledge of the nuclear phenomenon in the life-history of the Ascomycetes.

Material. The fungus under investigation was first found at Oxshott, Surrey, in October 1922, on a foray held by the British Mycological Society for students of the London Colleges. Since that time it has been found repeatedly at Oxshott, on Farnham Common and Stoke Common, Bucks., and at Mitcham, Surrey. Invariably it has been found associated with *Epilobium angustifolium*, most frequently being found immediately beneath it. It is of a beautiful salmon-pink colour, though occasionally, in a much younger state, and, very rarely, in well-developed cups, it is somewhat paler. Its nearest ally is *Humaria bolaris* Bresadela ('Fungi Tridentini', ii, p. 73 t.; cxciii, f. 1, 1898), which it resembles in colour and in microscopic structure, but differs from in the size of the parts; for example, it has smaller spores. The generic name is used in the sense of Boudier ('Histoire et Classification des Discomycetes de l'Europe').

II. Methods.

Some of the more common fixatives were first employed, their dilutions as well as the time for which they were used being carefully worked out. Flemming's strong fluid, Flemming's strong fluid diluted with equal parts of water, and Flemming's weak fluid produced more or less the same effect on the chromatin, when the time exposure in case of the weaker fluids was almost doubled. The cytoplasm was not, however, fixed equally well in both the cases. The stronger fluids made the cytoplasm coarser, which was detected even by staining with haematoxylin, while the two weaker solutions gave it a finer appearance.

Hermann's fluid diluted with equal parts of water was found very satisfactory when the time allowed for exposure was extended to from twenty-four to thirty hours. Later on, it was found that the osmic acid in that solution could be reduced to half its normal quantity without affecting the fixation at all, while at the same time the material was less blackened. It is very difficult to clear the thick cytoplasm of the ascus from the osmic acid, and, unless the minimum amount of it is used, it always leaves a greyish colour, even after the use of hydrogen peroxide for a considerable time.

The difference in the behaviour of two lots of material, one fixed in Hermann's fluid diluted to half and the other in Flemming's weak fluid, was then compared. In a precipitation stain like Heidenhain's haematoxylin the difference was not so very appreciable, and as a matter of fact the cytoplasm of the material fixed in weak Flemming's was finer, while the chromatin appeared equally homogeneous ; but when the material was subjected to a transparent stain, like Breinl or the triple combination of Flemming, the difference was clearly apparent. It was found that chromatin appeared very uniform, and the differentiation of chromatin and linin is much better seen in the material fixed in Hermann's fluid. The cytoplasm, on the other hand, took an intermediate position between the coarseness of material fixed with Flemming's strong fluid and the fineness of that fixed with weak Flemming's fluid. A balance was thus achieved between the precipitation of chromatin and linin inside and the cytoplasm outside the nucleus. The material fixed in half-strength Hermann's fluid, with half the normal quantity of osmic acid, was then restricted to the latter part of the work. The material was fixed mostly in the field, with the aid of an air-pump to secure proper infiltration of the fixing fluid ; while a vast quantity of very young material was brought in the evening into the laboratory on large sods. This was kept under a bell-jar in the greenhouse, and was fixed at intervals of an hour during the night in order to ascertain the state of the nuclei at that time.

The older apothecia were taken through different grades of alcohol, cleared in xylol, and embedded in paraffin in the ordinary way recommended for cytological investigations. The young ascocarps were taken through 10 per cent. glycerin and slowly evaporated to pure, as recommended by Blackman (6) and Miss Digby (28). From pure glycerin they were taken through four grades of glycerin-alcohol mixture to absolute alcohol, and were cleared in cedar-wood oil. The whole process of clearing and embedding was completed within four to five hours' time.

Though Heidenhain's haematoxylin-stained preparations, with or without a counter-stain of either orange G or erythrosine in clove oil, were used in drawing many of the figures of the prophase stage, the results were always checked by a Breinl or Flemming's triple-stain preparation. These preparations were used throughout the investigation in counting the number of chromosomes. When Gram's iodine was used as mordant the chromatin took a deeper violet and the linin retained a considerable amount of blue stain of the methylene polychrome, thus giving a good contrast. This was found useful, as the chromosomes are exceedingly small, and this violet shade was convenient for counting them. The safranin used was matured with a few drops of anilin oil ; this method helped the chromatin to retain the safranin longer, as anilin oil was used for the process of clearing the Breinl preparation.

As the greater portion of the nucleus could be included in a 10 μ thick section, the prophase figures when not otherwise stated have been drawn from 10 and 8 μ sections. Figures of first division of the ascus have been drawn from 8 and 6 μ sections, figures of the second division from 6 μ sections, while 5 and 4 μ sections have been used in counting chromosomes of the second and third telophase and for the study of the spore formation.

III. The Heterotype or First Meiotic Division.[1]

(a) *First Contraction.*

Soon after the fusion of the two gametic nuclei, the definitive nucleus of the ascus, which is the result of this union, passes into a contraction phase. The definitive nucleus at this stage presents a character which resembles in all essential details that of phanerogams and higher cryptogams. The chromatin element is embedded in a condensed matrix of linin and is balled into a conglomerate mass (Pl. V, Fig. 1, *a*). In a favourably stained Breinl preparation, the chromosomes can be differentiated from the linin matrix, though it seems to be impossible to ascertain their number correctly. The nucleolus is of perfectly spherical shape and is very prominent at this stage. It stains deeply, and is attached to one side of the chromatin aggregate and is not in any way hidden inside it. The chromatic 'ball' moves to a side of the nucleus and is separated from the delicate nuclear membrane on three sides by clear space, while it is attached to it on one side: in other words, the chromatin is polarized. The cytoplasm of the ascus is fine-grained, and is vacuolated towards the upper part of the ascus. A few faintly stained extruded chromatin bodies are seen passing out of this mass and are degenerating in the vacuolated cytoplasm of the ascus.

The stage of first contraction when the nuclear element is massed into a tight knot lasts only for a short time. This, perhaps, explains why it has so often been overlooked by other workers on the cytology of the Ascomycetes. Fraser (37) is of opinion that in *Humaria rutilans* the nuclei undergo first contraction before they unite to form a definitive nucleus; while in *Lachnea stercorea* (41) the definitive nucleus passes into the first contraction phase after the fusion in the ascus, and this is followed by second contraction.

[1] In order to describe this phenomenon, it seems advisable to follow the conventional terminology used to explain the meiotic phases of animals and plants in the strict sense; it will be seen when following the nuclear history that the sequence of events of different stages in *Pustularia* follows closely the corresponding stages of the phenomenon in the higher plants and animals. The terms *thread*, *filament*, *association*, *dissociation*, *conjunction*, and *disjunction* are used in the same sense in which Miss Digby (28) defined them in her paper on *Osmunda*. The word *reticulum* has been used in a general sense where the spireme loses its definite character and thus cannot be clearly traced.

Occasionally during the early prophase stages cases of bilateral massing of the spireme, as shown by Pl. V, Fig. 2, have been noticed. They resemble Fraser's Fig. 53 for *Humaria rutilans* (37). In such cases the two separate spiremes which come together after the nuclear fusion lie in the nuclear cavity like two coils of thread bridged over by cross-connexions. Nuclei showing this kind of irregularity have been seen only three or four times and have not therefore given sufficient opportunity for further observation. As they have not been seen in any other transition stages, it seems quite possible that they may have lost this peculiarity as soon as the spireme opens out. They show only delayed fusion of the nuclear contents.

(b) *Synapsis and Hollow-spireme Stage.*

As the nucleus comes out of the first contraction stage, there appear open spaces in the linin substratum. The spireme opens out in distinct parallel threads, which are bounded here and there by the vacuolated space thus formed. The chromosomes which are embedded in the parallel linin gradually become separated from one another. Their number at the earliest stage of the opening of the knot can be determined in well-stained Breinl preparations. There are over twenty-eight bean-shaped chromosomes strung in a broad band of linin thread which can be more or less accurately counted in the stage shown by the bigger ascus nucleus of Pl. V, Fig. 1, *b*. From this stage onwards there is a gradual enlargement of the nuclear cavity.

As the spireme opens out farther, the length of the loops increases and the parallelism of the thread becomes increasingly evident. The uniformly bean-shaped chromosomes split up into smaller chromatin beads of unequal size and shape, which are strung serially on the parallel thread. When the loop has attained a considerable length, it undergoes a twist in the middle and the head of the loop bends over the parallel arms on to the twist (Pl. V, Fig. 3). The nucleolus at this stage moves to the side remotest from the main mass of chromatin. As soon as the folding of the first loop is completed, others, which are visible in the initial stage in the main mass of the spireme, begin to open out. The number of chromatin beads increases as the process of formation of these loops advances. The spireme emerges out from the tangle of synapsis, and the lumpy mass is reduced to a condensed aggregation of short loops which retain a polarized appearance for a considerable time (Pl. V, Fig. 4). The nucleolus becomes entangled inside the loops, and it can be seen distinctly from Pl. V, Fig. 4 that the spireme forms a continuous series of loops without any free end.

The synaptic knot continues to unfold and the loops distribute themselves in the nuclear cavity in parallel series (Pl. V, Fig. 5). The chromatin beads are separated farther apart from each other than they were during

the earlier stages of the opening of the spireme. The nucleus attains its maximum size at this stage. The opening of the spireme does not keep pace with the extension of the nuclear cavity, consequently the spireme is not uniformly distributed in the beginning. The nucleus has an oval shape, and the first-formed loop occupies the long axis of the nucleus, while the others distribute themselves on both sides of it. A fully opened spireme after contraction is shown by Pl. V, Fig. 6. At this stage, the spireme is uniformly distributed in the nuclear cavity. The loops retain the parallel arrangement of their distribution in a more or less elongated area of the nucleus. There are about seven loops at this stage. Two of them in Pl. V, Fig. 6 are to be seen in the same focus. They are more prominent than the others, and appear as if they were interlocked. One of them, slightly the bigger of the two, *a*, has undergone a twist, while the other, *b*, has only formed an open loop with crossed arms, but has not undergone any twisting. This shows that the process of looping and twisting may not take place simultaneously. The two arms run parallel almost the whole length of the nucleus, and towards the lower side they are covered by the nucleolus and cannot be farther traced.

The chromatin beads are arranged in a linear series in a thick and rather broad band of linin which completely encloses them. The size and shape of the individual beads can be more readily determined at this stage. Their shape varies a good deal. Some are bean-shaped, others appear slightly oblong or rod-shaped, while a third kind approximate to the triangular. These last, when they occur at the bends of the loops, protrude from the linin band and are conical in form. The spacing of the beads along the spireme is much more irregular, and it will be seen later that this irregularity increases in the different stages in the course of synapsis. At this stage they are more regular than at any other. Though they are well differentiated by safranin or by Breinl stain from the band of linin, the overlying threads of the spireme which cross each other in different directions render any accurate estimate of the number of these beads impossible. It is very clearly seen at this stage that there is no nuclear membrane. The spireme merges into the cytoplasm. The cytoplasm is uniformly thick towards the upper part of the nucleus and extends to the apex of the ascus, while it is vacuolated and loosely spongy towards the lower part of the nucleus, and lower down the ascus tube it forms only a limiting layer. The extrusion of the chromatin bodies increases considerably at this stage. These bodies are seen to come out from the spireme in masses of big blobs as well as in the shape of small beads. They appear as if carried on a sling of linin and are slowly ejected into cytoplasm outside the nuclear cavity, where, surrounded by a clear space in the cytoplasm, they disintegrate.

The next series of changes that takes place in the spireme is manifested

by its rearrangement. It has been seen that the spireme forms a series of complete loops with a twist in the middle, in which place they generally bend. There is no split to be seen in the thread, and it will be evident from subsequent events that this parallelism in *Pustularia* corresponds with the parallelism of the early heterotype prophase of the higher plants. The spireme rearranges itself in the nuclear cavity in such a fashion that these parallel threads of the loops, which are at this stage far apart, may be brought into association. These series of loops, which have hitherto occupied a parallel position in the nucleus, arrange themselves in such a fashion that the heads of the loops come against the nuclear membrane, while the parallel arms take more or less radial positions. Pl. V, Fig. 7 shows a nucleus in which this new order of arrangement has just begun; Pl. V, Fig. 8 shows an advanced state of this arrangement; Pl. V, Fig. 9 exhibits diagrammatically this peripheral looping with radial arms. The nucleus presents an appearance of the open spireme stage of heterotype prophase of the vascular plants, but it is not quite the same. From the nature of the individual chromatin beads it can be readily perceived to be an earlier stage of that series. The association of the chromatin beads has just begun at the twist, while the beads on the parallel arms show a tendency to approach towards each other.

The nucleolus occupies a geometrical centre of the nucleus. The spireme can be easily recognized as an endless one. During the long duration of the heterotype prophase this is the only stage in which the spireme distributes itself uniformly in the nuclear cavity, arranging itself symmetrically with reference to the central nucleolus. It is clearly seen at this stage that the spireme forms seven continuous loops, one of which has a double head. This particular loop, *a*, which appears to be more prominent than the others, can be frequently identified during the later stage of synapsis.

(c) *Later Hollow-spireme Stage.*

The order of arrangement of the spireme when the symmetry of the nuclear element is maintained lasts only for a short time, which is evident from the relatively small number of nuclei showing this stage; after this, the spireme shows a tendency to mass on one side of the nucleus. The loops are again attached to the nucleolus by their parallel arms (Pl. V, Fig. 10). The shape of the nucleus is changed at this stage. It begins to elongate. The maximum elongation of the nuclear cavity is shown by Pl. V, Fig. 11. It is evident from this figure that the loops which are attached to the nucleolus are under a longitudinal strain or pull. The sides of the loops are drawn closer under the action of this pull. The heads of the loops under these circumstances appear to be overlapping each other. It is quite possible that such behaviour of the spireme is due to a physical phenomenon which is obviously helping the arms of the loop to associate.

The associated beads at the twist can be well recognized, and the association of the chromatin beads in the arms is more marked than at any previous stage. The attachment of the spireme to the nucleolus at this stage not only assists this, but at the same time appears to allow the spireme to take up reserve chromatin which is stored in the nucleolus. The chromatin beads increase in size and colour intensity when the spireme is connected with the nucleolus, and this again is followed by an increased discharge of extruded chromatin into the cytoplasm.

A careful examination of the individual beads of the arms shows that, towards the lower part, where the arms are attached to the nucleolus, the fission between the two beds occasionally remains open. The united beads, on the other hand, become slightly elongated in the direction of the pull. This strain lasts only for a short time. Pl. V, Fig. 12 shows that the longitudinal fission opens out again as the spireme is relieved of the strain. The position of the beads in the loops as well as in the parallel arms at this stage is shown in the more thinly cut nucleus of Pl. V, Fig. 13. Each chromatin bead, whether in the loops or in the arms, faces its *complementary half* of the same size and shape in the corresponding part of the spireme threads, which are at this stage rather far apart.

The spireme undergoes condensation preparatory to the second contraction. This gradually brings about the closing up of the split and consequently the reassociation of the beads. This stage is shown by Pl. V, Fig. 14 in an uncut nucleus. The chromatin beads, which will play an important part later on in a series of stages in the formation of bivalent chromosomes, become very prominent at this stage. They show a very remarkably paired arrangement. The paired beads, which have been considered as univalent, can be seen in that part of the spireme which has undergone association, and are very easily distinguished at this stage from the unpaired half-univalent ones. For instance, the beads *a* and *a'* at the crossing of the double-headed loop are two whole univalent beads, each of which has resulted from the association of two half-univalent beads. The beads *b* and *b'* are in the process of association. They are to be seen in all intermediate stages of union and are accommodated on a wider band of linin. The average counts of the chromatin beads in an uncut nucleus, such as is shown in Pl. V, Fig. 14, show that there are about ten united and about forty-four half-univalent beads, or there are approximately sixty-four chromatin beads of half-univalent nature.

A more fortunately cut thin section is shown on Pl. V, Fig. 15. It has a remarkable similarity to Fig. 20 of *Humaria rutilans*, which Fraser (37) has described as a case of second contraction. But though the nature of the spireme in both cases has a close resemblance, the sequence of events which these two figures represent is quite different. According to Fraser's description, that figure shows the loops of the second contraction in which

the longitudinal split between the half-univalent threads has become obliterated, each arm of the loop thus representing a full somatic chromosome. According to our contention, it is at this stage that the premeiotic split is closing up, consequently each arm represents half-univalent or somatic thread only. In other words, her figure showing the pairing of the univalent during the second contraction corresponds, in our seriation of *Pustularia*, with the heterotype prophase when the early parallelism of the spireme is closing up.

The fission and association of the threads give to the spireme the appearance of a longitudinal split. It is evident from our figure that the optical plane of the arms is at right angles to that of the loops; only the parallel three pairs of arms are shown on the lower side of the nucleus. The heads of the loops have been carried away in another section. Each pair of arms is terminated by a blob of chromatin, which represents the whole univalent bead, the junction of the loop and the arm. Towards the upper part of the nucleus there is one complete loop and parts of other three. Pl. V, Fig. 16 shows almost the same stage, displaying more of the marginal loops than of the radial arms. Pl. V, Fig. 17 represents a thin medium section of the nucleus at this stage.

(d) *Second Contraction.*

During the next stage the hollow spireme draws itself together more closely. As this process advances the union of the parallel arms becomes more intimate, but the fission in the loops still remains open (Pl. V, Fig. 18). The nuclear cavity decreases considerably. At a later stage (Pl. V, Fig. 19), when the closing up of the fission in the loops advances a step farther, the chromatin and linin elements undergo the maximum amount of condensation. It is difficult to trace the spireme at this stage. The loops retain their connexion with the nucleolus.

The fission in the loops is almost obliterated and the association of the univalent halves of the beads has been almost completely secured at this stage. This is indicated by the greater number of big univalent beads, though at the bends of the loops two parallel beads may now and then be observed still remaining apart. In the lower part of the nucleus some beads stained less intensely may be seen carried by a loose matrix of linin. It is quite possible that these beads are afterwards ejected out of the nuclear cavity.

The fission opens out again (Pl. V, Fig. 20), at first gradually, keeping the uniformity of the spireme intact, but as this process advances (Pl. V, Fig. 21) this uniformity is soon lost; the beads become less and less chromatic. In the same part of the thread a very darkly stained pair of beads is observed, followed by another pair of less strongly stained beads, apparently without any order of arrangement. In other words, the chromatin

becomes very 'rugged' in appearance. That this is a very characteristic stage leading to second contraction has been confirmed by many students of the cytology of higher plants. Lewis (61) found a rugged appearance of spireme in *Pinus* and *Thuja* just before the second contraction, while others have described this phenomenon as a display of conspicuous longitudinal fission by the spireme during the hollow-spireme stage.

A similar process, prior to second contraction, has been described in most of the Ascomycetes whose cytology has been fully investigated. Maire (63) in *Galactinia succosa*, *Morchella esculenta*, Guilliermond (49, 51) in *Humaria rutilans* and *Peziza vesiculosa*, Fraser (37) in *Humaria rutilans*, Fraser and Welsford (43), Fraser and Brooks (41), have all observed the phenomenon of the spireme undergoing longitudinal fission before entering on second contraction.

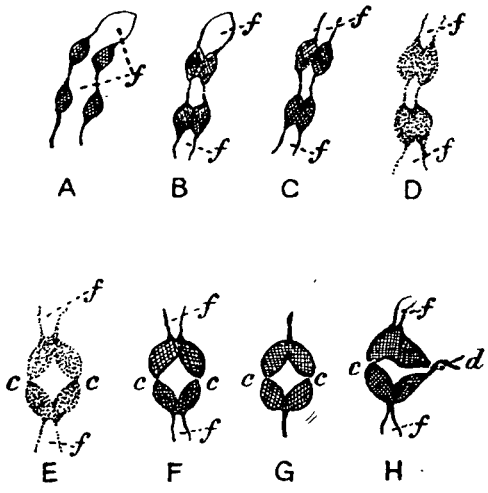
The stage presenting fission in the spireme before second contraction has been shown by Fraser (37) in *Humaria rutilans* (Fig. 19) and by Guilliermond (51) in the same fungus (Fig. 56). The post-synaptic fission in *Humaria* has been shown by Fraser in Fig. 22, while an identical stage has been described by Guilliermond (Fig. 57) as synapsis or second contraction. Guilliermond's Fig. 57 is similar to our Pl. VI, Fig. 27 of second contraction in this respect, that while the chromatin is massed on one side of the nuclear cavity, the bare linin below shows no fission. It seems as if here there is a want of harmony in sequence between these two accounts of the second contraction of the same fungus. We have already remarked that Fraser's second contraction stage corresponds to the hollow-spireme stage of *Pustularia*. It is evident, since it shows very marked longitudinal fission and polarization of chromatin, that her Fig. 22 really represents an earlier stage than that shown by our Pls. V and VI, Figs. 24 to 26. It is quite possible that, after missing the first contraction phase, which she supposes the paired nuclei to have passed through independently before their fusion, the subsequent events which she has followed so critically have been, consequently, pushed forward a stage beyond their true sequences. Such a misinterpretation would easily occur, since in *Humaria*, unlike *Pustularia*, there are no regular beads of chromatin to guide the true seriation of successive events. In *Humaria* the chromatin is granular and, as such, it is more or less uniformly distributed on the linin-matrix, while in *Pustularia* definite bodies are passed on from stage to stage, and a change in their arrangement and nature signalizes a definite change in the general history of the nucleus.

The chromatin loses its stainable capacity rapidly, and the spireme is soon reduced to a skeleton after this stage (Pl. V, Fig. 22). As the dechromatization of the spireme advances, the details of the nuclear constituents are almost lost. Nothing of the old spireme can be recognized except a confused maze of linin, speckled with faintly stainable beads. The reticulum appears almost hyaline, and great difficulty is experienced in tracing it, even

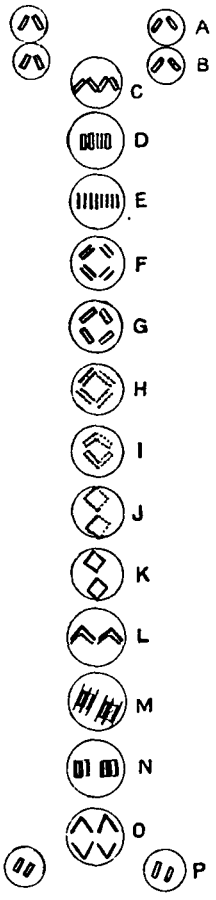
with well-differentiated preparations. Towards the periphery of the nucleus the reticulum displays a split here and there which can be detected under a careful focus, though it seems impossible to trace the split any distance. It cannot, therefore, be determined whether the spireme displays the split throughout or not. The distribution of the reticulum is not uniform; it is more thickly distributed towards the upper part of the nucleolus. Towards the lower part of the nucleus, below the nucleolus, where the reticulum is sparsely distributed, one can easily detect free ends. Pl. V, Fig. 22 shows a favourable nucleus of this stage, indicating that the destruction of the old spireme is almost complete, and that at the same time the reconstruction preparatory for the bivalent state has just begun. The evidence in support of the above statement can be found in a careful analysis of the constituents of this nucleus. In the upper left quadrant of the nucleus there is a small portion of the old spireme which can be identified by its former colour intensity. In the lower left quadrant there is a group of four faintly stained beads in the form of a close ring; and the right upper quadrant shows the same kind of ring, but formed of more deeply stained beads. Here, then, is the evidence in support of the view that a new arrangement of chromatin beads is taking place in the spireme preparatory to second contraction. In following the reconstruction stages, it will be seen that this grouping of chromatin beads on a linin framework in the form of a ring increases, and each group provides the building material for the construction of bivalents. As a matter of fact, here is the most convincing evidence for the theoretical starting-point of the formation of the bivalents with a fresh grouping of chromatin.

As the nucleus evolves out of this network towards the reconstruction stage, the outline of the underlying structure once again becomes visible. A fine uncut nucleus of this transition period is shown by Pl. V, Fig. 23. The linin is split longitudinally, though the fission is not continuous, being united at different points of the reticulum. The points of union are often marked by a pair of beads attached to one another, and grouping with the pair next to them. The line of original fission between the half-univalents opens out partially from one side and the two beads keep their attachment at the point of association, while by the opened-out ends they are conjoined to the similar pair of half-univalents facing them (Text-fig. 1). The space thus formed by the fission between the two pairs of half-univalents together with the conjunction of the two univalents is enclosed within the ring. The early indication of grouping to form a close ring has been shown in Pl. V, Fig. 22. In Pl. V, Fig. 23 there are three such complete groups, and two others in the initial stage. The distribution of these chromatin groups shows a certain amount of polarity.

The tetrad-like grouping of chromatin proceeds, and we get an arrangement as in Pl. V, Fig. 24. The individual chromatin beads of the tetrads



TEXT-FIG. 1.



TEXT-FIG. 2.

TEXT-FIG. 1. Diagrammatic representation of the chromatin beads showing the formation of the tetrad chromosomes.

A. Early heterotype prophase. B. Early hollow-spireme stage. C. Later hollow-spireme stage. D and E. Disorganization of the spireme prior to second contraction. F. Early stage of second contraction; fission still visible in linin framework. G. Second contraction; disappearance of fission from linin framework. H. Bivalent, showing distortion of tetrad figure, reappearance of fission in the linin framework. *f*, fission; *c*, conjunction; *d*, disjunction.

For the convenience of representation, red and black have been used for the two chromosomes forming the bivalent combination.

TEXT-FIG. 2. Diagrammatic representation of the distribution of chromatin from the ascogonium to the telophase of the first division.

A. Ascogonium. B. Ascogenous hyphae. C. Fusion in the young ascus. D (Pl. V, Fig. 1). Definitive nucleus showing first contraction. E (Figs. 3 to 6). Opening out of first contraction with wide fission. F (Figs. 7 to 9). Early hollow spireme; rearrangement of spireme; sorting of half-univalents. G (Figs. 10 to 19). Later hollow spireme; association of half-univalents. H (Figs. 20 to 22). Reappearance of longitudinal fission; disorganization of spireme prior to second contraction. I and J (Figs. 23 to 25). Conjunctions of univalents. K (Figs. 26 to 27). Second contraction; conjunction and fission; appearance of tetrad figures. L (Fig. 28). Early diakinesis; folding of bivalents. M (Figs. 29 to 37). Late diakinesis: twisting and condensation of chromosomes; fission in linin endings visible. N (Fig. 38). Heterotype prophase; equatorial plate stage. O (Figs. 39 to 42). Metaphase and anaphase; appearance of split in the univalents receding towards the pole. P (Figs. 43 to 46). Late anaphase and telophase; disappearance of the split.

For the convenience of representation, red and black have been used for the two sets of chromosomes, each set consisting of two chromosomes.

enlarge, and consequently the two beads of the pair unite, or they may occasionally undergo a slight elongation in one direction. This kind of variation leads to a certain amount of distortion of the ring. Thus it may be noticed that pairs of elongated and blob-like masses of chromatin are facing each other; they tend to form a distinct zone on the linin framework. This, to a certain extent, is due to the condensation of chromatin, and also to the tendency of the individual chromatin beads to group together. As a result of this arrangement the linin framework is laid bare on one side of the nucleus, where it shows the longitudinal split very clearly.

The next stage in advance is shown by Pl. VI, Fig. 25; here the chromatin groups are closer to each other, the blob-like character being accentuated. The nucleolus shows the earliest sign of vacuolization. The zonation of the groups of chromatin becomes more pronounced as the second contraction reaches its maximum (Pl. VI, Fig. 26). In this stage the fusion and condensation of the chromatin beads have increased to such an extent that the space in the ring has become almost obliterated. The half-univalent beads, as they coalesce with each other, enlarge and become extremely blob-like, and cannot therefore be recognized at this stage. Owing to close massing in a comparatively small area, the groups overlie each other. It becomes difficult to identify them at the central region of the nucleus, but when focused on the periphery or towards the linin support they are easily recognizable. The chromatin masses, each stained as deeply as its neighbour, stand out of the reticulum in such a way as to present an impression that these bodies are independent of one another.

Just before the groups separate from one another, the parallel linin unites, and the fission, carried on so far, then disappears entirely. The linin appears as uniformly thick spokes (Pl. VI, Fig. 27) holding out the cluster of tetrads, which are again tied to one another by similar connexions of linin thread. The almost diagrammatic regularity in the form of the tetrads is very noticeable in this drawing, and has not been at all exaggerated. The nucleolus shows a group of vacuoles coalesced together, forming a system of short-branched vacuoles.

(*e*) *Chromosome Formation and Diakinesis.*

As the nucleus reaches the highest stage of development of the second contraction, the limit and orientation of the chromosome bivalents can be more readily determined. The apparently quadrivalent arrangement of the chromatin beads at this stage shows the striking regularity with which the pairs are arranged, in an end-to-end fashion, to form a bivalent (Pl. VI, Fig. 27). Though the fission is obliterated at this stage and cannot be seen until the chromosomes begin to separate, the disjunction or the line of separation of the bivalent into two entire univalents is very clearly seen in most of the groups.

In some of the groups it seems that the enclosed space in the tetrads has become almost obscured through the swelling of the beads, and the bivalents appear as a homogeneous mass. But when such groups are carefully focused, the centre of these apparently homogeneous bodies always reveals a more lightly stained area. The chromosome at the upper extremity of the nucleus (Pl. VI, Fig. 27) and that lying below the nucleolus show these characteristics. In other groups, where the disjunction is widely displayed, the arrangement is slightly different. Here it appears as if the two univalents are conjoined at one end only. The other two ends of the pair run parallel for a short distance, and undergo twisting by their linin endings. This phenomenon is shown by two chromosomes of the right inferior quadrant ; cf. Pl. VI, Fig. 27.

It has been observed in the heterotype prophase of the Angiosperms, as well as in that of the vascular Cryptogams, that some of the chromosomes at least appear in definite form when the spireme emerges from second contraction. The general history of the chromosome evolution in *Pustularia* in no way differs from that of higher plants. A characteristic nucleus of this stage is shown by Pl. VI, Fig. 28. The fission in the spireme opens out again as the chromatin concentrates, but from the nature of the fission it is rather difficult to distinguish the post-synaptic from the presynaptic stage, which in these fungi rapidly succeed each other, while the highest stage of development of the second contraction phase intercalated between the two is of much shorter duration. Moreover, the nuclei in these fungi are so small and give such a small amount of chromatin to guide one in working out the true sequence of events that, unless other important characteristics are taken into account, there is a risk of misinterpretation of these two stages which have entirely different significance in the history of chromosome evolution. The chromatin spireme which is coming out of second contraction no longer appears as a simple condensation of homogeneous chromatin granules or an aggregation of beads, as is generally the case prior to second contraction, but always presents a heterogeneity of form. The bivalents, some of them at all events, are seen to be sorting out of the second contraction.

A detailed study of a nucleus at this stage (Pl. VI, Fig. 28) will support the above statement. The figure shows that some of the chromosomes are present in definite forms at the end of the second contraction stage. Two chromosomes, occupying the left superior and inferior quadrants, have just moved away from the main body of the spireme; they have an elongated appearance and are twisted in the middle. There are three others, two lying below the nucleolus and the third above it, which have retained the tetrad arrangement. The rest are clearly in the transition stage, from the ring-shaped grouping of the tetrad to the rather elongated form of the chromosomes. During this stage of progressive differentiation of the chro-

mosomes the nuclear cavity undergoes extension, and this helps the chromosomes to move apart from one another. The linin framework which holds the chromosomes together displays considerable thinness here and there.

The process of segmentation of the chromosomes is accompanied by the phenomenon of torsion (Pl. VI, Fig. 29). The double spireme, in which the limits of the chromosome bivalents have already been laid down, shows a remarkable tendency to undergo folding and twisting. The strain of twisting is transmitted to the linin connexions, so much so, that the connecting string becomes twisted as well as elongated, and is forced to give way in regions which become visibly thin.

As the spireme segments, the chromosomes, which are attached to one another by a loose connexion of linin, show a tendency to move towards the periphery. The chromosome (Pl. VI, Fig. 29) in the left upper quadrant has almost severed its connexion with the main spireme. The character of the chromatin shows clearly as four masses of chromatin placed on the double linin, which is again twisted in the middle. The chromosomes in the lower half of the nucleus show this phenomenon of twisting in all degrees. The same figure also shows that some of the chromosomes retain their regular tetrad form remarkably well, without undergoing any distortion. They can be recognized in almost every nucleus in which chromosomes are forming out of the second contraction spireme till later diakinesis. These chromosomes evidently pass off unchanged, and are to be seen as tetrads during diakinesis.

As the chromatin concentrates, the underlying framework of linin, which quite clearly is split, can be more readily studied. The chromosomes at this stage display very definitely four linin endings. The chromosome occupying the upper left quadrant of Pl. VI, Fig. 29 shows two long and two short ends. But at a later stage (Pl. VI, Fig. 30), when the concentration of chromatin has proceeded a step farther, one has little doubt as to the significance of the four threads of linin coming out of the chromosomes which occupy the upper left quadrant of the nucleus. The fission of the prophase—which emerged between the widely separated parallel arms and loops, was temporarily associated in the later hollow spireme, reopened at the time of derangement of the spireme prior to second contraction, and reassociated during the second contraction—is visible once more at this stage. The split that has been observed in the higher plants in the loop of the post-synaptic spireme, simulating the appearance of true longitudinal fission, can be seen between the thickly twisted arms of the same pair of chromosomes. It is also apparent from the behaviour of this pair of chromosomes that the univalents have united in the plane of the twist.

As the progressive differentiation and condensation of chromosomes advances, the bivalents show a tendency to distribute themselves towards

the periphery (Pl. VI, Fig. 31). It is possible to identify at this stage sixteen pairs of chromosomes. At a later stage the bivalents, as most characteristic of early diakinesis, are distributed uniformly throughout the nuclear cavity. The chromosomes during the early diakinesis present very varied shapes and forms, such as are often to be seen in the Phanerogams and higher Cryptogams, but when mature they appear as bean-shaped or short rod-shaped bodies with moderate variation in size. What appears most striking during this stage is the occurrence of some of them as a close group of chromosome tetrads. These tetrads can be identified through different stages of condensation. The appearance of such tetrads during the early diakinesis is shown on Pl. VI, Fig. 31. Evidently it is one of the constant tetrads which we have observed before, and remains true to its form when the other chromosomes differentiate out from the second contraction. The further modifications of these tetrads are quite simple. Owing to subsequent condensation of linin the chromatin beads approach close to one another (Pl. VI, Fig. 32), and the rectangular form of the pair is occasionally transformed into a close ring. At a still later stage (Pl. VI, Fig. 33) these bivalents appear in the form, so well known to cytologists, of tetrads of the heterotype chromosomes, when four distinct chromatin beads, which are fixed on a square frame of linin and are attached to one another, present themselves in a quadrivalent appearance. Further identification of these tetrads during the later diakinetic stage was not possible; they might have ended in solid oblong pairs of chromosomes. Of the other forms, **V** and **Y**, with widely separated arms and a pair of dumb-bell-shaped chromosomes, are easily recognized through the successive stages of condensation.

The formation of tetrad chromosomes in plants, though not a very common phenomenon, has, nevertheless, been noticed in very widely separated members of different families. They are rather common in the Bryophytes as well as in the Pteridophytes. Moore (66) has figured tetrads in the heterotype division of *Pallavicinia Lyellii*, and Melin (64) in *Sphagnum squarrosum*, and Florin (35) in *Chilocyphus polyanthus*. In the Pteridophytes, Osterhout (69) has observed chromosomes forming regular tetrads or groups of four ('Vierergruppen') during diakinesis in *Equisetum limosum*. Calkins (12) has described the 'ring type of tetrads' in *Pteris* and *Adiantum*. Sarbadhikari (76) has noticed similar forms in *Doodia*. In Phanerogams, tetrads appearing as close rings have been described by von Stomps (80) in *Spinacia oleracia*; and Miss Digby (26) has observed undoubted tetrads in *Primula kewensis*, which appear as quadrivalent arrangements of chromatin.

As has been observed in the case of vascular plants, the two individuals of the bivalent remain widely separated even to a very late stage of diakinesis. The linin connexions between the bivalents become longer and

more delicate, and last to a very late stage of diakinesis (Pl. VI, Figs. 31 to 34). Gradually they become loose, and ultimately become invisible when the centrosomes appear.

(f) *Metaphase, Anaphase, and Telophase.*

Spindle formation in the Ascomycetes has been studied in great detail by Harper in *Erysiphe* (55) and *Phyllactinia* (58), by Guilliermond in *Peziza rutilans* and in *Pustularia vesiculosa* (49), by Maire in *Galactinia succosa* (63), by Fraser in *Humaria rutilans* (37), and by Claussen in *Pyronema confluens* (16). They all agree as to the intranuclear origin of the centrosome. In *Pustularia* the centrosome is minute and has the shape of a very small, not at all prominent, disc. Unless the preparation is rather overstained the centrosomes cannot easily be detected; very often they can only be traced by following the converging rays of the spindle. It could not, therefore, be determined whether they originated independently or by division of one centrosome, as observed by Maire (63) and Guilliermond (51) in *Galactinia succosa*, and by Harper in *Erysiphe* (55) and *Phyllactinia* (58). They appear, however, very near each other (Pl. VII, Fig. 35) and throw out a cone of rays. The chromosomes, which are dispersed uniformly in the nuclear cavity, arrange themselves in the form of a wreath in the region between the centrosome radiations; and at the same time some of the delicate rays are seen to approach them. The centrosomes later on move apart from each other (Pl. VI, Fig. 36), when connecting rays appear between them. The chromosomes close up and their shape becomes more uniform; the split in the bivalents almost disappears at this stage. The centrosomes move farther apart and a 'Hermann spindle' is formed between them, and the chromosomes appear to be laterally placed on it (Pl. VI, Fig. 37). At a still later stage (Pl. VI, Fig. 38), when the centrosomes move farthest apart, the chromosomes are drawn into the middle of the spindle to form an equatorial plate. The chromosomes in the equatorial plate stage present the shapes of fat beans or oblong blocks, and slightly elongated rods; they stain uniformly and show no split.

The metaphase is a stage of long duration, and therefore one can get a sufficient number of nuclei in every slide to follow the separation of the univalents. In Pl. VI, Fig. 39 about five bivalents can be recognized, and the rest are univalents moving towards the poles. At a later stage, when the chromosomes advance towards the poles, a second longitudinal split can be detected in them. The chromosomes split up lengthwise and remain attached to one another by a short or slightly drawn out connexion, and occasionally present a V-shaped appearance (Pl. VI, Figs. 39 to 41). During the anaphase stage (Pl. VII, Fig. 42), as the chromosomes approach nearer to the poles, the halves of the univalent unite; and at the late anaphase (Pl. VI, Fig. 43), when the chromosomes migrate to the poles in two

batches, they appear again as regular bean-shaped bodies, and the split is seldom visible. The chromosomes do not arrive at the poles all in a body; at the telophase (Pl. VI, Figs. 44 and 45) one notices some of them lagging behind, and some of these slowly moving chromosomes are to be seen near the middle of the spindle and in all other intermediate positions (Pl. VI, Figs. 44 and 45). No split is visible in them. At the same time, those that arrive at the poles in advance become aggregated; they swell up and occasionally become attached to one another to form a compact mass. Thus, once they arrive at the poles, during the telophase, their apparent individuality is lost.

On Pl. VI, Fig. 44, which has been drawn from a very well stained Breinl preparation, thirteen chromatic masses can be counted at one pole and fourteen at the other. Of these thirteen bodies one is evidently twice as large as the others; while at a still later stage (Pl. VI, Fig. 45) any accurate counting of these chromatic masses seems impossible, and an attempt to suggest the nature of their union would be equally unwise. An oblique polar view of a late telophase is shown on Pl. VI, Fig. 46. It shows clearly that the identity of some of these chromosomes can, to a certain extent, be traced to those lumps, but most of them are in different stages of disorganization. The two arms of the chromatic masses are therefore not two arms of a **V**-shaped chromosome, nor is the opening between them the split in the univalent. One of these lumps, which is the largest of all, obviously represents a swollen chromosome on the point of disintegration. The achromatic spindle forms a thick sheaf of fibres connecting the conglomerate mass at the two poles. The nucleolus becomes elongated and discharges chromatin bodies, which are all scattered in the neighbourhood. It does not stain uniformly and has a thick central core which thins out towards the margin (Pl. VI, Fig. 47). Vacuoles appear in the cytoplasm, which gradually spread out and help the connecting fibres to dissolve (Pl. VI, Fig. 48).

IV. The Second and Third Divisions.

(a) *The Reconstruction of Daughter Nuclei.*

Simultaneously with the thinning out of the achromatic fibres of the spindle there appear two vacuolated regions facing the chromatic masses (Pl. VII, Fig. 48). At this stage, a set of fine linin threads emerging from the periphery of the chromatic masses gradually encloses these vacuolated areas (Pl. VI, Fig. 48 and Pl. VII, Fig. 62). A delicate nuclear membrane is thus formed. The linin thread opens out and forms a fine network in the nuclear cavity (Pl. VI, Fig. 49 and Pl. VII, Fig. 63). At the same time the chromatic mass

breaks up into fine granules, which are eventually distributed in the interstices of the reticulum (Pl. VII, Figs. 50 and 64). At a later stage, when this process advances, the chromatin becomes more or less uniformly distributed in the reticulum (Pl. VII, Figs. 51 and 64). The size of the chromatin masses varies at first, owing to conditions of growth, but when they are fully developed, they show a certain amount of variation in shape and size (Pl. VII, Figs. 51 and 65). The youngest nucleus lies generally uppermost in the ascus, while the intermediates, in the third prophase, range between them. This is the most frequent order of development during the prophase stages; but later on, when spindle-formation takes place, this order is often seen to be reversed.

(b) *The Prophase of the Second and Third Divisions.*

The fine reticulum of the early prophases of the second and third division is gradually transformed into a more or less uniform but coarse spireme (Pl. VII, Figs. 51 and 64). The chromatin beads, which are connected by linin thread, become prominent at this stage. At a later stage (Pl. VII, Fig. 51, lower nucleus) these beads show a tendency to pair. This process of pairing of the beads as a preparation for a contraction phase is very clearly shown by the upper nucleus of Pl. VII, Fig. 52. The prophase of the second division is quickly over, while that of the third division is a long-lasting one; consequently, one can readily observe in the four nuclei of the third division a complete series showing the beads in different degrees of union prior to contraction. Otherwise the process in both the prophases is the same and is subject to the same interpretation.

The development from a uniformly coarse spireme with prominent chromatin beads to a stage of approaching contraction, with the intermediate stages, can be seen in a single ascus (Pl. VII, Fig. 65). The number of the chromatin beads which can be more or less accurately determined at this stage is over thirty. They are strung on a broad band of linin and gradually approach one another to undergo association. At a later stage (Pl. VII, Figs. 52 and 66) the parallel threads of linin carrying the half-univalent beads of chromatin come closer together, and this process gradually leads to a remarkable contraction phenomenon. The two nuclei of Pl. VII, Fig. 52 and the four nuclei of Pl. VII, Fig. 66 show the process of association of these beads. The uppermost nucleus of Fig. 66 is in an earlier stage than the lower three nuclei. The three lower ones of Fig. 66 and the one of Pl. VII, Fig. 52 are at the height of contraction. The lower two nuclei of Pl. VII, Fig. 66 show contraction, a fact which clearly demonstrates the nature of the spireme at this stage; it forms a knot with an open end.

The spireme breaks up and the chromosomes, which are sixteen in number (Pl. VII, Fig. 53), are seen to arrange themselves in a wreath-like

fashion in the nuclear cavity. The centrosomes are visible at this stage. To begin with, they appear close to each other, and then throw out a cone of radiations like the first meiotic division. Later on they move apart and the interconnecting fibres appear; they then move farther apart and a regular spindle is formed. The chromosomes are drawn to the equatorial plate of the metaphase (Pl. VII, Fig. 54). An exactly similar phenomenon takes place when the spireme divides into sixteen chromosomes after the contraction of the third prophase, only in this case it passes slowly, and one can thus follow more easily the details of the process. The chromosomes are distributed uniformly in the nuclear cavity (Pl. VII, Fig. 67), imitating the diakinetic stage of the first division (Pl. VI, Fig. 34).

The chromosomes at this stage present an appearance similar to that of the diakinetic chromosomes of the first division, and have wide splits between their halves. The significance of this split is, however, entirely different in the two cases. In the first division, the split represents the line of *transverse division* of two *entire* chromosomes, which have evolved through a distinct phase of second contraction when tetrads were formed by telosynaptic conjunction of the whole univalents together with the fission between the half-univalents. The split is closed up later on, and the chromosomes become longitudinally united. The significance of the apparently diakinetic figure of the third division, which is followed by a single contraction phase, is easy to interpret, for, like the premeiotic contraction phase, it merely brings about the association of the half-univalents. The split, consequently, represents the line of *longitudinal fission* of the *half-univalent beads* of chromatin which are to function during the succeeding metaphases. The split between the half-univalents closes up gradually and the stages of union can be seen in Pl. VII, Fig. 67. During the union of the two halves they form V-shaped chromosomes with different angles of divergence between the halves. There exists a certain amount of linin fibre loosely attached to these chromosomes at this stage. Later on, very faint centrosomic corpuscles appear (Pl. VIII, Fig. 68) and the chromosomes are drawn on the achromatic spindle like the first and second divisions. The splits in the chromosomes entirely disappear and the chromosomes present thick bean-shaped forms.

Guilliermond (49) has observed the formation of the chromatic knot in the prophase of second division in *Peziza* (*Humaria*) *rutilans*, when the chromosomes are united to form a mass in the centre of the nucleus out of which sixteen V-shaped chromosomes arise. Maire (63) has noticed the formation of a chromatic knot in the prophases of the second and third divisions in *Galactinia succosa* and *Peziza vesiculosa*. A stage of contraction prior to the third division has been noticed by Fraser and Welsford (43) in *Otidia aurantia*. In *Peziza vesiculosa* the same authors have again observed this phenomenon in both the second and third divisions. Fraser

and Brooks (41) have described a similar phenomenon of contraction in the prophase of the second and third divisions in *Ascobolus furfuraceus*, which they have compared 'to the so-called first contraction of meiosis'.

(d) *Metaphase, Anaphase, and Telophase.*

The number of chromosomes at the prophases of the last two divisions is sixteen. They appear uniformly as bean-shaped bodies. The spindle is symmetrical, like that of the first division, and consists of fine fibres (Pl. VII, Fig. 54 and Pl. VIII, Fig. 69).

During the metaphase stage of the second division, the chromosomes split up along the line of union of the half-univalent beads. The process of division of the chromosomes can be readily followed. Pl. VII, Fig. 55 represents such a stage and gives a longitudinal view of the spindle. The upper nucleus of Pl. VII, Fig. 56 presents a polar view of such a stage; the chromosomes in this nucleus are in all different degrees of fission. To begin with, as the chromosomes split up into beads, the beads form an acute **V**; the beads move farther apart from one another, making a wider angle between them. Just before their complete separation they appear as two short rod-shaped or slightly bead-shaped bodies connected by a narrow portion. Pl. VII, Fig. 57 shows the late metaphase stage, when the chromosomes are uniformly distributed all over the spindle.

As the chromosomes move towards the poles, a second longitudinal fission appears in them (Pl. VII, Figs. 55, 58, and 59), which soon closes up as they advance towards the pole. The chromosomes divide into two batches, and each batch, consisting of sixteen chromosomes, moves towards the poles. The passage of the chromosomes towards the poles during the anaphase stage is slow and exhibits a certain amount of irregularity. It is seen that when one batch of chromosomes is nearing the pole some of the chromosomes of the other batch are lagging behind in the neighbourhood of the equatorial plate. This kind of irregularity, as a matter of fact, gives a better opportunity for the study of the character of the chromosomes during the anaphase and telophase stages. From the slowly moving group of chromosomes of Pl. VII, Fig. 58, it is evident that during the anaphase stage the chromosomes again appear to be split up longitudinally, and the two halves are joined by a narrow region presenting a **V**-shaped appearance with a wide angle between the arms, while at the other end of the spindle, where the chromosomes present a later stage, no split is to be seen in them. The halves have obviously united to form bean-shaped chromosomes again. Even during the late anaphase stage (Pl. VII, Fig. 59) one occasionally notices a pair of beads connected together.

As in the telophase of the first division, the chromosomes during the telophase of the second as well as of the third divisions become very closely aggregated together as soon as they arrive at the poles, and at the same

time a process of disintegration sets in. Consequently their identity is soon lost. Pl. VII, Fig. 60 shows four groups of chromosomes on two spindles in different stages from anaphase to telophase. In the upper nucleus the spindle is curved; the ends, therefore, present a polar view. A telophase stage on a similarly curved spindle is shown on Pl. VII, Fig. 61. The end presenting a polar aspect shows that the chromosomes are very closely massed together. They are in different degrees of disintegration and the number of these bodies appears, approximately, to be twelve. The reconstruction of the daughter nuclei after the second telophase takes place in the same way as after the first division.

The third division merely repeats the second division in every detail. The separation of the halves during the metaphase takes place on a plan similar to that of the second division. The metaphase is shown in a longitudinal view on Pl. VIII, Fig. 70. The chromosomes separate longitudinally along the line of fission of the half-univalents, which closed up during the telophase of the second division, and opened again during the prophase of the third division. The polar view of a similar stage is shown on Pl. VIII, Fig. 71, which is merely a repetition of Pl. VII, Fig. 56 of the second division. Pl. VIII, Fig. 72 represents a stage of metaphase which is comparable to Pl. VII, Fig. 57 of the second division. The separation of the halves is complete, while a subsequent fission is visible in the chromosomes which are approaching the poles. This fission is closed up quickly during the late anaphase (Pl. VIII, Fig. 73), when one can count two batches, each of sixteen bean-shaped chromosomes, moving towards the poles. The process of movement of the chromosomes towards the poles, as well as the process of disorganization of the chromosomes at the poles, is much more rapid than in the second division. But as it is often accompanied by a certain amount of irregularity on the part of the chromosomes moving towards the poles, one therefore can find sufficient cases to prove that there is no reduction in the number after the third division. The uppermost nucleus of Pl. VIII, Fig. 74 shows chromosomes at the poles appearing as regular bean-shaped bodies, and the number is about fourteen. The nucleus next below is at a little later stage, and the rest are in the telophase and late telophase stages. Pl. VIII, Fig. 75 shows the polar view of a nucleus at a late telophase stage which can be compared with Pl. VI, Fig. 46 of the first and Pl. VII, Fig. 61 of the second divisions.

V. Spore Formation.

The daughter nuclei are formed in a manner similar to that observed after the first and second divisions. The connecting fibres of the spindle gradually dissolve away and vacuolated spaces appear facing the chromatic lump (Pl. VIII, Fig. 76). At this stage very faint astral radiations are

visible emerging from the periphery of the chromatic lump; the centrosome is not visible at this stage. It seems to be hidden in the chromatin mass and the emanation of astral rays signifies its presence there. At a later stage the chromatin mass gradually fragments into a number of small chromatic granules which are connected to each other by a delicate linin network (Pl. VIII, Fig. 77). The astral rays become more numerous and prominent. The centrosomes are clearly seen at this stage; they are apparently much bigger than before, and are disc-shaped. The number of rays increases, and a delicate membrane is formed round the nucleus (Pl. VIII, Fig. 78). The cytoplasm gathers round the nucleus in a rather dense mass, while its distribution becomes considerably thinner in the intervening region. The nuclear membrane is gradually pulled out into a characteristic nuclear beak. At a later stage (Pl. VIII, Fig. 79), when the spores are completely delimited from the hyaline cytoplasm, one can more readily study the mechanism of the underlying process of spore formation. The centrosome throws out a set of rays which are recurved to form an outer and inner series. The outer series opens out as umbrella-like radiations and encloses the cytoplasm as sporoplasm. The inner series of rays are fine and fibrous; they form the nuclear membrane. The delimitation of the spores is helped by the appearance of vacuoles bordering the spore wall. The shape of the spore is uniformly round at this stage. The nuclear beak is very prominent and the centrosome appears as a small condensed disc and is situated at the apex of the beak.

The method of spore formation in the Ascomycetes has been a subject of divided opinion among the workers on the cytology of the Ascomycetes. Harper (52), who first studied the spore formation in this group of fungi, is of opinion that the spores are delimited by astral rays. He has concluded that the spore wall is formed by the lateral fusion of the astral rays, while the recurved ends of the fibres fuse again in a similar fashion to form the nuclear membrane. In his later work (58) on *Phyllactinia corylea*, as well as on *Erysiphe cichoracearum*, he has confirmed his previous observations on the spore formation. The whole spore body is formed out of undifferentiated cytoplasm of the ascus by the formation of a plasma membrane derived by the lateral fusion of the fibres without the deposition of a cellulose wall. In a recent paper (59) he has again expressed his opinion that the centrosomes in the Ascomycetes originate in the region of the cell where the chromatin and the cytoplasm come into specific contact and 'where fibrillar kinoplasm is formed and passes out to form the plasma membrane of the young daughter-cell, the ascospore'.

Faull (33, 34) denies Harper's conclusion that the ascospore wall originates from the lateral fusion of the astral rays. He concludes that the spores are delimited by the differentiation of a limiting layer of hyaline or finely granular protoplasm. This differentiation begins adjacent to the

centrosome and continues progressively to the other side of the pole. The delimitation of the spore, according to Faull, takes place at the expense of kinoplasm or altered cytoplasm. Faull, in his account, attempts to establish a connexion between the sporangia of the Phycomycetes and the ascus.

Fraser (37, 41, 43) has suggested that though the spores are delimited by astral rays, the lines of rays represent the flow of an enzyme, the centrosome being the centre of these enzymic activities. In *Humaria rutilans* (37), for example, the spore is delimited by astral rays, as Harper (52, 58) suggested, but the nature of these rays suggests the flow of currents set up in the neighbourhood of the centrosome; this she has confirmed later on by her study on the spore formation in *Peziza vesiculosa* (43). In *Ascobolus furfuraceus* Fraser and Brooks (41) have noticed vacuolated areas or line of cleavage delimiting the spore, a phenomenon essentially comparable with that observed by Faull (33) in *Neotiella*. *Lachnea stercorea*, in which the astral rays are well marked, again approaches the forms studied by Harper.

The spores in *Pustularia* are delimited by the astral rays emanating from the centrosome, as Harper (52, 58, 59) stated. The incurved rays which form the nuclear membrane are fine and fibrous, and are to be seen for a very short time. How far they are transformed into nuclear membrane could not therefore be determined. Vacuole formation undoubtedly plays an important part in delimitation of spores. The sporoplasm is as finely granular as the original cytoplasm enclosed by the astral rays, except that it forms a dense layer round the nucleus. No difference could therefore be made out between the sporoplasm and the limiting cytoplasm. Again, from the character of the cytoplasm and from the changing shape of the spore as shown by Pl. VIII, Figs. 78 and 81, it seems quite possible, as Fraser and Brooks (41) have remarked, that a new tension is set up in the neighbourhood of the centrosomes which in a great measure accounts for the formation of a cleavage line, and ultimately for the vacuole formation in the cytoplasm delimiting the spore.

The nuclear beak becomes further elongated, and with it the spore elongates (Pl. VIII, Fig. 80). At a later stage (Pl. VIII, Fig. 81) the number of chromatin granules in the reticulum increases, when one can readily count more than twenty-four of them. The beak disappears, and the nucleus takes a rounded shape; the chromatin beads increase in size and number, and become more chromatic (Pl. VIII, Fig. 82). Their number at this stage is over thirty; they are short rod-shaped bodies (Pl. VIII, Fig. 83), as has been seen in the prophase of the second and third divisions, just before contraction. At a later stage (Pl. VIII, Fig. 84) these beads are again connected by a spireme. At the same time the beads are seen to approach one another to form pairs. At first the paired beads form a wide, and then an acute, angle between them, as the union of these beads becomes closer. The split between the two beads forming a **V** closes

up gradually (Pl. VIII, Fig. 85), and the bean-shaped chromosomes become apparent once more. The chromatin is deposited on the linin connexion, and the nuclear cavity gradually begins to decrease. At a later stage (Pl. VIII, Fig. 86) the spireme character becomes more prominent, and the chromosomes appear as swollen nodes on the spireme. The number of the chromosomes at this stage is about sixteen. The thick-noded spireme (Pl. VIII, Fig. 87) gradually contracts away from the nuclear membrane. The sporoplasm thins out on both sides of the nucleus at this stage and the oil glands appear, first on one side of the nucleus and then on the other. The oil cavities increase in size, and the nucleus, which lies in the middle of the spore, becomes elongated in the direction of the shortest diameter of the spore. The sporoplasm becomes a thin limiting layer on both sides of the nucleus facing the oil drops. The spireme, now almost deformed, loses its staining power, and in the mature spore (Pl. VIII, Fig. 89) the resting nucleus often moves to an eccentric position facing the spore wall. The number of the chromosomes of a resting nucleus cannot be determined. The size of the spore varies from $10 \times 14\,\mu$ to $14 \times 20\,\mu$.

VI. Discussion.

(a) *General Considerations.*

In *Pustularia* we face once more some of the most controversial problems of modern cytology, the most outstanding of which is the significance of parallel threads arising from the first contraction knot, as well as the nature of the chromatin beads which are strung on these parallel threads. In *Lilium,* as well as in a large number of other plants, these parallel threads present an appearance of longitudinal fission in the spireme. This longitudinal fission has received at the hands of various investigators very different interpretations, which can be divided into two main groups.

One school of cytologists, notably Farmer and Moore (31), Mottier (67), Schaffner (77), Miss Digby (25, 26, 27, 28), and other botanists, interpret this split as a precocious division of the spireme, which only reaches its consummation during the homotype division of the chromosomes. According to their view, this split is not to be confused with the subsequent split formed by the looping over of the entire spireme during the second contraction. The most potent evidence on which some of the investigators of this school have based their interpretation, is the establishment of the identity of the parallel threads of the last premeiotic division of the sporogenous tissue with the parallel threads of the heterotype prophase. According to these investigators, this premeiotic fission, though occasionally masked by temporary association, is carried through the prophase of the heterotype,

and the final separation leads to the quantitative division of chromatin at the homotype metaphase.

Following the first contraction, the nucleus enters upon a second contraction phenomenon. As the spireme passes into this phase, the longitudinal fission becomes almost obscured, and this is followed by a confusion of the chromatin spireme when the primary fission appears again. As, however, the spireme evolves out of the second contraction, it is thrown out into a number of loops, between the arms of which a second fission appears resembling the true or primary longitudinal fission; but the original longitudinal fission can be occasionally seen in the sides of the loops. Through further condensation the arms of the loops become closely approximated. The loops separate from one another, and the two limbs tend to become twisted about each other. Each arm of the loop consequently corresponds to a single chromosome, and by their approximation the arms form a bivalent chromosome. According to Farmer and Moore, 'the side of the loops represents not the longitudinal halves of a split thread, but the approximation of serially distinct regions of the spireme as a whole'.

The other school of investigators, chiefly supported by Grégoire (47, 48), Berghs (4, 5), Strasburger (82, 83), Allen (1, 2), Miyaki (65), Rosenberg (73 to 75), Overton (71), and others, regard this fission as the longitudinal approximation and conjugation of independent threads of paternal and maternal origin. Berghs (4), in his investigation of the formation of heterotype chromosome in *Allium fistulosum*, has observed that this dual nature of the filament is due to a longitudinal approximation of two filaments, which lead to the formation of a thick spireme. In his earlier works, Strasburger (81) remarked that the explanation of the parallelism of the spireme is to be looked for in the telophase of the last premeiotic division. This interpretation, as a matter of fact, supported the view held by the first school; but later on (82) he changed his opinion. He has subsequently (83) interpreted the double nature of the spireme during heterotype mitosis as a parallel conjugation, and not as an early splitting. He has further stated that the explanation of the reduction from the bivalent to the univalent number is to be found in this parallel conjugation. It follows as a corollary to the above theory that the investigators belonging to the later school do not admit the importance of second contraction in the formation of bivalent chromosomes.

It has already been stated in the introduction that immediately after the union of the gametic nuclei in Ascomycetes, the definitive nucleus which results from this union enters upon the first contraction stage of the heterotype prophase, like the spore mother-cells of higher plants. Consequently here is no independent sporophytic generation in the life-history of such fungi. On the other hand, as the sporophytic nucleus enters abruptly into

the prophase of the heterotype, the series of events, such as premeiotic divisions and complete resting interphases which are noticed in the life-history of most of the Cormophytes, is cut short in the history of the sporophytic nucleus. Thus to follow the parallel threads from a preceding division in the sporogenous tissue is impossible. What we are concerned with in *Pustularia* is the later behaviour of the parallel spireme after first contraction, and our interpretation in this matter is to be based upon the subsequent sequence of events in the history of the parallel spireme.

In order to determine what evidence *Pustularia* presents for the significance of the duality of the spireme in question, it seems advisable to give here a concise account of the behaviour of the chromatin beads from synapsis to the subsequent division stages.

The first contraction knot (Pl. V, Fig. 1) carries the full number of somatic and univalent chromosomes. The knot opens out (Pl. V, Fig. 1) into parallel threads carrying these chromosomes along with them; and during the earliest stage of the opening of the knot the somatic number can be counted. The threads, as they grow, undergo twisting in the middle, thus forming loops with parallel arms (Pl. V, Figs. 3 to 6). As the process of the opening of the loop advances, the chromosomes split up longitudinally, forming chromatin beads. From the fully opened spireme after the first contraction till the hollow-spireme stage (Pl. V, Figs. 6 to 13), the nuclear activity is manifested by a process of rearrangements of the loops to effect the pairing of the *half*-chromatin beads, which, during the process of opening of the knot, have become split up and have been carried away in the loop far apart. During the early hollow-spireme stage the chromatin beads show a tendency to undergo association, and this phenomenon becomes more accentuated during the later hollow-spireme stages (Pl. V, Figs. 14 to 17).

The association of the threads is gradually completed (Pl. V, Fig. 18) and the fission is obliterated (Pl. V, Fig. 19); and this is rapidly followed by the reopening of the split (Pl. V, Figs. 20 and 21). The chromatin beads lose their staining power, and the spireme presents a rugged appearance. The loss of chromatin by the spireme, together with the reappearance of the original longitudinal fission, lead to its complete disorganization before the second contraction (Pl. V, Fig. 22). The reconstruction of the bivalents begins very rapidly from this confusion. The beads become increasingly chromatic (Pl. V, Fig. 23). The most characteristic feature of the nucleus entering on the second contraction is the formation of regular tetrad chromosomes from these beads (Pl. V, Fig. 24 and Pl. VI, Figs. 25 to 27). During the subsequent stages of segmentation and condensation, up to the early diakinesis (Pl. VI, Figs. 28 to 33), the tetrads appear in the form of rectangles or rings. The univalents separate during the heterotype metaphase (Pl. VI, Fig. 39). During the early anaphase the split is visible again, though for

a short time, in the chromosomes advancing towards the poles. The daughter nuclei are reconstructed during the late telophases, after which a period of rest follows.

The chromatic lump is entirely dissolved away during the period of rest, except for a few minute granules which are to be seen in the hyaline matrix of linin. The spireme is reconstructed during the interphase (Pls. VI and VII, Figs. 49 to 51). The spireme presents again the parallelism which is so characteristic of the heterotype prophase (Pl. VII, Fig. 52). The split is closed up by a simple process of contraction and the halves are again associated together and present the whole univalent chromosome on the homotype spindle (Pl. VII, Figs. 53 and 54). The separation of these halves is effected during the metaphase of the homotype (Pl. VII, Figs. 55 to 58), and the meiotic phase of the nucleus is completed.

During the prophase of the post-meiotic division of the nucleus, the spireme presents once more the phenomenon of parallelism (Pl. VII, Figs. 64 to 66) identical with that of the heterotype prophase and that of the homotype prophase. The split is closed, and the chromatin beads of the spireme are longitudinally associated in pairs by a remarkable contraction phase. The spireme segments and the number of whole univalent chromosomes becomes apparent again during the diakinetic stage (Pl. VII, Fig. 67 and Pl. VIII, Fig. 68) and on the equatorial plate stage of the third division (Pl. VIII, Fig. 69). The split appears again in the chromosomes during the early anaphase (Pl. VIII, Figs. 70 to 72), and during the late telophase the full number of half-univalent beads can again be counted in the spore nuclei (Pl. VIII, Figs. 81 to 84).

The occurrence of the post-meiotic contraction phase, which takes place in the same cell and soon after the heterotype reduction has been completed, is a unique phenomenon in the Ascomycetes which is hardly known in any other organism, and an equivalent for this should be found, if anywhere, in the premeiotic and early heterotype prophase of higher plants. The process involved in these post-synaptic contractions is a simple one, and consequently calls for a simple interpretation. On the other hand, if the explanation of the heterotype reduction, as suggested by Grégoire, Berghs, Strasburger, Allen, Miyaki, Overton, and others, is to be found in the phenomenon of early parallelism of the heterotype prophase (when by the lateral conjugation of parallel threads an apparent numerical reduction of chromosomes to half their number is brought about), there is no substantial reason why this process should be repeated in the history of the daughter nucleus, after its aim has already been achieved.

The above description of the nature of the spireme and the behaviour of the chromatin beads in the spireme during the heterotype prophase, as well as during the succeeding interkinetic phases, presents us with an opportunity of discussing the significance of prochromosomes, and the part they

play in the formation of bivalents. A considerable mass of information has accumulated on this subject. The literature of the discussion has often been quoted in most of the works on cytology where the spireme is described as presenting this aspect. But as, in the case of *Pustularia*, the interpretation of the sequences has been entirely based on the nature and behaviour of these chromatin beads, we are consequently led to a very different conclusion from that put forward, as to the significance of the prochromosomes. Thus it seems well worth our while to bring forward some of the most noted views on the subject.

Strasburger (82), in connexion with the formation of heterotype chromosomes in *Lilium*, held the view that the pairing of the homologous maternal and paternal chromosomes takes place in the form of gamosomes. The gamosomes which are present in the somatic cells of *Galtonia* correspond to the somatic number of chromosomes. Miyaki (65), in his investigation of the pollen-formation of certain Monocotyledons, has found concentrated chromatin masses in the presynaptic stage which are comparable to the gamosomes of Strasburger. The gamosomes pair to form zygosomes during the heterotype prophase. Overton (71) has observed prochromosomes in the somatic nuclei as well as in the resting germ-cells of *Thalictrum purpureus* and *Calycanthus floridus*. They are arranged in parallel pairs. He concluded that double spiremes, which are associated during fertilization, remain side by side, and actual conjugation occurs during synapsis or the associated stages. Rosenberg (73) has observed prochromosomes in the form of chromatic masses in *Hieracium* and *Tanacetum* during the early heterotype prophase of the pollen mother-cells which are present in somatic numbers. They unite in parallel pairs during synapsis to form gamosome pairs, each member of which is a univalent chromosome. In *Crepis virens* (74) he has noticed a paired arrangement of prochromosomes in the premeiotic resting nucleus, when they are present in diploid number, while in the resting tetrads they are present in haploid number. In the resting somatic cells of *Nuphar* and *Helianthus* (75) he has again observed prochromosomes in the form of chromatic masses which are apparent in somatic number.

Maire (63) has described the formation of granular chromatin bodies in *Galactinia succosa* and *Peziza vesiculosa*, which he has termed protochromosomes. These bodies generally unite two by two, in the prophases of the three divisions, by forming a knot out of which are formed bigger bodies of half the number of these chromatin granules. The protochromosomes which appear in the prophases of *Galactinia* in the form of granules are rather variable and transitory formations. As a matter of fact, Guilliermond (51), in a later research on *Galactinia*, refuses to accept Maire's observation of the union of these bodies to form chromosomes.

Fresh light has been thrown by Miss Digby (27) on the question of the

presence of definite chromatin bodies as prochromosomes in the resting pre-meiotic as well as in the resting tetrad nucleus of *Crepis virens*. She has explained that in *Crepis* there may be stages of complete disintegration of chromatin in the resting nucleus, leading to a distribution of chromatin in the reticulum in very fine form, or the disintegration may be so slight as to allow of the identification of the chromatic aggregations as prochromosomes. As to the numerical relation of these aggregations with the chromosomes, she has found that the numbers of these bodies are inconsistent and variable. Consequently the rigid hypothesis of the permanency of these bodies from one cell generation to another, as put forward by Rosenberg, does not hold good. The chromosomes lose their identity during the interkinetal rest. The reconstruction of the chromatin beads during the early prophase of the second and third divisions indicates a phase of reawakening of the resting nucleus to an active stage. The number of these chromatin beads during the early prophase of the heterotype approximately corresponds to twice the somatic number. Consequently, in view of present research, each bead is equivalent to a half somatic or univalent chromosome. The apparent numerical relation will be further supported by the second part of the discussion. The distribution of the chromatin beads from the first contraction of the early heterotype prophase to the telophase of the first division has been diagrammatically shown by Text-fig. 2.

The occurrence of these bodies in the reticulum of the resting somatic cell or in the prophase of the heterotype division, whether they are in the forms of elongated threads, definite beads, condensed chromatic aggregation, cloudy masses, or otherwise, raises the question of the subsequent arrangement of these bodies in the spireme of the early heterotype prophase and the part they play later on in the formation of the bivalent combination. Miss Digby (25) has made this point clear in her paper on *Galtonia*, and Farmer (29) has emphasized the fact that the main difference of opinion between the two schools lies in the question of the pairing of the univalents, while the rest are merely secondary parts of this main problem.

Having stated our opinion in relation to the significance of the parallelism of the heterotype, we are now able to discuss the importance of second contraction, which, according to the first school of cytologists, is one of the most important corollaries of the main proposition. Farmer and Moore (31) consider this phase as 'essentially a synaptic one', and hold that it is thus primarily involved in the pairing of the homologous portions of the univalent spireme to form the bivalents. This view has been upheld by Lewis (61), Miss Digby (25, &c.), Nothnagel (68), and others. In *Galtonia* (25) and *Osmunda* (28) Miss Digby has observed that the pairing of the entire univalent chromosomes takes place during the second contraction.

In *Pustularia* the second contraction has a marked significance in the

formation of the bivalents. The most striking feature which characterizes the setting in of the second contraction is the gradual sorting out of the chromatin beads into the form of tetrads. Prior to the appearance of the tetrads, the spireme is distinguished by the reopening of the longitudinal fission. This process, together with the telosynaptic conjunction of the univalents, gives rise to tetrad figures. The mode of tetrad formation during the second contraction is a straightforward process. We have been able to show that some of the later modifications of these tetrads during the evolution of heterotype chromosomes are as simple as their origin.

In clearing up the most obscure and difficult problem in the significance of the parallelism of the heterotype prophase in *Osmunda* (28)—as to which interpretations differed so widely—Miss Digby lays emphasis on the point that the separation between two *half-univalent threads* and *conjunction* between two *whole univalents* are two different phenomena which should not be confused. In *Osmunda* the spireme, before entering on the second contraction, shows the phenomenon of conjunction or union of whole univalents very remarkably, but fission in the conjoining filaments is completely obscured. Sarbadhikari (76) has observed in *Doodia* that the vestiges of fission remain apparent throughout the second contraction, and can be traced in the chromosomes of advanced diakinesis, when the fission can be seen in the conjoining univalents of each bivalent combination. *Pustularia*, in this respect, presents an intermediate aspect. Though the split cannot be traced in the linin framework during the second contraction, yet in the chromatin spireme the simultaneous occurrence of partial fission in the half-univalent beads and conjunction between two homologous univalents leads to the formation of tetrads. Calkins (12) has made a similar observation in his investigation of chromatin reduction and tetrad formation in Pteridophytes. According to his description the tetrad is derived by a longitudinal fission of the spireme segments, together with the transverse union of two chromosomes.

Farmer and Moore (31) considered the process of the separation of these tetrads as easier to interpret than their formation. They have explained that these tetrad chromosomes separate as pairs of dyads in the heterotype, whilst in the homotype mitosis each dyad further divides into monads, which are thus distributed between the daughter nuclei of the second division; while Grégoire and others regard these forms as due to quadripartite rather than quadrivalent arrangement of chromatids. According to the latter, a slight constriction in the conjugated univalents may lead to the quadripartite appearance, and as such it has nothing to do with the reduction phenomenon.

It is not difficult to realize how the formation of tetrads in plants during the heterotype prophase strikes at the very root of the arguments of Grégoire and others in favour of the early parasynaptic conjugation of the spireme. Those who maintain that the longitudinal fission of the early

heterotype prophase in plants does not completely close up, but ends in the separation of the univalents on the heterotype spindle, will have to modify their hypothesis with regard to the appearance of a simultaneous split in the spireme at right angles to the conjugating plane that is functioning in the homotype mitosis. Otherwise there will be an insurmountable difficulty in reconciling their hypothesis with the fact of the appearance of tetrad chromosomes in the heterotype prophase. In *Pustularia* the split in question has been traced from the early prophase, and its identity has been established with the parallelism of the early prophase. On this ground, again, their theory to establish the early pairing of the chromosomes by the criterion of parallelism of the heterotype prophase breaks down.

It is interesting to record at this stage an intermediate view held by a number of prominent cytologists, Gates (45, 46), Lawson (60), and Fraser (38) amongst them, who have in recent years worked on the pollen formations. Gates (44) has observed in *Oenothera rubinervis* that chromosomes which are stout, short, and sausage-shaped, come out of the first contraction in pairs, and are arranged in an end-to-end fashion. The spireme segments into pairs and the members of each pair come to lie side by side to form bivalents. In *Lactuca* (46), again, there is no indication of pairing of threads in the early prophase, but the delicate univalent spireme of the first contraction gradually condenses to form the short and thick spireme of the second contraction. The chromosomes are arranged end to end, as in *Oenothera*, and the method of reduction is in accordance with the scheme of Farmer and Moore. Gates is of opinion that looping over can only take place when the chromosomes are long and thread-like, as in *Lactuca* and *Galtonia*. In such cases a phase of second contraction is intercalated to bring about their approximation. In *Oenothera*, on the other hand, there is no need for a phase of second contraction. Though the early heterotype spireme in *Pustularia* presents a remarkable similarity to that of *Oenothera*, the subsequent phases leading to the formation of bivalents differ greatly. The chromosomes, though very small, short, and bean-shaped, nevertheless pass through a very distinct stage of second contraction when the pairing of the homologous paternal and maternal chromosomes is brought about. Fraser (38), though essentially in agreement with Farmer and his supporters on the significance of the parallelism of the early prophase, has found evidence of both parasynaptic and telosynaptic pairing of the thread in the later stages of second contraction in the pollen mother-cells of *Vicia Faba*.

There appears to exist a more general agreement as to the nature and significance of synapsis, as well as the method and formation of bivalents, from the evidence furnished by the Ascomycetes hitherto cytologically investigated. Guilliermond (49) regards the first division in the ascus as heterotype. He has worked out the cytology of *Peziza vesiculosa*, *Humaria*

rutilans, *Galactinia succosa*, and *Peziza catinus* in great detail. The spireme often showed a paired arrangement of filaments. The synaptic stages are very distinctly characterized. The chromatin spireme condenses and forms a knot on one side of the nucleus. The chromosomes are formed out of this synaptic knot by side-by-side union of the threads. The heterotype chromosomes during diakinesis present the forms of **V**, **U**, **8**, **O**, and other forms seen during the heterotype prophase of higher plants. In the metaphase the chromosomes separate in a more or less longitudinal plane, forming hollow lozenges. On their advance towards the pole during anaphase a second longitudinal split appears in them, and the chromosomes appear **V**-shaped again. In his earlier account (49) Guilliermond regarded this phenomenon of reduction as essentially the same as that of pollen formation studied by Grégoire, Berghs, and Strasburger (82). But in a later work (51) he has completed his investigation on the cytology of *Humaria rutilans* by adding some of the most essential figures of the synaptic stages, and has accepted the interpretation of the phenomenon as advanced by Fraser (37).

Maire (63) is inclined to accept the interpretation of the reduction phenomenon in the Ascomycetes as put forward by Strasburger (81) and Farmer and Moore (31). In *Galactinia succosa* the true longitudinal fission closes up, and the union is followed by a folding of the filament and longitudinal fusion which renders the chromatic filament twice as thick as before. The filaments coil themselves into a compact knot which segments into a number of chromosomes.

Fraser and her colleagues (37, &c.) have been able to establish definitely the presence of both the fissions in the heterotype spireme of the Ascomycetes. In *Humaria rutilans*, *Otidia aurantia*, *Peziza vesiculosa*, and other forms which they have so critically studied, they have traced the first or true fission till the second contraction. During the second contraction a process of looping over follows. In the arms of these loops, before and after segmentation, they have detected this primary fission, while the fission between the loops represents the line of approximation to the univalents, which are arranged in the spireme in an end-to-end fashion. Some of the early diakinetic bivalents in *Humaria* (Figs. 24 *b* and *c*) present a remarkable similarity to those of *Pustularia* (Pl. VI, Fig. 30). Though occasionally broken by unavoidable gaps, her sequence in *Humaria* is in a general way in agreement with that of *Pustularia*. The evidence she has obtained from *Humaria* and other forms strongly supports the view held by Farmer and Moore. She has remarked that the main difference in the interpretations of the phenomenon advocated by the two aforesaid schools is due to a difference in the true seriation of events. It would seem that this is also, to a certain extent, due to want of harmony in correlating the observed sequences.

(b) *The Second and Third Divisions.*

Passing on to the succeeding divisions from the telophase of the heterotype, we are confronted with certain phenomena peculiar to these fungi, owing to the intercalated resting stages, but, nevertheless, the processes are comparatively simple. The chromosomes in most of the vascular plants, after telophase, maintain their individuality to a certain extent, and often remain almost intact throughout the process of interkinesis. They may occasionally undergo partial dispersal of their chromatin substance. When the chromosomes are long or thready and of good size, they undergo vacuolization, and thus form semi-reticulated or irregular alveolized bands. A complete rest is seldom seen in the higher plants. Consequently, fewer steps are required for their reconstruction.

In *Pustularia*, as the nuclei undergo a period of rest during interkinesis, which evidently lasts for a considerable time, the process of reconstruction is not quite so simple. The daughter chromosomes cannot be traced farther at the telophase. The chromosomes which are distributed between the daughter nuclei form a chromatin mass which completely dissolves away during the period of rest. Consequently the succeeding phases do not occur till after a considerable period. During the early prophase, the chromatin becomes concentrated in the interstices of the linin, and forms a fine reticulum. The reticulum is transformed into a coarse spireme and the split of the heterotype becomes again evident in the parallel thread of the spireme. The split is closed up and the beads are again associated through a simple process of contraction.

There seems to exist a certain amount of obscurity as to the significance of the phenomenon of contraction just referred to which has been observed in some other Ascomycetes. The interpretation put forward by some authors to explain this post-meiotic contraction phase has interfered with the views as to the nature and significance of the second division in these fungi, hitherto accepted without question as homotypic in strict agreement with the interpretation of this division in animals and in plants. This phenomenon of contraction, as well as a subsequent one prior to the third division, has been partially dealt with in the first part of the discussion in connexion with the general trend of the heterotype prophase. But from the point of view of the cytology of the Ascomycetes, as this phenomenon presents us with a matter of further controversy, it seems desirable to discuss the question from this aspect.

According to the observations of Guilliermond (49, 51) in *Peziza* (*Humaria*) *rutilans*, *Peziza catinus*, and *Pustularia* (*Peziza*) *vesiculosa*, the chromosomes which separate out of the chromatic knot present the character of those of the first division, the only difference being that they do not

form the hollow lozenges of the first division at the metaphase. There is a transverse division of the V-shaped chromosomes as in the homotype division of the Phanerogams. Maire (63) regards the second division as essentially homotype, which brings about the separation of the half-chromosome thread formed at the metaphase of the preceding division. According to Claussen (16) the chromosome relation in *Pyronema confluens* in the second and third divisions is the same as in the first. The first division differs from the other two by the presence of synapsis and diakinesis of the heterotype. Similar observation has been recorded by Brooks (11) and Faull (34) from their investigation of other groups of Ascomycetes.

Fraser in her work on the cytology of *Humaria rutilans* (37) upheld Guilliermond's interpretation of the second division. But the subsequent discovery of a contraction phenomenon during the prophase of the second division in *Peziza vesiculosa* (43) and *Ascobolus furfuraceus* (41), and the numerical reduction of chromosomes at the telophase, led her to change considerably the interpretation of this division. She has attached importance to this contraction phase combined with a subsequent one preceding the third division, as connected with some kind of pairing arrangement of the allelomorphs, so that these contraction phases are thus ultimately responsible for bringing about the brachymeiotic reduction between the last two divisions. The second division in these forms, therefore, according to her contention, is not a straightforward homotype, but more or less a reducing one.

Our knowledge as to the significance of the first contraction, of the parallelism of the heterotype as well as that of the second contraction, is fortunately definite in this case. At the same time, our observations of this phenomenon during the succeeding phases and the processes which are connected with them are without any break, so that nothing is left obscure. The split has been traced through the interphases to the subsequent contraction. The number of chromatin beads left over from the early prophases and the second and the third divisions (Pl. VII, Figs. 51 and 65) is the same; while the number of chromosomes in the anaphase of the second division (Pl. VII, Fig. 59) agrees with that of the late prophase of the third division (Pl. VII, Fig. 67 and Pl. VIII, Figs. 68 and 69). With these evidences at our disposal we are unable to accept the contention of Fraser and her colleagues that the contraction before the second division is in any way connected with the pairing of the allelomorphs. The second division is homotypic, and is associated with the longitudinal separation of sister halves of the univalent chromosomes, which are represented by these beads precociously united during the prophase of the heterotype. From the extraordinary way in which these beads undergo premature separation in the early prophase spireme after an interkinetic rest, it is obvious that the attachment between the two halves of the somatic chromosomes is not firm enough to hold them together. In these organisms in which these beads are peculiarly liable to

separate from one another it is not unexpected to find a simple contrivance, such as a process of contraction, intercalated to bring about their association.

The third division has been regarded by a considerable number of investigators as equivalent to a vegetative division, like the second, to effect quantitative division of chromatin, which is to be distributed between the spores. On the other hand, Fraser and her colleagues hold that this division is essentially a reducing one, which they have defined as *brachymeiosis*. The controversy on the nature of this division arose out of cytological investigation of *Humaria rutilans*, which has been worked out by Guilliermond (49) as well as by Fraser (37). Guilliermond has observed sixteen straight chromosomes at the telophase of the third division, while Fraser has regarded them as sixteen ends of eight **V**-shaped chromosomes. Unfortunately, the chromosomes in *Humaria* after the third division become extremely thready, and consequently none of these authors has been able to follow the mode of separation of the chromosome during the metaphase, nor have they obtained a clear view of the anaphase. The interpretation of Fraser and her colleagues of this division in other forms of Ascomycetes investigated by them differs widely from the views of Guilliermond and Maire, who studied the same fungi.

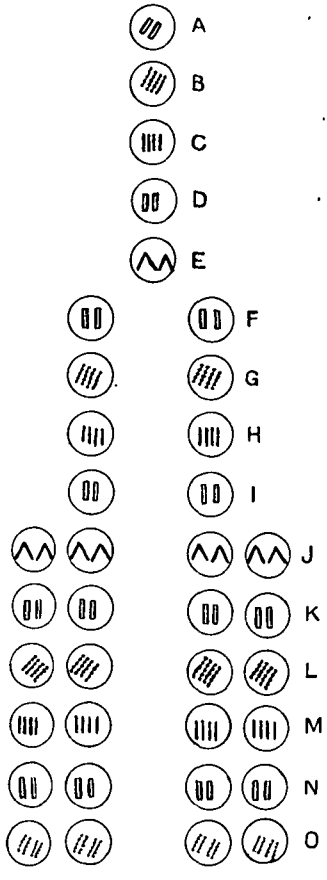
The brachymeiotic reduction, according to Fraser and her colleagues, is a very variable phenomenon, but its most essential feature seems to be to bring about a numerical reduction, when half the number of chromosomes passes to each daughter nucleus. The variability is not only shown by the shifting period through which this reduction takes place, but also by the mechanism by which the reduction is brought about. For instance, this reduction may be effected only during the third division, without any evident pairing by the allelomorphs (*Humaria rutilans*); or it may be preceded by a pairing of allelomorphs which is effected by a contraction phase (*Otidia aurantia*); or such pairing may take place in the prophases of both the second and third divisions (*Peziza vesiculosa* and *Ascobolus furfuraceus*); or the pairing may take place very early in the life-history, after fertilization, in which case the chromosomes appear in half the number in the division of each of the paired nuclei in the crosier (*Humaria granulata*). In the absence of any contraction phase which could be related to such pairing, this has been explained as a case of (partial) association of chromosomes. Lastly, the pairing of the allelomorphs may be not at all apparent at any period of the life-history of the nucleus, but association of chromosomes takes place from one division to another (*Phyllactinia corylea*). In such a case there is no change in the chromosome number throughout the life-history of the fungus. It has been argued that the chromosomes remain tetravalent till the first division and bivalent till the third, the association being so intimate as to leave no visible effect of their treble and double nature on the chromosomes.

Though the contraction phase before the third division is very conspicuous, we are unable to confirm any numerical reduction of the chromosomes at this division. The process of separation of the beads is very clear during the metaphase, and in the anaphase the late prophase number of chromosomes is repeated. Consequently, the contraction phenomenon here also is not associated with any allelomorphic pairing of the chromosomes. We gain further insight into the problem by studying the condition of the young spore nuclei. As a matter of fact, the early spireme condition of the spore nuclei shown on Pl. VIII, Figs. 81 to 84, according to the convention observed in flowering plants, represents a late telophase stage, when the chromatin beads are present again in the prophasic number of the third division, while the number of chromosomes in the mature spore corresponds to the number of chromosomes in the late prophase of the third division. The distribution of the chromatin beads from the telophase of the first division to the succeeding divisions, and in the spores, is diagrammatically shown in Text-fig. 3.

Finally, there only remains to be discussed her last contention, the evidence drawn from *Phyllactinia*, and partly also from *Humaria granulata*, in which there is no change in the chromosome number throughout the life-history, as a case of chromosome association rather than true pairing. In *Phyllactinia* (58) at the time of fertilization, the male and female chromosomes approximate in pairs. As each of these two nuclei carries eight chromatin strands and in the united nuclei eight strands of chromatin could be counted, it is assumed that in the absence of any apparent sign of pairing, the paternal and maternal chromosomes, represented by the chromatin strands, have become associated side by side, as the result of which the number remains the same but the valency has been doubled. The second or asexual fusion takes place in the young ascus when these double chromatin strands again become approximated in pairs. This is followed by the synapsis stage, when the whole chromatin mass begins to contract and becomes more dense. After synapsis the chromosomes again appear as eight strands, and ultimately give rise to eight tetravalent chromosomes on the equatorial plate of the first division.

This Harper-Fraser hypothesis of chromosome association, even though it could be given a theoretical consideration, has its drawbacks. According to the above description of synapsis in *Phyllactinia*, one cannot overlook the importance Harper has attached to the process of synapsis that brings about the pairing of the chromosomes and thus causes the actual reduction of chromosome number. The point is how far it is justifiable to correlate this synaptic contraction with the sexual fusion. In fact, in *Phyllactinia* as well as in *Humaria granulata*, the fertilization is followed by no apparent disturbance of the nuclear substance, but the process of actual reduction and the synaptic stages that bring it about are postponed till a subsequent

asexual fusion has taken place. Following the analogy between the phenomenon of fertilization in animals and plants, in which the actual reduction process is related to the visible synaptic stages and the matura-



TEXT-FIG. 3. Diagrammatic representation of the distribution of chromatin beads from the telophase of the first division to the succeeding divisions and in the spore. A (Pl. VI, Figs. 43–46). Telophase of the first division. B (Figs. 49–50). Resting stage during interkinesis. C (Figs. 51–52). Prophase of the homotype, showing parallelism of the spireme. D (Figs. 52–54). Late prophases; contraction to equatorial stage; association of half-univalents. E (Figs. 55–58). Metaphase to early anaphase; appearance of split in the chromosomes receding towards the pole. F (Figs. 59–61). Late anaphase and telophase. G (Fig. 63). Interkinetal rest. H (Figs. 64–66). Prophase of the third division, showing parallelism of the spireme. I (Figs. 66–69). Late prophases; contraction to equatorial stage; association of half-univalents. J (Figs. 70–73). Metaphase to anaphase; split visible in some of the chromosomes receding towards the pole. K (Figs. 74, 75). Anaphase to telophase. L and M (Figs. 76–84). Late telophase in the immature spore nucleus. N (Figs. 85, 86). Mature spore nucleus. O (Fig. 89). Resting spore nucleus.

tion divisions succeeding them, and the phenomenon of asexual fusion, in which (as defined by Fraser), without any visible indication of pairing, an intimate association of nuclear substance takes place, which again is followed by a separation of unaltered chromosomes, it appears quite reasonable to

suggest that the two phenomena in question in the life-history of *Phyllactinia* and *Humaria granulata* may have been reversed. If this deduction is correct, then Claussen's hypothesis (16) of association followed by true fusion of the sexual nuclei holds good. In fact, lately Claussen's observation has received confirmation by Shikorra (78) and Ramlow (72), who undertook to rework this part of the problem on other members of the Ascomycetes.

The phenomenon of pairing of the homologous chromosomes and the association of the complementary halves of the univalents are distinctly marked out processes in *Pustularia*. Consequently, the interpretation of *Phyllactinia*, be it as it may, cannot be applied to *Pustularia*.

Claussen (16) has aptly remarked that such a phenomenon as the occurrence of more than two divisions of the spore mother-cells, is not uncommon in plants. Farmer and Williams (32) have stated that reduction of Fucaceae takes place during the first division of the nucleus. The second division follows the first, and after the third or final oogonial mitosis eight eggs are found. Yamanouchi (85) has closely followed the reduction process in *Fucus*. Reduction takes place during the first division of antheridial and oogonial mitosis, after which each of the antheridial nuclei undergoes five more divisions, resulting in the formation of sixty-four sperm mother-cells, while the oogonial nucleus, as Farmer and Williams observed, divides twice and forms eight egg-cells. Similar examples in which spore mother-cells undergo more than two divisions can be quoted from higher plants. In Phanerogams this is seen in the embryo sac of *Lilium*, in which, after heterotype and homotype divisions, there follows rapidly a third division. There is no need to accumulate instances. Besides, some of the spores of the sixteen-spored ascus (Pl. VIII, Fig. 90) appear to possess quite normal nuclei. It is absurd to suggest that in such cases there have been, three times, fusions of the nuclei, which have made the definitive nucleus of the ascus octavalent, and consequently that there have been three reductions to form univalent ascospores.

The above facts and the evidence at our disposal from the study of *Pustularia* forbid us to enter into any such generalization as that the third division is essentially a reducing one. On the other hand, we are of the same opinion as Guilliermond, Claussen, Brooks, Faull, and others, that in all the three divisions the chromosomes are present in the same numerical relation; the first division is heterotype, the second is homotype, and the third is equivalent to a vegetative division. If, however, the question of a second reduction is purely a matter of interpretation, such a difference resolves itself into an unimportant one.

VII. Summary.

1. The definitive nucleus in *Pustularia* presents a very marked contraction phenomenon, wherein the chromatins of the two fused nuclei retain their individuality to a certain extent, as in the case of the first contraction of higher plants.

2. The first contraction knot opens out as a parallel spireme, when the chromosomes split up, and are serially arranged in the linin thread, forming loops. During the subsequent stages of the early heterotype prophase a rearrangement of the spireme is shown by a development which finally brings about the union of the chromatin beads thus split up. The function of the parallel threads of the early heterotype prophase is, therefore, to bring about the association of the chromatin beads which are of a half-univalent nature.

3. Prior to the second contraction, the fission opens out again, and the chromatin spireme undergoes disorganization; but reconstruction of the spireme takes place speedily, and as the nucleus enters upon the second contraction stage, the fission in the linin frame-work closes up completely, but that in the chromatin beads remains partially open. The process of second contraction is very remarkable in *Pustularia*, and brings about the formation of regular tetrad bivalents owing to fission in half-univalent beads, together with an end-to-end conjunction of the whole univalent.

4. There are sixteen bivalent chromosomes in the prophase of the first division. The first division is heterotype, and the chromosome reduction follows essentially the scheme laid down by Farmer and Moore.

5. The second division is homotype, and it is succeeded by an interphase and a contraction phenomenon whose function is to bring about the reassociation of the half-univalent beads, which in these fungi are liable to undergo precocious separation.

6. The second division is followed by an interphase, when the nucleus appears to undergo a state of complete rest. The spireme is reconstructed, and the half-univalent beads again appear in full but reduced number.

7. The nuclei present a phenomenon of post-meiotic parallelism, which can be interpreted in the light of the parallelism of early heterotype prophase, which brings about the association of half-univalents. The first contraction phase, as well as those prior to the last two divisions, is not, therefore, associated with any kind of pairing of full somatic chromosomes.

8. The third division is equivalent to a vegetative one, in which the chromosome divides like a somatic division, and the chromatin is distributed in the nuclei of eight spores.

9. The number of chromosomes is the same in the prophases as well as in the telophases of all the three divisions. There is no second reduction in

Pustularia, nor is there any evidence that an association of whole somatic chromosomes has taken place from one nuclear division to another. Owing to the presence of prominent contraction phases during the prophase of every division, such an hypothesis does not apply to *Pustularia*.

10. The number of chromatin beads remains apparently the same in the prophases of the last two divisions, as well as in the young spore nucleus which represents the late telophase stage of the third division. As the spore matures the chromatin beads reunite when the chromosomes appear in the full number of the third prophase. In a resting spore nucleus the chromosomes become disorganized and their individuality is apparently lost.

11. When the spores are formed, the centrosomes play an essential part, throwing out rays which are recurved to form an inner and an outer series. The inner series is transformed into a nuclear membrane, and the outer series encloses cytoplasm.

12. 'Chromatin bodies' are extruded from the nuclear framework. During the heterotype prophase, connexion of the spireme is established with the nucleolus several times, when the extrusion of these bodies reaches its maximum, and occasionally a portion of the spireme is seen to be thrown out into the cytoplasm.

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IX. EXPLANATION OF PLATES V–VIII.

Illustrating Mr. Bagchee's paper on *Pustularia bolarioides* Ramsb.

All the figures were drawn with the aid of an Abbe camera lucida under 2 mm. apochr. hom. imm. Zeiss N.A. 1–40 with comp. oc. 18. × about 2250.

PLATE V.

Heterotype Division.

Fig. 1. Definitive nucleus of ascus: *a*, nucleus, showing first meiotic contraction; massing of the nuclear substance on one side of the nucleus; chromosomes are embedded in the linin matrix; *b*, slightly later stage, showing the opening of the knot; appearance of vacuolated space in the linin matrix.

Fig. 2. Ascus containing single nucleus in which the spireme is concentrated into two masses which are bridged over by cross-connexions.

Fig. 3. Nucleus showing the loosening of the first contraction knot and appearance of the first loop with parallel arms; while others are in the initial stage in the main body of the knot.

Fig. 4. Further stage in loosening of the knot as wide loops, which are formed of beaded chromatin strung in a broad band of linin. The others are concentrated on one side of the nucleus.

Fig. 5. An almost completely opened synaptic knot, showing the endless nature of the spireme, which consists of simple and twisted loops.

Fig. 6. Fully opened spireme after first contraction. Nucleus showing two prominent loops, *a* and *b*, in the same focus; the loop *a* is twisted in the middle, and the loop *b* is a simple loop without any twist.

Fig. 7. Synapsis showing a peripheral arrangement of the loops.

Fig. 8. Early 'hollow-spireme' stage, showing a further stage during the rearrangement of the spireme. The spireme shows the earliest stage of association of the beads at the place of twist in the loops.

Fig. 9. Early 'hollow-spireme' stage, showing the association of the beads has become more pronounced at the twist. *a*, double-headed loop with associated bead at the twist.

Fig. 10. A slightly later stage. Nucleus showing a rearrangement of the spireme. The continuity of the spireme is broken and the loops are attached to the nucleolus. Association of the beads of the arms is apparent.

Fig. 11. Nucleus showing an advanced 'hollow-spireme' stage. Note the strained and elongated appearance of the loops; the united beads have become elongated in the direction of the pull.

Fig. 12. 'Hollow spireme.' The spireme is relieved of the strain. The beads have again undergone fission, but each of them faces its complementary half. Note the migration of chromatin spireme into the cytoplasm.

Fig. 13. Superficial section of a nucleus of the same stage as Fig. 12. Note the parallel arrangement of the complementary beads.

Fig. 14. Later 'hollow-spireme' stage. Nucleus showing accentuated association of the beads. The univalents are very marked at the twist of the double-headed loop, *a* and *a'*, and the two pairs, *b* and *b'*, are in the process of association.

Fig. 15. Superficial section of a nucleus of late 'hollow-spireme' stage. Nucleus shows more of the parallel arms than of the loops. Fission is closing up. Note *a* and *a'*, the univalent beads at the point of junction of the loop and the arms.

Fig. 16. Another superficial section of a nucleus of the same stage, showing more of the loops than of the parallel arms. Note that the fission of the loops is closing up.

Fig. 17. A thin medium section of a nucleus of the same stage, showing a closer association of the beads.

Fig. 18. Massing of the spireme before the second contraction. The spiremes show closer association of the beads. Note the increase in the number of large-sized beads.

Fig. 19. Slightly later stage, showing that the association of the beads is almost complete. Note that nearly all the beads are large sized.

Fig. 20. Nucleus showing the reopening of the fission before the second contraction. Note the split-up beads at the curve of the loops.

Fig. 21. Nucleus showing a further stage of advance in the process of reopening of the fission. The spireme presents a 'rugged' appearance.

Fig. 22. Nucleus showing a complete disorganization of the spireme prior to second contraction. Note *a*, a portion of the old spireme, and *b* and *b'*, grouping of the chromatin beads as tetrads.

Fig. 23. Commencement of the second contraction stage. The spireme now reconstructed shows fission and pronounced grouping of the beads to form tetrads.

Fig. 24. Nucleus entering upon the second contraction stage. Note the zonation of chromatin on linin. The linin framework shows fission.

PLATE VI.

Fig. 25. Slightly later stage. Nucleus shows an accentuated grouping of the chromatin beads as tetrads.

Fig. 26. Further advance in the second contraction stage. Note the close grouping of the tetrads. Fission in the linin framework is still visible.

Fig. 27. Nucleus in the height of second contraction stage. The fission in the linin framework has completely disappeared. The chromatin tetrads are distinctly polarized. Note the bivalent segments showing fission in the individual beads together with conjunction with the next pair to form a close ring.

Fig. 28. Nucleus showing spireme emerging from the second contraction stage. Note the transformation of the tetrads into intermediate forms of chromosomes.

Fig. 29. Loosening of the second contraction. Note the segmentation of the chromosomes is accompanied by twist and torsion.

Fig. 30. Early diakinesis. Note the original fission visible again between the pairs of linin endings and that the conjoined pairs are twisted in the middle.

Fig. 31. Early diakinesis, showing gradual evolution of the chromosomes after the complete segmentation of the spireme. Note the close ring-form of tetrads, *a*.

Figs. 32 and 33. Later stages of concentration of the chromosomes. Sixteen pairs of chromosomes can be identified. Some of them have already taken up the mature form of bean and the others are in tetrads, *a*, and intermediate forms.

Fig. 34. Diakinesis. Split between the univalent halves of the bivalent combination is still open.

Fig. 35. Diakinesis, showing early stage of spindle formation. Two centrosomes lie close together with a cone of fibres radiating from them.

Fig. 36. Spindle formation. Centrosomes move farther apart from one another. The spindle is established.

Fig. 37. Spindle formation. Appearance of 'Hermann spindle' with laterally placed chromosomes. Note that the split between the univalents has completely disappeared.

Fig. 38. Mature spindle. Chromosomes on the equatorial plate stage. Sixteen mature bivalent chromosomes.

Fig. 39. Early metaphase, showing five bivalents; the rest are univalents. Note the appearance of fission in some of the univalents moving towards the poles.

Fig. 40. Advanced stage of metaphase. Note the fission in some of the univalents.

Fig. 41. Slightly later stage. As the chromosomes move towards the poles in two batches, fission widely separates the halves.

Fig. 42. Early anaphase. Fission, though visible in some of the chromosomes, is rapidly disappearing.

Fig. 43. Anaphase. The fission is no longer visible in the retiring chromosomes.

Fig. 44. Early lophase, showing disorganization of the chromosomes at the poles.

Fig. 45. Telophase. Chromosomes forming a compact mass at the poles.

Fig. 46. Telophase. Oblique polar view of the chromosomes. Notice the different ways these chromosomes disorganize and lose their individuality at the poles during the telophase.

Fig. 47. Telophase. Achromatic fibres forming a bundle-sheaf. Appearance of vacuoles between the chromatic aggregations.

Fig. 48. Later telophase, showing the formation of daughter nuclei.

Second Division.

Fig. 49. Later telophase, showing the reconstruction of the chromatin spireme.

PLATE VII.

Fig. 50. 'Resting' nucleus to fine spireme stage.

Fig. 51. Early prophase. The lower nucleus shows the chromatin beads undergoing association during the coarse spireme stage.

Fig. 52. Advanced prophase. The nuclei show the approach of contraction phase. The association of the beads is clearly shown in the upper nucleus.

Fig. 53. Spindle formation. Centrosomes are near each other in one of the nuclei; the other shows the formation of 'Hermann spindle'.

Fig. 54. Mature spindle. Chromosomes on the equatorial plate, showing polar and longitudinal views.

Fig. 55. Early metaphase. The nucleus shows the separation of chromosomes in longitudinal view. Note the appearance of longitudinal fission in the chromosomes advancing towards the poles.

Fig. 56. The same stage as Fig. 55, showing the separation of chromosomes in polar view. Note the appearance of wide fission during the separation of the chromosomes.

Fig. 57. Metaphase, showing chromosomes distributed all over the spindle.

Fig. 58. Early anaphase. The chromosomes moving towards the poles in two batches. Note the reappearance of wide fission in the chromosomes of the slowly moving batch.

Fig. 59. Anaphase stage, showing fission in the chromosomes has disappeared.

Fig. 60. Anaphase to telophase. Note the disorganization of the chromosomes during the telophase stage.

Fig. 61. Polar view of the telophase, showing chromosomes at different stages of disorganization.

Fig. 62. Late telophase stage, showing the formation of the daughter nuclei.

Third Division.

Fig. 63. 'Resting' nuclei.

Fig. 64. 'Resting' nucleus to fine spireme stage.

Fig. 65. Early prophase stage, showing coarse spireme stage with prominent beads.

Fig. 66. Early prophase stage, showing post-meiotic parallelism of the spireme and contraction phase.

Fig. 67. Prophase, showing the distribution of sixteen chromosomes uniformly in the nuclear cavity. Note that some of the chromosomes present a wide fission and appear as V-shaped bodies.

PLATE VIII.

Fig. 68. Spindle formation. Centrosomes are near one another. Note the bean-shaped form of the mature chromosomes, and that there is no fission in them.

Fig. 69. Prophase, showing chromosomes on the equatorial plate stage in longitudinal, polar, and oblique views.

Fig. 70. Early metaphase stage, showing the separation of the chromosomes in longitudinal view.

Fig. 71. The same stage as Fig. 70, showing the separation of the chromosomes in polar view.

Fig. 72. Metaphase stage, showing the distribution of the chromosomes all over the spindle. Note that some of the chromosomes approaching towards the poles present a wide fission and appear as two beads connected together by a narrow portion.

Fig. 73. Anaphase, showing the chromosomes are separated into two batches. No fission is visible in them.

Fig. 74. Ascus, showing nuclei from anaphase to telophase. Note the disorganization of the chromosomes during the telophase.

Fig. 75. An oblique view of the telophase.

Spore Formation.

Fig. 76. Telophase stage, showing the emanation of astral rays from the periphery of the chromatin mass.

Fig. 77. Late telophase stage, showing the fragmentation of the chromatin mass into beads. Centrosome is separated from the chromatin mass.

Fig. 78. Early stage of spore delimitation. Formation of nuclear beak. Note the accumulation of cytoplasm round nuclei.

Fig. 79. Spore delimitation, later stage. Note the umbrella-like radiation, the elongated nuclear beak, and the fibrous nature of the nuclear membrane.

Fig. 80. Very young spore. Nuclei showing fine chromatin spireme.

Fig. 81. Slightly later stage of the young spore. Nucleus showing a coarse chromatin spireme. Spore-wall shows distinctly fibrous nature.

Fig. 82. Young spore. Nucleus showing chromatin beads.

Fig. 83. Young spore, another stage.

Fig. 84. Young spore, showing association of the beads and gradual formation of the chromatin spireme in the spore nucleus.

Fig. 85. Later stage of young spore. Nucleus showing the association of the beads is complete.

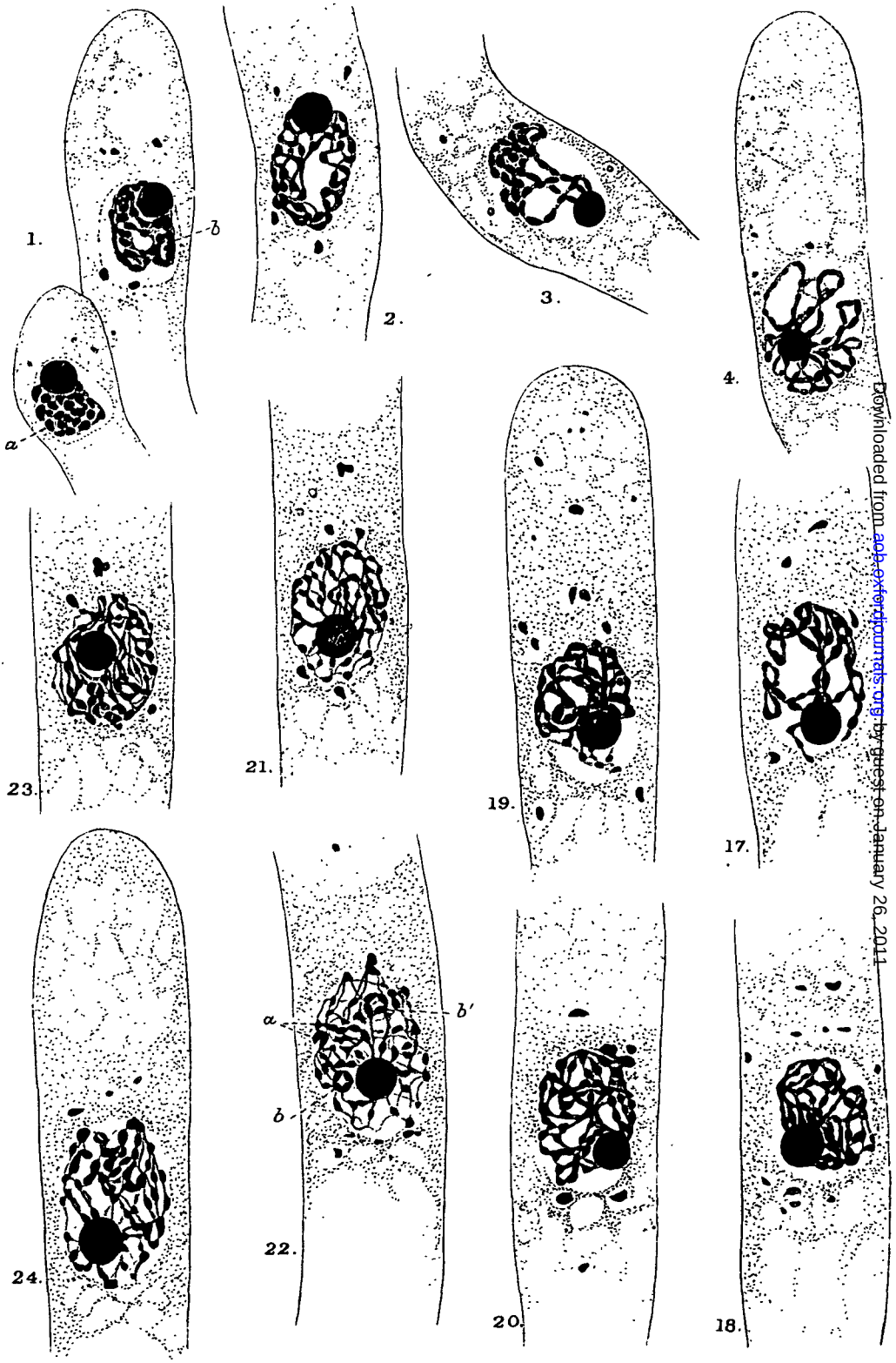
Fig. 86. Immature spore. Nucleus showing a thick spireme with noded chromatin.

Fig. 87. Immature spore in fine section, showing the formation of oil cavities. Nucleus with deformed spireme.

Fig. 88. Mature spore with fully developed oil cavities. Nucleus showing a skeleton spireme.

Fig. 89. Mature spore with 'resting' nucleus.

Fig. 90. Spores from an abnormal sixteen-spored ascus.

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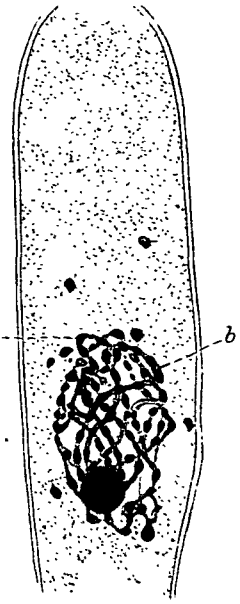

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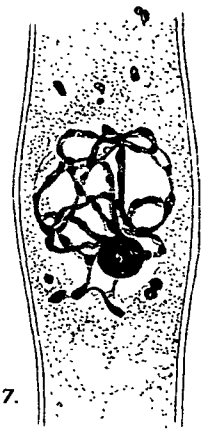
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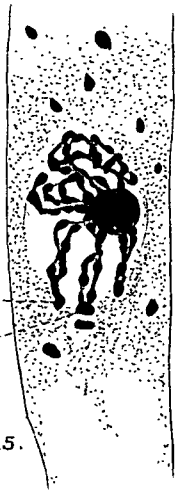

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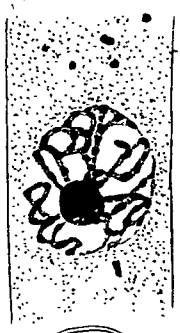
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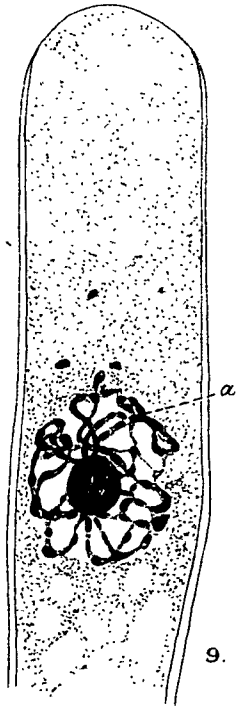

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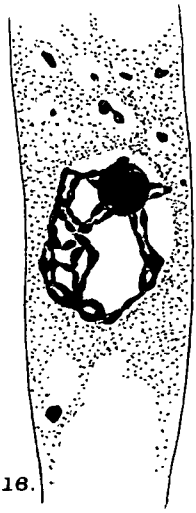
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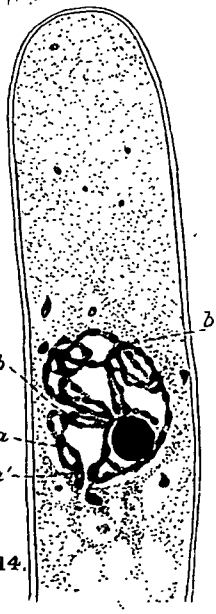

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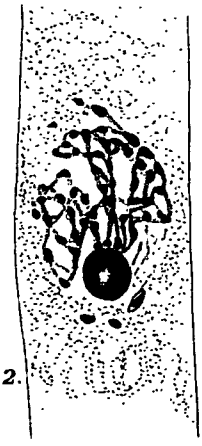
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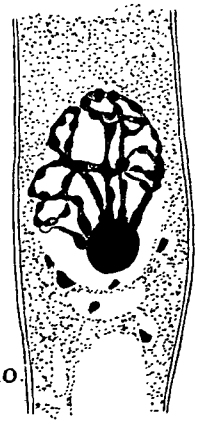
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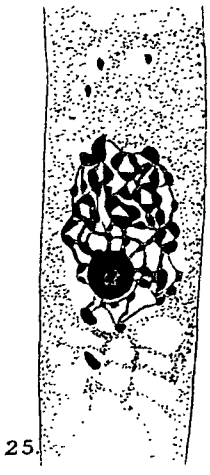
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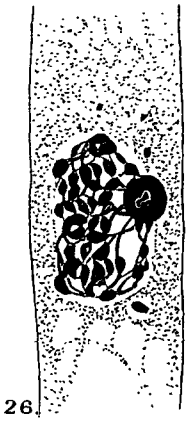
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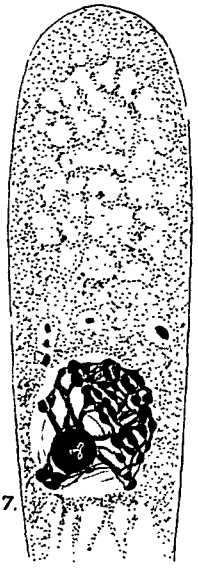
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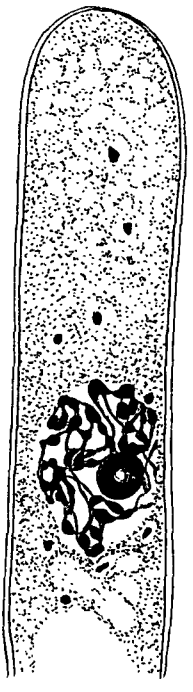
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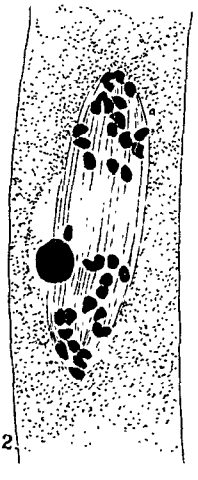
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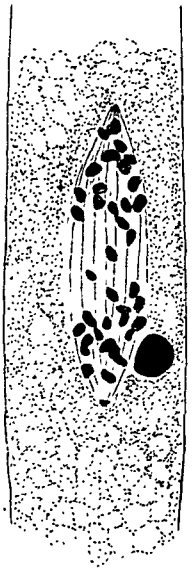
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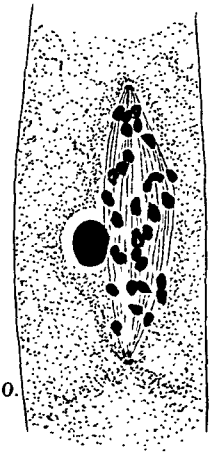
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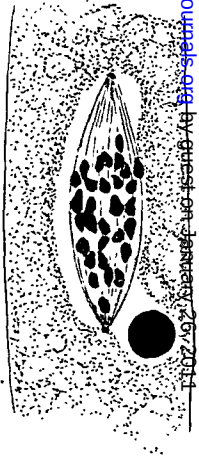
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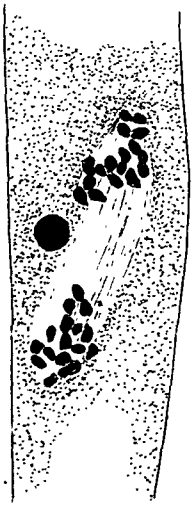
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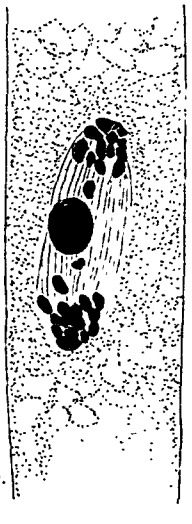
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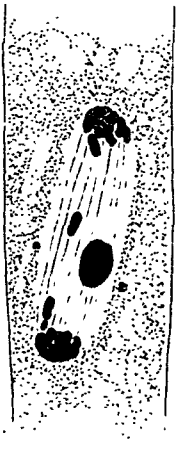
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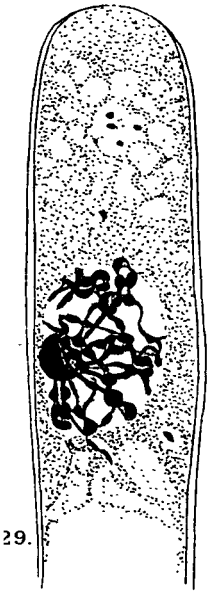
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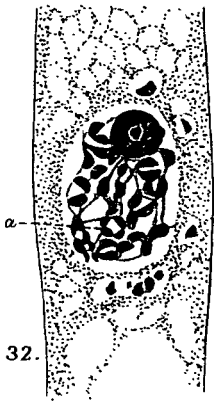

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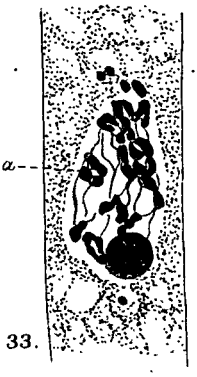

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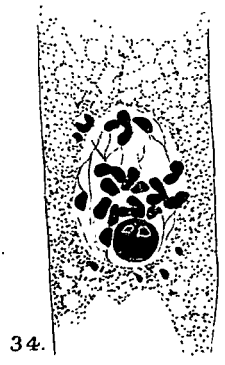
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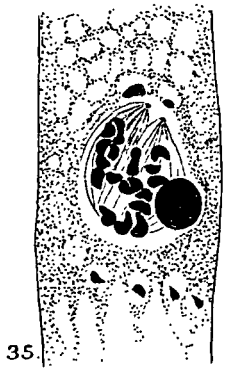
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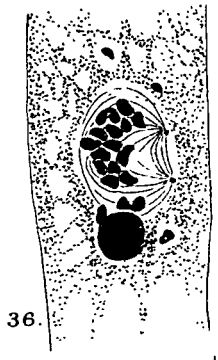
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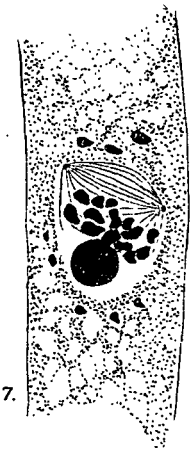
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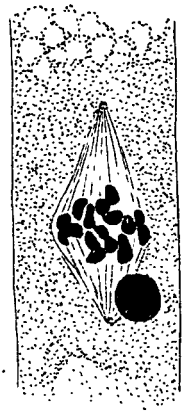
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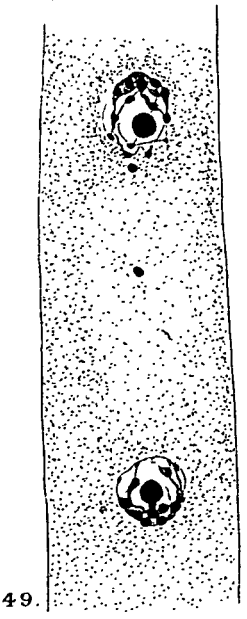
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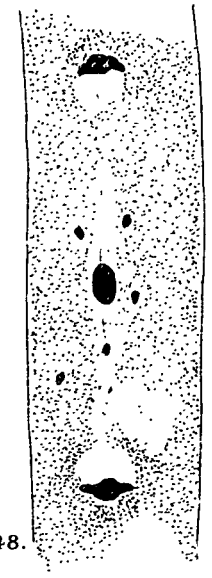
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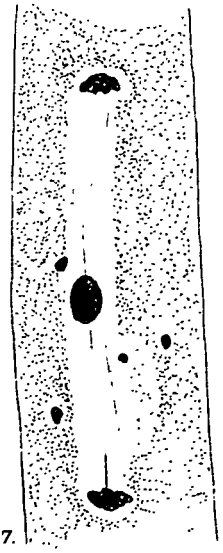
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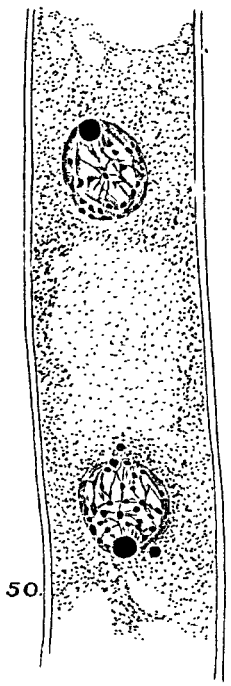

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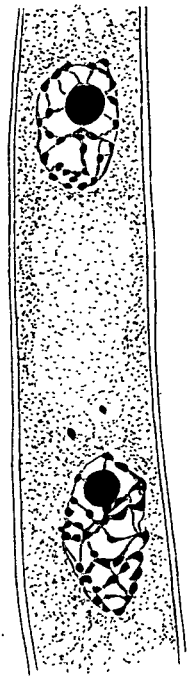

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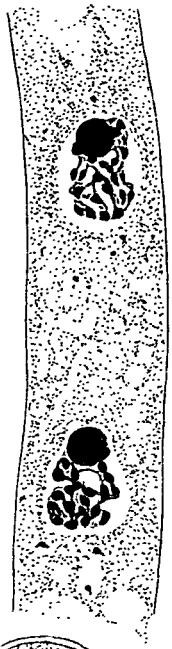

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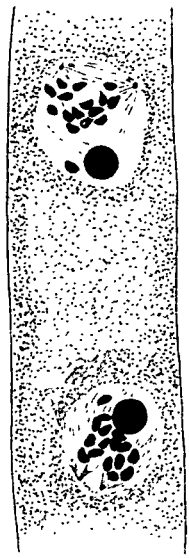

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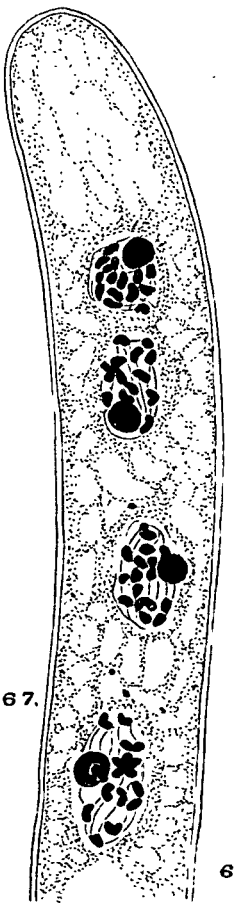

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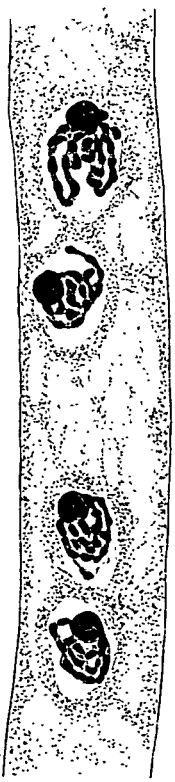

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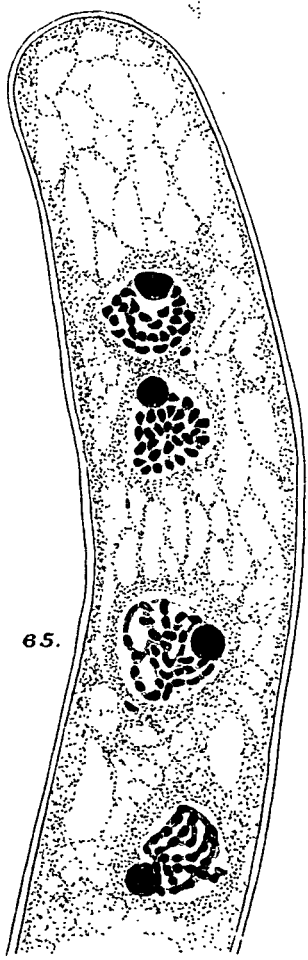

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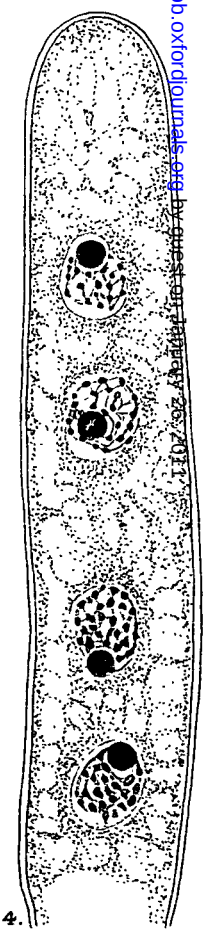

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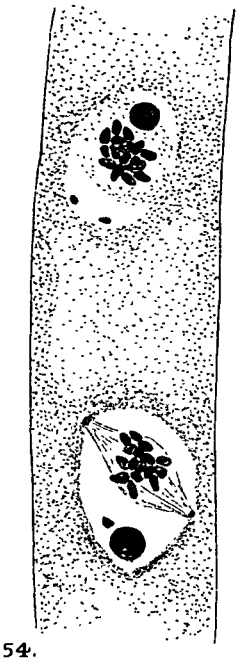
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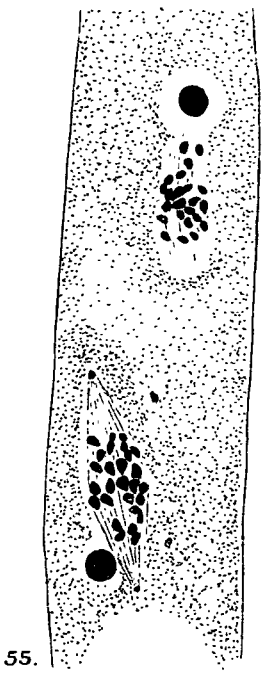
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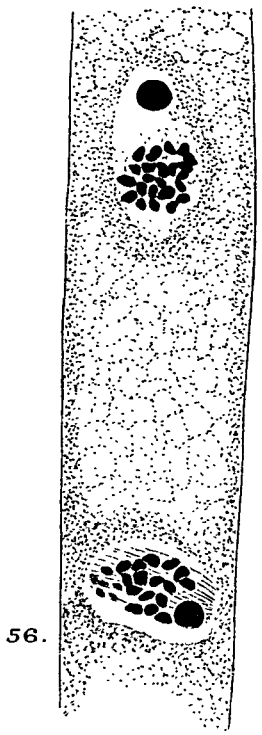
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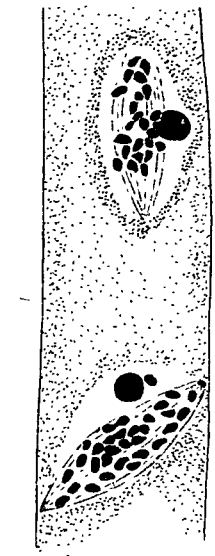
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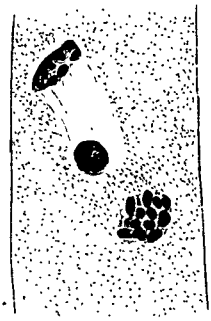
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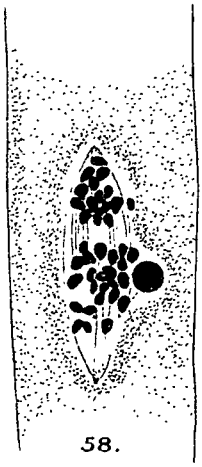
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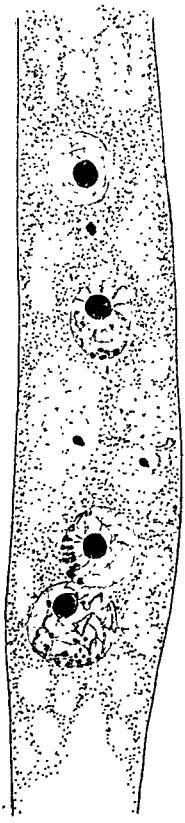
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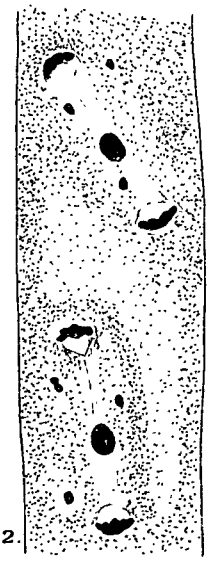
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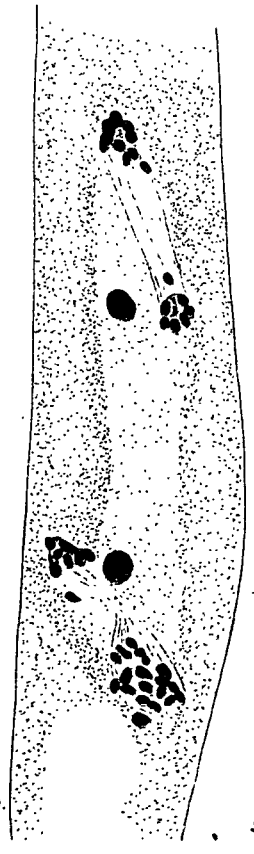
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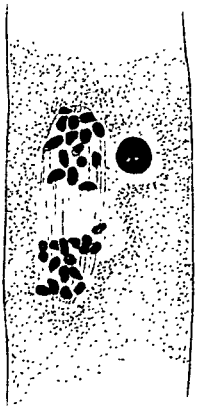
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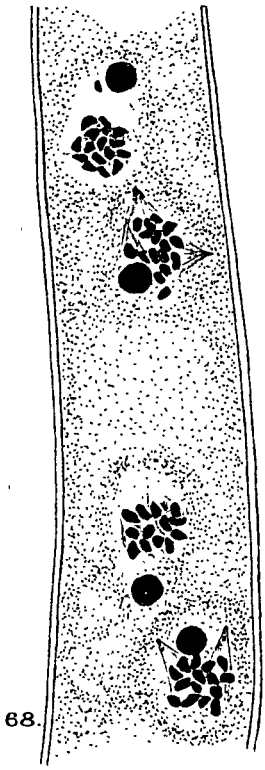

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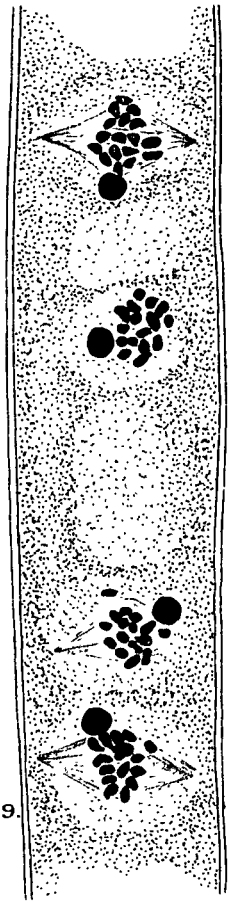

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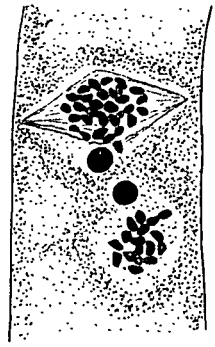

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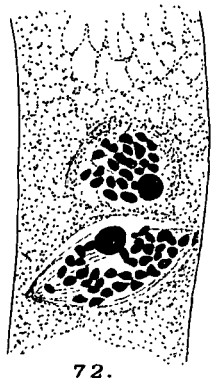

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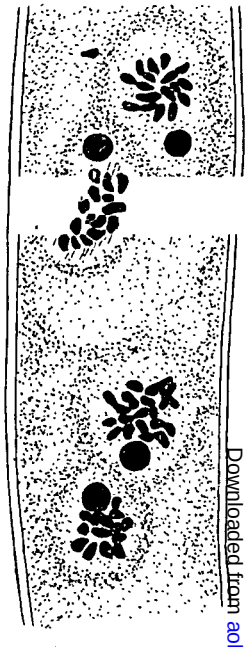

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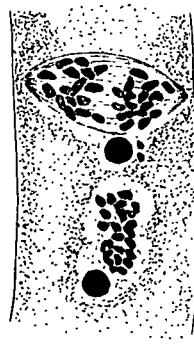

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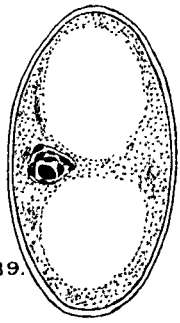

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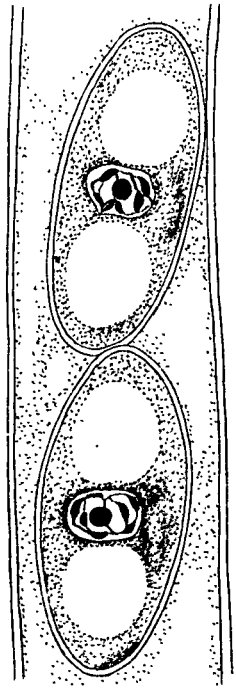

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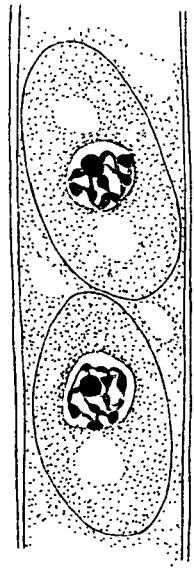

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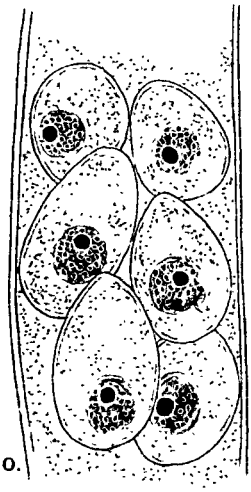

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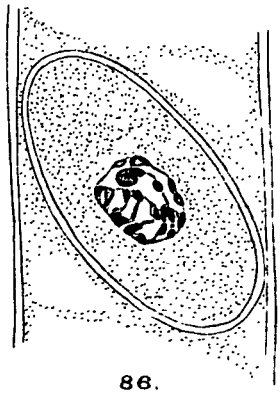

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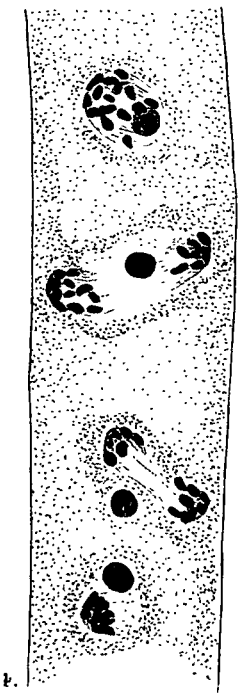

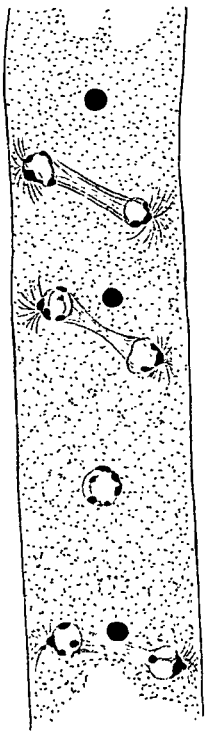

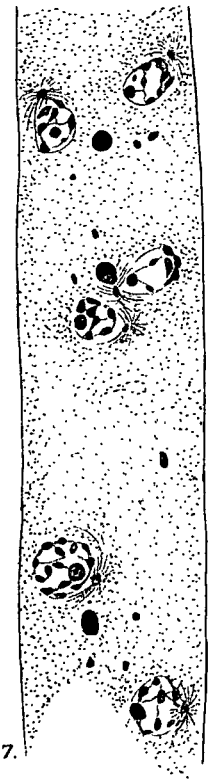

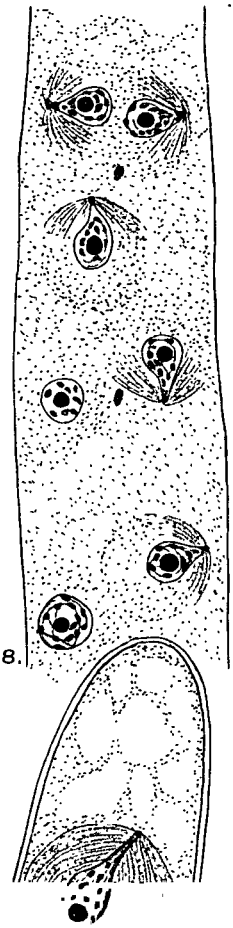

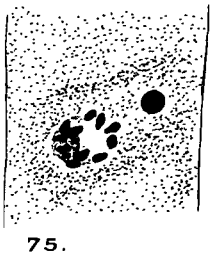

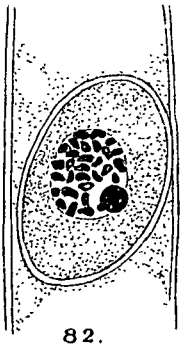

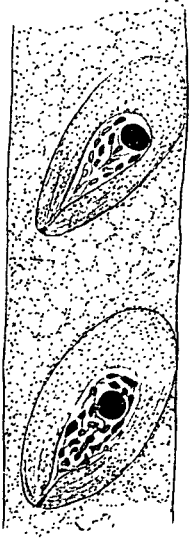

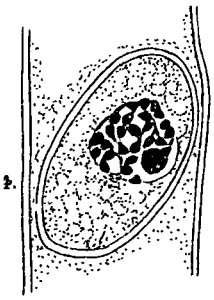

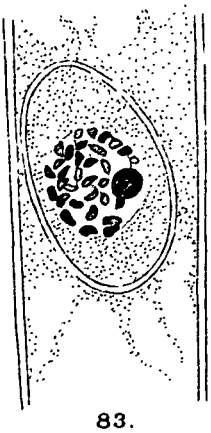

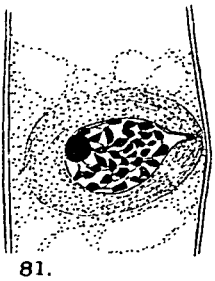

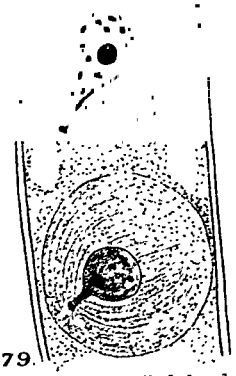

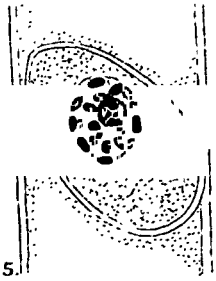

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