Contributions to the Cytology of Humaria rutilans, Fries.[1]

BY

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With Plates IV and V.

HUMARIA rutilans,[2] Fries (*Peziza rutilans*, Fries) is an orange Discomycete 0·5–1 cm. in diameter, occurring in abundance on sandy soil among moss.

Material was collected during the autumn and winter of 1905 and 1906, and was fixed in the field, chiefly in Flemming's weaker fluid. It was embedded either through chloroform or through cedar oil, and was allowed to remain in the bath for from half an hour to two days at a temperature of about 54° C. Material did not appear to be adversely affected by the longer time, but it cut readily after half an hour, and this period was employed during the latter part of the work.

Sections were cut from 4 to 20 μ in thickness, and were stained either with Flemming's triple stain or with Heidenhain's iron Haematoxylin and a solution of erythrosin in clove oil.

I have to thank Miss H. S. Chambers for valuable help in the preparation of material during part of the work.

Development of the Ascocarp.

The young ascocarp is first distinguishable as a small knot of septate hyphae; sometimes one cell is larger than the others (Pl. IV, Fig. 1), but the nuclei are quite similar, and any distinction in size is soon lost. The outer cells are distinguished by their rather thick walls, the inner by their richer protoplasmic contents (Fig. 2).

Great difficulty was experienced in obtaining the very young stages; specimens, even, in which the asci had begun to form can hardly be dis-

[1] Thesis approved for the Degree of Doctor of Science in the University of London.

[2] In naming this fungus I have followed the diagnosis given by Rabenhorst's Kryptogamenflora; my specimens probably belong to the variety *vivida* of Nylsen. The species appears to be the same as that investigated by Guillermond, who, however, does not give the authority. I wish to thank Miss A. Lorrain Smith for her kindness in confirming my identification.

tinguished, under a lens, from the white or yellow sand grains among which they grow, and the youngest stages were only secured when they remained attached to an older ascocarp after the sand had been cleaned away.

The hypothecium is formed as a loose tangle of septate hyphae, which show somewhat scanty cytoplasm and a few granules. Each cell contains one or a few nuclei. These show a reticulum with conspicuous net-knots; a nucleolus may or may not be present; evidence of the occurrence of a central-body, as described by Harper (31) for *Phyllactinia*, or of the attachment of the chromatin filaments at a particular point, was not obtained.

Nuclear divisions in the hypothecium are karyokinetic (Fig. 3); the chromosomes are densely massed on the spindle and could not be counted; early prophases were not identified with certainty.

The nuclei at this time are of two sizes, and the smaller nuclei are seen to *fuse in pairs*, thus giving rise to the larger. Such fusions are very readily observed in all but the youngest ascocarps, and show the usual series of dumb-bell shaped figures (Figs. 4, 5). Migration of a nucleus from one cell to another occasionally takes place (Fig. 6), no doubt in connexion with these fusions; but, whereas more than twenty fusions were counted, migration was only twice observed. It seems likely, therefore, that it is not of general occurrence, and that the two nuclei which fuse have often been present in the same cell since their formation.

Ascus Formation.

The paraphyses are now differentiated, and, soon after, the first asci appear. In the subhymenial layer nuclei of two sizes are present, the larger being in the ascogenous hyphae, the smaller in the paraphyses and the cells from which they arise. The nuclei of the ascogenous hyphae at first resemble the fusion nuclei of the hypothecium in size and general structure (Fig. 7 *a*), and there seems no reason to doubt that they correspond to them, and that the nuclei in the paraphyses are to be related to the nuclei which have not undergone fusion.

As the ascogenous hyphae develop, their nuclei increase in size, and become vacuolate in structure (Fig. 7 *b*). Eventually the two terminal nuclei of the hyphae undergo simultaneous karyokinetic division. This may take place, either before (Fig. 10), or after (Figs. 8, 11), the hypha bends over to form the crozier first described by Dangeard (11) in 1894. In the early prophases of this division the nucleus shows a definite spireme, and large, centrosome-like bodies are present (Fig. 8). The spireme breaks up into about sixteen curved chromosomes (Fig. 9). These become densely massed on the spindle (Fig. 10), and eventually pass to the poles (Fig. 11). A terminal, uninucleate and a penultimate, binucleate, cell are formed, the two nuclei in the latter being sisters respectively of those in the terminal cell and the stalk-cell.

The ascus-cell, or cell from which the spore-containing part of the ascus grows out, is semicircular in section. A small projection, rich in cytoplasm, is formed from it, and grows actively, pushing up among the paraphyses and the bases of the older asci. Often the convex surface of the ascus-cell faces to one side or directly towards the hypothecium, and the projection may become much curved. Finally it assumes the characteristic shape of the ascus, and either before or after fusion the nuclei of the ascus cell pass into it.

Frequently the terminal cell cut off from the ascogenous hypha continues its growth (Fig. 12), giving rise, in the usual way, to another ascus, the terminal cell connected with which may in turn develop further (Fig. 13). It at first appeared that, in such cases, the nuclei of the new ascus were necessarily of the relationship of cousins. But, since the publication of my preliminary note on *H. rutilans* (20), another process has been observed. The growing terminal cell often lies in contact with the stalk-cell of the same ascus, and may become united to it by an H-connexion (Fig. 14). The nucleus of one of these cells may then migrate into the other (Fig. 15). If this takes place, the relationship of the nuclei of the new ascus is not necessarily close. It was not possible to ascertain whether such migration is of general occurrence. It was only observed once, and, in that case, the nucleus was passing from the terminal cell into the stalk-cell (Fig. 15); but, where proliferation has taken place, an H-piece was often found, and it was usually impossible to identify a nucleus in the stalk-cell of the corresponding ascus. Sometimes it is from the stalk-cell that the new hypha arises (Fig. 14).

The Meiotic Phase.

Very soon after the young ascus-cell has been cut off, its two nuclei enter independently upon the prophases of the first division. The stainable material of the nucleus forms a fine thread-work and becomes aggregated towards one side of the nuclear membrane forming the first contraction figure (Fig. 16), as has been described for the spore mother-cells of higher plants.

As this contraction passes off the thread thickens, and becomes more or less equally distributed (Fig. 17). A certain degree of polarity is sometimes observed (Fig. 18), the emptier part of the nucleus being remote from the region of the previous contraction.

As the thread distributes itself over the nuclear cavity, indications of a longitudinal split are here and there observed (Fig. 17). This becomes increasingly evident (Fig. 18), until the thread appears to be double along its whole length (Fig. 19).

Throughout these mitoses, chromomeres could not be distinguished, though the thread has frequently a granular appearance which no doubt indicates the usual arrangement of its constituents.

Before the longitudinal fission is complete, the two nuclei fuse, forming

the definitive nucleus of the ascus (Fig. 18). The nuclei lie against each other, either in the ascus or in the cell from which it originates, and appear simply to flow together; the two nucleoli are visible for a time, then they also fuse. The two spiremes mingle and cannot be distinguished after the first stages of fusion.

The synapsis or second contraction now sets in (Fig. 20). The chromatin filament thickens, the longitudinal split is more or less obliterated, and the whole thread, except a few loops which run out from the main mass to the periphery, becomes aggregated towards one side of the nucleus. The synaptic stage apparently persists for some time, then, as the contraction loosens, the loops running out from the central mass become more obvious, especially as shown in transverse section of the ascus (Fig. 21), and the longitudinal fission is once more apparent (Fig. 22).

The spireme next breaks up into its constituent loops, each of these forming a bivalent chromosome (Fig. 23), in the limbs of which the longitudinal fission can still be distinguished. The limbs may be twisted on each other, or united at both ends, forming a ring-shaped figure, or they may diverge very considerably (Fig. 24). Most of the forms described for the heterotype chromosomes of Phanerogams have been observed both at this and at later stages. All the chromosomes are not necessarily formed from loops, but their limbs are always derived from different portions of the spireme.

The chromosomes now shorten and thicken, and the longitudinal split is, for the most part, obliterated, though it may still be distinguished in favourable cases. During this process the chromosomes become arranged about the periphery of the nucleus, appearing to undergo a mutual repulsion. As the contraction of the chromosomes proceeds, it becomes possible to count them; they appear, as first stated by Guillermond (24) in 1904, to be sixteen in number (Fig. 25).

During synapsis the nucleolus becomes closely pressed against the nuclear membrane, and assumes a characteristic sickle shape (Fig. 22). When the synaptic contraction loosens it remains close to the wall, and either retains its irregular shape or becomes once more rounded. By the time the chromosomes are fully formed, it is seen to be vacuolate in structure, but it persists, though diminished in size, till the late telophases of the division (Fig. 33).

Spindle formation follows closely the process described by Harper (28) for *Erysiphe*, but the fibres are very delicate. The first stage to be recognized with certainty shows the two discoid centrosomes lying rather close together, with a separate cone of fibres radiating from each (Fig. 26). The centrosomes move apart, a spindle is established between them (Fig. 27); the radiating fibres become attached to the chromosomes, and have the appearance of drawing these on to the spindle (Fig. 28). A little

later the centrosomes reach opposite ends of the nucleus, and the monaster is formed.

Up to this time the membrane has been perfectly distinct and the space enclosed by it almost free from granules, but, from now onwards, the nuclear area becomes much less evident, though it can still be traced for some time.

The aster is demonstrated only with difficulty, but in favourable cases faint radiations may be seen passing away from the centrosome to be lost in the cytoplasmic reticulum.

The mature chromosomes, though so much contracted that the fact is more or less disguised, are typically V-shaped bodies, both limbs of which have undergone longitudinal fission throughout their length. Each chromosome lies on the spindle in such a way that one of its limbs is directed to either pole; the two limbs then separate, the bivalent chromosome being thus *transversely* divided. In the meantime, the longitudinal split becomes evident (Fig. 30), and each half of the bivalent chromosome thus passes to the pole as a V-shaped structure (Fig. 31). Sometimes one limb of the daughter chromosome remains attached to its fellow on the opposite half of the spindle after the other has broken away; the attached limb becomes considerably drawn out, and irregular figures are thus formed (Fig. 30 *a*).

The chromosomes become closely aggregated at the poles (Fig. 32), and reconstruction of the daughter nuclei begins. During this process the outline of the chromosomes can for some time be distinguished (Figs. 33, 34).

In the prophase of the second division, sixteen stout V-shaped chromosomes, no doubt representing those of the first telophase, reappear. They become attached to the spindle either at the apex of the V, or, more usually, about half way along its limbs (Fig. 35). The limbs separate and pass to opposite poles, being straight or bent according to the position in which they are attached to the spindle; the bent chromosomes are of much the more common occurrence (Fig. 36).

The longitudinal fission begun in the prophase of the first division, before the formation of the definitive nucleus, is thus completed.

Spindle formation takes place as in the first division, and the centrosomes, here also, are large and discoid.

The four nuclei of the ascus now pass into a stage of rest which, as it was frequently encountered, probably lasts for some time.

The Third Mitosis.

At the beginning of the third division the chromatin of each nucleus forms a delicate spireme (Fig. 37), which breaks up into sixteen curved chromosomes (Figs. 40, 41). Rounded bodies are present either near together (Fig. 38), or at opposite ends of the nucleus (Fig. 39); they

resemble the bodies observed during the divisions in the ascogenous hyphae; definite radiations could not be traced from them.

A spindle is formed and the V-shaped chromosomes become aggregated on it. The metaphases (Fig. 42) and anaphases (Fig. 43) of this division are difficult to obtain, and no doubt take place with rapidity; but the telophase was very frequently observed, and, in polar view, the number of chromosome ends radiating from the centrosome could be very readily determined. These ends were counted in some eleven cases, and were found to be always sixteen in number (Figs. 44, 45). During the anaphases and telophases the chromosomes are rather closely massed, but individuals were frequently traced throughout their length, and were always found to be curved, usually V-shaped structures (Fig. 43, 45). The sixteen ends counted in the telophase therefore represent *eight bent chromosomes*, their apices towards and their limbs radiating from the pole of the spindle.

An appearance in the telophase such as that shown in Fig. 44 might be due to the presence of sixteen rod-shaped bodies produced either by the transverse fission, or by the longitudinal fission, straightening and considerable contraction of the sixteen chromosomes of the prophase. No evidence in support of such a conclusion has been obtained, and the presence of V-shaped chromosomes in the later stages of division is against it. Guillermond (25) has recently stated that sixteen chromosomes pass to each pole in the third mitosis; but he neither figures the full number nor gives a detailed description of their appearance.

According to the present observations the sixteen chromosomes do not undergo fission in the metaphase, but half their number pass bodily to each pole of the spindle. The chromosomes of the prophase may either have arisen (1) by the breaking up of the spireme directly into sixteen parts, in which case the sixteen chromosomes would be presumably of different value, and the two daughter-nuclei would differ; or (2) by the breaking up of the spireme into eight parts, each of which, either before or after its separation from the others, undergoes longitudinal fission. The chromosomes would then form eight pairs of duplicates and the two daughter-nuclei would be equivalent. Occasionally the spireme shows two portions of the thread running parallel for a little distance (Fig. 37), but there is no evidence that this is due to a longitudinal split rather than to a chance looping of the thread. It thus appears that, in the third mitosis, sixteen diverse chromosomes are formed, and eight of these pass to each pole of the spindle.

Spore Formation. Towards the end of the third mitosis the cytoplasm becomes rather densely massed at the poles of the spindles (Fig. 46) and shows an indication of faint lines radiating from the centrosome. The daughter-nuclei, as they separate, have the appearance of pushing actively into these masses, the cytoplasm seeming to flow back on each side of the nuclear beak (Fig. 47).

A little later the beak reaches the periphery of the mass and the outline of the latter is defined, in section, by a line passing in both directions away from the centrosome (Fig. 48). Frequently this line cannot be traced to the lower part of the spore, and this portion appears to be delimited by the sides of neighbouring vacuoles. The wall of the spore becomes increasingly definite and the nuclear beak continues to elongate, remaining attached to the inner membrane of the spore (Fig. 49). The nucleus then becomes rounded up and the beak disappears.

One or two vacuoles are always enclosed in the spore plasm, and become very conspicuous in the mature spore. They contain oil.

Abnormalities. Certain abnormalities in the development of the ascus are fairly common ; trinucleate (Fig. 50) and tetranucleate (Fig. 51) asci are sometimes formed ; their fate could not be determined. In three or four cases asci were found containing two nuclei, each a good deal smaller than the ordinary definitive nucleus, and each undergoing synapsis (Fig. 52), and, in one case, a single nucleus of ordinary size was seen in which the contracted thread was aggregated into two separate masses (Fig. 53).

Occasionally after the second division, and more rarely after the first, two of the nuclei in the ascus undergo fusion (Fig. 54). It seems probable that such asci degenerate; they were never observed at a later stage of development.

Two or more nuclei are sometimes observed within one spore (Fig. 55), binucleate spores being of fairly common occurrence.

Sexuality of the Ascomycetes.

The most important of the earlier work on this subject is due to De Bary (14, 15), who described the sexual organs of *Sphaerotheca*, *Aspergillus* and *Erysiphe*. From these and similar discoveries he inferred that a sexual process was of general occurrence among the Ascomycetes. In 1884, however, in view of his own and his pupils' more extended work, he (16) reached the conclusion that, while some of the Ascomycetes are undoubtedly sexual, yet others are parthenogenetic, the archicarp being present but developing without ordinary fertilization, or apogamous, the sexual organs having entirely disappeared.

These conclusions have been largely borne out by subsequent discoveries.

Harper (27), in 1895, observed the fusion of the sexual nuclei in *Sphaerotheca* and in 1896 in *Erysiphe*. The former discovery, contradicted by Dangeard (12) in 1897, was subsequently confirmed (Blackman and Fraser (6)) in 1905.

In 1900 Harper (30) described the coenocytic ascogonium and antheridium of *Pyronema* and the fusion of the numerous sexual nuclei in pairs.

In 1905 Claussen (10) announced fertilization in *Boudiera*, and in the same year Harper (31) gave a very full account of the sexuality and general cytology of *Phyllactinia*.

Besides these forms, in which the fusion of the sexual pronuclei was actually observed, several cases have been described among the lichens, the Laboulbeniaceae and the Ascomycetes generally, in which considerable evidence of such a process has been obtained.

In 1906 the behaviour of the nuclei in a 'parthenogenetic' form, *Humaria granulata*, was observed (Blackman and Fraser (8)). An ascogonium but no antheridium is developed, and the female nuclei fuse in pairs before passing into the ascogenous hyphae.

In 1907 a similar process was described by Welsford (4) for *Ascobolus furfuraceus*, and in the same year *Lachnea stercorea* (Fraser (21)) was added to the 'parthenogenetic' or homoiogamous forms.[1] In this case, however, reduction has progressed rather less far than in the others, since an antheridium is still present, though its nuclei degenerate *in situ*.

In all these forms, whether normal or reduced, the ascogenous hyphae arise from the ascogonium only, and the nuclei which pass into them are formed by the fusion of postmeiotic nuclei in pairs. They constitute the sporophyte generation and develop parasitically at the expense of the gametophyte.

The ascocarp of *Humaria rutilans* reaches maturity without the formation of sexual organs. In the hypothecium fusion of nuclei in pairs takes place. Ascogenous hyphae are then developed altogether similar to those which form the sporophyte in species with normal sexuality and moreover containing, as will be shown later, nuclei with the premeiotic number of chromosomes.

Thus it appears that here also the ascogenous hyphae form the sporophyte and that the fusions in the hypothecium constitute a reduced sexual process comparable to that observed by Farmer and Digby (17) in the prothalli of *Lastrea pseudomas* vars. *polydactyla*. In *Lastrea* the cells are uninucleate and the nuclear fusion is preceded by migration; this also is sometimes the case in *Humaria*, though, since the cells may be multinucleate, it perhaps does not always occur.

Humaria rutilans is thus an example of the so-called apogamous development of the ascocarp, or pseudogamy.

Probably a similar fusion of vegetative nuclei takes place in other Ascomycetes, such as *Cordyceps* and *Claviceps*, which have been described

[1] The various forms of reduced fertilization are grouped by Farmer and Digby (17) under the general term *pseudapogamy*. The diversity of such processes, among Fungi at any rate, having appeared to render desirable a further subdivision, the following terminology was recently (Fraser and Chambers (21 *a*)) suggested :—Fusion of two sexual nuclei of the same kind—*homoiogamy*. Fusion of one sexual and one vegetative nucleus—*hylogamy*. Fusion of two vegetative nuclei—*pseudogamy*.

as reaching maturity without the formation of sexual organs. The small size of the nuclei will in many cases, no doubt, render the actual observation of this process very difficult.

Formation of the Ascus.

De Bary (14, 16) first observed the presence of a single nucleus in the comparatively young ascus. He discovered that it underwent three successive divisions and that, about each of the eight resultant nuclei, a spore is normally organized. His observations were confirmed by Strasburger (39), by Schmitz and by Gjurasin (22), who also discovered in *Peziza vesiculosa* that the nuclear divisions are karyokinetic, and that well-marked asters are present.

In 1894 Dangeard, investigating *P. vesiculosa* and some other forms, made the important observation that the ascus originates from the binucleate, penultimate cell of an ascogenous hypha, and that the two nuclei of this cell fuse to form the definitive nucleus of the ascus. The bending over of the ascogenous hypha, the simultaneous karyokinetic division of the two terminal nuclei in such a way that the nuclei enclosed in the ascus shall not be of the relationship of sisters, and the fusion in the ascus have since been confirmed in a number of other species.

Recent research, however, has brought to light various modifications of this method. Maire (32), in 1903, discovered that, in *Galactinia succosa*, the two or three end cells of the ascogenous hypha are binucleate, the ascus being formed from the terminal cell; here also the two nuclei of the ascus are not sisters.

In *Peziza Catinus*, according to Guillermond (25), the bending over of the ascogenous hypha does not take place, otherwise development is typical.

Faull (19), in 1905, described, in *Genea hispidula* and a number of other species, the outgrowth of the ascus from the curved terminal cell of the ascogenous hypha; he regards this process as differing from the typical one only in the absence of a wall cutting off the recurved tip of the hypha. He makes no suggestion, however, as to the fate of the nucleus usually contained in the tip. He records the further interesting observation that in *Verpa bohemica* the ascus may grow out from the terminal cell of the hypha or it may arise from the second, third or fourth cell from the end, the growth of the hypha continuing beyond it. In one or two other forms the ascus may apparently spring from any cell whatever. Faull finds that the nuclei which fuse in the ascus, though not sisters, may be the daughters of sister nuclei.

Harper (31), both in *Erysiphe* and in *Phyllactinia*, found that the asci arise from binucleate cells of the ascogenous hyphae. There is no process of bending over, and no provision to prevent the inclusion of sister nuclei.

In *Humaria rutilans*, as in *Verpa bohemica*, the ascogenous hypha may

continue its growth, and it has been ascertained to give rise to other asci. I am inclined to think that the formation of the ascus from the subterminal cell of the ascogenous hypha is nothing more than a provision to allow the further growth of the latter, the asci developing, as is well shown in Fig. 13, and in Faull's Fig. 75, as lateral branches of the hypha. Probably the conjugate division of the two terminal nuclei of the ascogenous hypha, is a convenient method of providing two nuclei in the ascus and one in the terminal cell which is to continue its growth, rather than an arrangement to prevent the near relationship of the ascus nuclei. Where a cell contains more than one nucleus, simultaneous division seems to be the rule; it occurs, for instance, in the oogonium of the Oomycetes, in the embryo-sac of Phanerogams, and in the ascus itself.

The development of the ascus has been shown to be liable to considerable variation, the only constant process being the inclusion and subsequent fusion of two nuclei; even this is subject to exception, since in *H. rutilans* young trinucleate and tetranucleate asci are found.

The fusion in the ascus is regarded by Dangeard (12, 13) as sexual, and he has consistently denied the occurrence of a fertilization at any other stage in the life history of the Ascomycetes.

Recent research, however, has proved that, in a number of cases, a typical or reduced fertilization precedes the formation of the ascogenous hyphae. This in itself, unless the occurrence of two fertilizations in a single life history be regarded as possible, serves to disprove the sexual nature of the fusions in the ascus. In *Humaria rutilans* the fact that the reducing division begins before this fusion takes place, constitutes further evidence in the same direction.

For Harper (31) the fusion in the ascus serves to adjust the nucleo-cytoplasmic equilibrium. This indeed would account for the presence of more than one nucleus, since the ascus is to become the largest cell of a typically multinucleate mycelium, but it does not appear to explain their fusion.

In *Humaria rutilans* the nuclei lie in contact, and have entered on the prophases of division before fusion occurs. It may be suggested that, at this stage, the nuclear membrane becomes increasingly delicate, and the nuclei simply flow into each other, as is the case when nuclei artificially separated from their cytoplasm are brought into contact.

The Meiotic Phase.

Considerable additions to our knowledge of chromosome reduction among animals and higher plants have been made during recent years. In 1905, Allen (1) investigated the reducing division in *Lilium canadense*; he described the approximation and fusion in pairs of chromatin strands in the early prophase of the heterotype division. Synapsis then takes place, and is followed by a uniform distribution of the spireme.

A longitudinal split in the filament becomes evident, and was regarded by Allen as a separation of the threads which had previously fused. The spireme breaks up into a number of chromosomes which shorten and thicken while, at the same time, a second longitudinal split is distinguished. The limbs of the heterotype chromosome thus originate by a longitudinal split representing the separation of two fused, but originally independent filaments. The limbs of the chromosomes separate in the heterotype metaphase, and the second longitudinal split is completed in the homotype.

A similar account is given by Grégoire (23) and by Berghs (3) for various other Angiosperms, and by Allen (2) for *Coleochaete.*

In 1905 Farmer and Moore (18) published a full account of the reducing divisions or meiotic phase as studied by them in a number of animals and plants.

They observed stages corresponding to those seen by Allen, Grégoire and Berghs, but they were also able to distinguish various others, and have interpreted them quite differently.

According to these investigators, the heterotype division is initiated by the aggregation of the newly formed spireme towards one side of the nuclear area, forming the first contraction figure. As this loosens, the spireme undergoes longitudinal fission, but the split becomes more or less obliterated when the thread shortens and thickens in the synaptic or second contraction. During this process the nucleolus becomes vacuolate, and is regarded as giving up its substance to the chromatin element of the spireme.

Loops extend from the contracted mass to the nuclear wall, and in these the longitudinal split is still obvious. The sides of the loops become drawn into parallel positions; they are often twisted, and simulate the appearance of a single longitudinally split thread. This appearance is illusionary, since the earlier stages have been fully traced, and moreover the true longitudinal fission is still seen in the parallel sides. The synaptic tangle loosens, and the thread breaks up into chromosomes; each of these represents a loop or similar segment of the spireme, and the two limbs of each bivalent chromosome are thus derived from diverse portions of the thread which have been bent towards one another. The original longitudinal fission is still visible, but disappears as the chromosomes shorten and thicken. During this process the chromosomes pass to the periphery, appearing to act under the influence of mutual repulsion.

On the spindle the chromosomes break apart at their angle, the daughter chromosomes thus representing different portions of the spireme. In them the longitudinal split becomes once more apparent, and it takes effect on the spindle of the homotype division.

The meiotic phase is thus regarded as a peculiar series of events resulting in the reduction of the chromosome number and interpolated

between the longitudinal fission, characteristic of somatic divisions, in the first prophase, and the final separation of the split halves in the second metaphase.

The description of Farmer and Moore is in agreement with that given by Schaffner (38), and has been recently confirmed by Mottier (34).

The chief points in which these authors differ from Allen and Berghs are, (1) the occurrence of two contraction figures, (2) the approximation of the arms of each of the loops formed during synapsis, (3) the breaking up of the spireme so that each loop (or some equivalent portion of the thread) forms a bivalent chromosome, (4) the consequent transverse fission of each chromosome on the spindle. To a great extent the difference in interpretation is due to a difference in the seriation of the stages observed.

It has been suggested by various authors that the divisions in the ascus probably correspond to those in the spore mother-cells of higher plants, and also that the occurrence of three divisions is in some way related to the two nuclear fusions in the life-history of Ascomycetes.

In 1905, Harper (31), in his account of the development of *Phyllactinia*, described the two ascus nuclei, at the time of fusion, as each containing eight or nine chromatin threads attached to a central body. The centres fuse and the chromatin systems become intimately mingled. Synapsis takes place, the chromatin becoming contracted towards the central body. Later a spireme of eight strands is formed, and gives rise to the eight chromosomes of the equatorial plate. The chromosomes divide, and eight go to each pole. Eight chromosomes appear in the second and third mitoses, and in each case divide so that eight daughter chromosomes pass to the poles. These divisions appear to resemble the first, but are not initiated by a synapsis.

Maire (33), in 1905, described the first divisions in the ascus, in *Galactinia succosa*, *Pustularia vesiculosa*, and some other species, as respectively heterotype and homotype.

Guillermond (25), in the same year, investigated the ascus divisions of various species, amongst which is *Humaria* (*Peziza*) *rutilans*. He describes the nucleus of the ascus as rich in chromatin and containing a large oval nucleolus. The spireme often shows paired filaments; it undergoes a characteristic synapsis in which the chromatin is condensed at one side of the nucleus. Subsequently sixteen chromosomes appear, having the forms of U's or V's, with short, thick branches; they become grouped in the centre of the nucleus, and an intra-nuclear spindle and centrosomes appear; the asters can only exceptionally be distinguished. In the metaphase the chromosomes split longitudinally, forming hollow 'lozenges'; the two halves separate, and the V-shaped daughter chromosomes pass to the poles. In the anaphase sixteen V- or hook-shaped chromosomes were counted at each end of the spindle. On reaching the poles they branch and become united end to end, and the daughter nuclei are reconstructed.

The second mitosis begins by the formation of sixteen chromosomes; these become grouped on the spindle but do not acquire the lozenge shape of the first division. V-shaped figures only are seen, and later much drawn out filaments; there seems reason to believe that the V breaks at its apex as in the homotype division of Phanerogams.

The third division differs sensibly from the other two. A fine spireme is formed and divides into sixteen curved chromosomes, which become grouped in an equatorial plate. Guillermond states that, owing to the delicacy and large number of the chromosomes, the method of splitting could not be observed. In the late metaphase and early anaphase the chromosomes are elongated along the threads of the spindle, and directed towards the poles. In this division, as in the first and second, the number of chromosomes at each pole is given as sixteen.

I have been able, in the present investigation, to confirm Guillermond's observations with regard to the first and second mitoses. In the early prophases of the first division, and between the synaptic contraction and the appearance of the mature chromosomes, as described by him, I have been able to observe some further stages, thus bringing these divisions into line with the meiotic processes as described by Farmer and Moore. The first contraction and the longitudinal fission of the spireme (in part at least) take place before the fusion in the ascus, and the changes thus begun continue, apparently without interruption, in the definitive nucleus. The difficult question of seriation is here specially clear; it seems obvious, for instance, that such a stage as Fig. 17, where the ascus nuclei each show a few paired threads, must precede that shown in Fig. 19, where the spireme of the definitive nucleus is double throughout its length. Synapsis sets in, loops are formed, their sides approximate, and the whole loop constitutes a bivalent chromosome. On the spindle of the first division the two limbs of the chromosome break apart, thus separating unlike portions of the spireme. In the second division the longitudinal split takes effect.

Thus here, as in *Lilium candidum* and the other forms described by Farmer and Moore, the first meiotic division is diaschistic, and brings about a reduction in the sense of Weismann.

The Third Mitosis.

The processes in the ascus are confused, as compared to the meiotic phase in other organisms, by the introduction of a nuclear fusion.

The number of chromosomes in the vegetative divisions directly preceeding meiosis has been constantly found to be the same as in the first division in the ascus. This was ascertained by Harper for *Pyronema* (30) and *Phyllactinia* (31), both forms with normal sexuality, and in the present instance for *Humaria rutilans*.

In *Humaria rutilans*, at least, the heterotype division is begun before

fusion takes place, and, since no evidence of the union of the two spiremes was obtained, it may be supposed that each continues its development separately, and separately breaks up into the reduced number of chromosomes. There are sixteen chromosomes in the divisions in the ascogenous hyphae, and the sixteen which appear in the heterotype prophase are thus made up of two sets of bivalent chromosomes, eight of which have been derived from each spireme; in the same way the definitive nucleus of the ascus is a double structure, representing two nuclei enclosed within one membrane. This view is further borne out by the occasional appearance of a nucleus in which the spireme, in the second contraction, is aggregated into two masses, or of an ascus containing two nuclei in synapsis, which have no doubt failed to fuse, but have nevertheless continued their development independently.

The third mitosis has been regarded by Maire and Guillermond as vegetative, and Guillermond states that in *H. rutilans* the number of chromosomes in the third telophase, as in the first and second, is sixteen.

If, however, the conclusions drawn from the present research be correct, the sixteen ends radiating from the pole at this stage represent, not sixteen rods, but *eight* V-shaped chromosomes. The postmeiotic number, that is to say, has become apparent, and the fusion in the ascus is compensated.

Nemeč (35), experimenting with the root-apices of Phanerogams, found that treatment with 0·75 per cent. of chloral hydrate produced degeneration of the spindle fibres; cell division is thus inhibited, but the daughter nuclei separate and binucleate cells appear; in these the two nuclei either fuse, subsequent divisions showing double the somatic number of chromosomes, or divide simultaneously; in the latter case three cells are formed, the middle one containing two nuclei; these may fuse and show the double number of chromosomes in their divisions.

After a few hours mitoses showing double the somatic number of chromosomes cannot be identified, reduction having apparently taken place. Nemeč observed in *Pisum* a large cell, quite like those which contain two nuclei or a nucleus with the double number of chromosomes, containing a nucleus in the late anaphase, which showed the ordinary somatic number. This he regards as a reducing division. It seems quite possible that such divisions correspond to the third division in the ascus. This division resembles an ordinary vegetative mitosis, but shows in the metaphase the double number of chromosomes, and in the anaphase the ordinary number (half that shown in the metaphase). The relation of the two stages would not, however, be obvious, except where, as in the ascus, their connexion could be recognized in some other way. The process, compensating as it does a vegetative or asexual fusion, is much less elaborate than the meiotic reduction.

Evidence of similar fusions and reductions in graft hybrids has

been adduced by Noll (36), and we may hope here to obtain some knowledge of the effect of this method of chromosome distribution in heredity.

In *Phyllactinia* the number of chromosomes, or chromatin strands radiating from the central body, is the same throughout the life history of the fungus. On the fusion of the sexual pronuclei these strands combine in pairs; thus the diplocytic nuclei contain not $2n$ chromosomes, but n bivalent chromosomes. A similar process takes place in the fusion in the ascus, and the eight chromatin strands of the definitive nucleus are thus regarded by Harper as tetravalent and as actually representing thirty-two somatic chromosomes. The meiotic divisions[1] then take place and four nuclei are formed; each contains eight bivalent chromosomes representing sixteen somatic chromosomes. In the third division the valency of each chromosome is again halved and eight somatic chromosomes appear.

The essential facts are thus the same in *Phyllactinia* and in *Humaria rutilans*. In each a sexual fusion takes place (normal in *Phyllactinia*, much reduced in *Humaria*), and the actual number of chromosomes is thus doubled. Owing to the association of the chromosomes in pairs, the apparent number remains unchanged in *Phyllactinia*; in *Humaria* sixteen chromosomes, the premeiotic number, can be counted in the ascogenous hyphae. In each case a fusion of two nuclei takes place in the ascus, resulting in the association, in *Phyllactinia*, of eight tetravalent chromosomes within one membrane, in *Humaria*, no doubt, of thirty-two univalent chromosomes. Meiosis occurs and four nuclei are formed, each of which, in the next prophase, shows, in *Phyllactinia* eight bivalent chromosomes, in *Humaria* sixteen univalent chromosomes, the reduced number for *two* nuclei. In the third division a further reduction occurs, compensating, no doubt, the fusion in the ascus, and in each of the two species eight univalent chromosomes are shown in the anaphase.

It may be suggested that, once a third division, compensating the fusion in the ascus, had been established, the latter would become a necessary process, and this may account for the extraordinary regularity of the appearance and fusion of the two nuclei, just as the occurrence of meiosis is held to render imperative some form of reduced fertilization if the normal sexual process be lost.

An interesting transition between the two arrangements detailed above perhaps occurs in *Pustularia vesiculosa* as described by Maire (33). He states that eight chromatin bodies appear in the prophase of each of the three divisions, but unite into four as they pass on to the spindle. In the first anaphase separation occurs on the spindle, so that eight chromatin

[1] Harper did not observe a first contraction of the chromatin in the heterotype prophase; the absence of this stage may probably be connected with the early pairing of the chromosomes.

bodies pass to each pole; in the second and third anaphases only four can be distinguished. It may perhaps be possible that these eight bodies are not, as suggested by Maire, protochromosomes, but rather true chromosomes, which become associated in pairs during a part of first and second mitoses, as is the case throughout these divisions in *Phyllactinia*. The four bodies which pass to the poles in the third division would be, as in both *Phyllactinia* and *Humaria*, the univalent, postmeiotic chromosomes.

It must be added that Guillermond (25) finds eight chromosomes throughout the ascus divisions in *P. vesiculosa*. Possibly, then, the grouping of the chromosomes in pairs does not always take place; such an hypothesis would account for the discordant results obtained by Maire and Guillermond. It seems not improbable, on the analogy of the present researches, that the eight structures counted by Guillermond in the third telophase represent only four chromosomes.

In *Galactinia succosa* Maire describes the association of the chromosomes as lasting rather longer than in *P. vesiculosa;* this species, therefore, shows a state of affairs in closer accordance with that obtaining in *Phyllactinia*.

In *Phyllactinia*, if Harper's suggestions and those set forth above be correct, it seems probable that the constituents of a given double chromosome will pass to different nuclei, and it may be that their association in pairs is a provision to ensure that end. A similar provision then is evident in *G. succosa* and *P. vesiculosa*, and it is likely that it exists in *Humaria rutilans*, although in this case it is not apparent. If this be so, a sorting of the chromosomes, analogous to that which occurs in the meiotic phase, would appear to take place in the third division in the ascus.

This division represents a type of reduction differing markedly from the meiotic phase, and to which the term brachymeiosis may be conveniently applied. It shows none of the characteristic features of meiotic reduction, it takes place in a single mitosis, and it is not preceded by a contraction of the nuclear material.

The absence of this contraction probably entails the most essential distinction between meiosis and brachymeiosis. Meiosis, while it brings about the separation of entire somatic chromosomes, yet, in its contraction, presumably allows a mingling of maternal and paternal chromatin. In brachymeiosis such a contraction does not appear, and the separation of entire chromosomes alone takes place. It is thus to be expected that the product of a brachymeiotic division should follow very exactly the law of Mendel allowing none of the minor variations which meiosis makes possible. On the other hand it is quite likely that in brachymeiosis the *entire* nuclei which united in asexual fusion separate from each other, and that no interchange of chromosomes takes place.

The existence of brachymeiosis suggests a new distinction between sexual and asexual fusions. The two fusions in the life-history of *Humaria rutilans* are very similar in character; both are fusions of apparently undifferentiated nuclei, and, in both, the nuclei which fuse have often been present in the same cell since their formation. Each fusion also results in a doubling of the number of chromosomes, and is compensated by a process of reduction.

The first fusion, however, occurs at the same stage in the life-history as a normal act of fertilization and initiates a similar development. The second fusion takes place after meiosis has begun. The syngamous fusion is related here, as in all other investigated organisms to a meiotic reduction. The asexual fusion in the ascus is followed by the simpler brachymeiotic process, and there is reason to believe that this method of reduction may compensate other asexual fusions also.

Association of Nuclei.

According to Maire (32, 33) the nuclei in the ascogenous hyphae of various Ascomycetes are associated in pairs, and constitute a synkaryon comparable to that observed by various authors in the Basidiomycetes.

I have not been able to observe such an arrangement in *H. rutilans*.

Harper (31) also has observed paired nuclei in *Phyllactinia*, and suggests that the fusion in the ascus is to be related to the fusion in the teleutospore and basidium, the association of nuclei in pairs having 'worked back' in the latter cases, to the stage of fertilization, and the occurrence of two fusions, as in the Ascomycetes, being therefore eliminated.

As, however, Blackman and others (5, 7, 9) have shown, the nuclear union in the teleutospore is a stage in the act of fertilization initiated in the aecidium (or at some equivalent point in the mycelium), and corresponds to the fusion of the sexual nuclei in other plants and animals, and therefore to the fusion which takes place typically in the female organ of Ascomycetes.

The fusion in the ascus follows the fusion of the sexual nuclei. It appears to be a peculiar process intercalated in the life-history of the Ascomycetes and is compensated by the third division in the ascus.

The ascus, however, resembles the teleutospore (or its outgrowth, the promycelium) and the basidium in being a spore mother-cell in which reducing divisions take place.

Spore-formation.

The details of spore-formation were first studied by Harper (26), who regards the spore as cut out by astral rays which fuse laterally to form a membrane.

This point, together with its bearing on the phylogeny of the Ascomycetes, has recently received full discussion from Faull (19), who describes

the spores as delimited by the differentiation of a finely granular protoplasm, and from Overton (37), who confirms the description of Harper.

Faull's account is of great interest, but it does not seem to satisfactorily explain either the persistence of the astral rays or the formation of the nuclear beak; the latter appears in *H. rutilans*, as in the forms described by Harper, before the spore is delimited.

The exact function of the radiations from the centrosome is, owing to their extreme tenuity, somewhat difficult to ascertain in *H. rutilans*.

Farmer and Moore (18) regard the spindle fibres rather as protoplasm modified by the forces at work in the cell than as actively growing entities. For them the spindle is a passive manifestation of the real operating agency.

In *H. rutilans*, the spore is delimited by the astral rays, but it would seem that these represent not cell organs of the nature of cilia, as suggested by Harper (31), but rather currents set up in the neighbourhood of the centrosome as it pushes into the dense cytoplasm near the pole. It is in accordance with such a point of view that the boundary of the spore should sometimes be partly defined by the walls of neighbouring vacuoles, or, as would seem to be the case in the abnormal ascus of Fig. 55, by the ordinary cytoplasm.

Summary.

1. The ascocarp of *Humaria rutilans* originates as a tangle of septate hyphae; sexual organs are not differentiated.

2. Fusions of nuclei in pairs occur in the hypothecium constituting a process of reduced fertilization or apogamy. The cells containing the fusion nuclei form ascogenous hyphae.

3. Divisions in these hyphae are karyokinetic, showing sixteen chromosomes.

4. The first and second divisions in the ascus are respectively heterotype and homotype. They show the stages observed by Farmer and Moore in the meiotic phase of certain animals and plants, and they bear the same interpretation.

5. During the first mitosis fusion of the two nuclei in the ascus occurs. At this time the spireme in each already shows evidence of longitudinal fission.

6. Sixteen chromosomes appear in the first two divisions in the ascus and in the prophase of the third. They are regarded as representing two sets of post-meiotic chromosomes united with one membrane.

7. In the telophase of the third division eight chromosomes only are seen at each pole. The two sets of post-meiotic chromosomes have thus separated, and the reduced number is apparent. To this type of reduction the name *brachymeiosis* is given.

8. The spores are outlined by radiations passing from the centrosome; near the base of the spore vacuoles may take part in the process.

July, 1907.

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EXPLANATION OF PLATES IV AND V.

Illustrating Dr. Fraser's paper on *Humaria rutilans*.

Fig. 1. Section through a very young ascocarp, lying against the wall of an older one. × 625.
Fig. 2. Section through a rather older ascocarp. × 625.
Fig. 3. Mitosis in hypothecium. × 1250.
Fig. 4. Apogamous fusion in the hypothecium. × 1250.
Fig. 5. The same, later stages. × 1250.
Fig. 6. Nuclear migration in the hypothecium; nuclei of two sizes are shown. × 1250.
Fig. 7. Part of the subhymenial layer, showing nuclei in young ascogenous hyphae at *a*, and in a hypha just before crozier formation at *b*. (From the same ascocarp as Fig. 4.) × 1250.
Fig. 8. Early prophase in the ascogenous hypha. × 1875.
Fig. 9. Prophase in ascogenous hypha, showing sixteen chromosomes. × 2808.
Fig. 10. Metaphase in ascogenous hypha. × 1875.
Fig. 11. Telophase in ascogenous hypha. × 1875.
Fig. 12. Proliferation of terminal cell of ascus. × 1250.
Fig. 13. An ascus (*a*), the terminal cell connected with which has continued its growth and given rise to another ascus (*b*); from the terminal cell of which a third ascus (*c*) has arisen. 1250.
Fig. 14. *H*, connexion between stalk and terminal cell. The nucleus of the latter has passed into the stalk-cell, from which a hypha is growing out. × 1250.
Fig. 15. Nucleus migrating from the terminal into the stalk-cell. × 1250.

FIRST DIVISION.

Fig. 16. Two nuclei in the ascus, each showing the first contraction-figure of the heterotype prophase. × 1875.
Fig. 17. Two nuclei in the ascus; the beginning of the longitudinal fission can be distinguished in the spireme of each. × 1875.
Fig. 18. Fusion in the ascus, the longitudinal fission is further advanced. × 1875.
Fig. 19. Definitive nucleus of ascus; longitudinal fission complete. × 1875.
Fig. 20. Synapsis. Longitudinal section of ascus. × 1875.

Fig. 21. Synapsis. Transverse section of ascus. The arms of each loop are closely approximated. The longitudinal fission can just be distinguished in places. × 1875.

Fig. 22. Ascus in longitudinal section. Nucleus passing out of synapsis. The longitudinal fission is once more obvious. × 1875.

Fig. 23. Spireme broken up into chromosomes, in the limbs of these the longitudinal fission can be seen. × 1875.

Fig. 24. Immature chromosomes. × 2808.

Fig. 25. Nucleus showing sixteen mature chromosomes. × 2808.

Fig. 26. Spindle formation; the two centrosomes lie close together with a cone of fibres radiating from each. × 1875.

Fig. 27. Later stage, spindle is established. × 1875.

Fig. 28. Passage of chromosomes on to spindle. × 1875.

Fig. 29. Chromosomes on spindle; centrosomes and asters visible. × 1875.

Fig. 30. Chromosomes on the spindle. × 2808.

Fig. 31. Passage of chromosomes to poles. × 1875.

Fig. 32. Chromosomes aggregated at the poles. × 1875.

Fig. 33. Reconstruction of daughter-nuclei. × 1875.

Fig. 34. Same; later stage. × 1875.

SECOND DIVISION.

Fig. 35. Passage of chromosomes on to spindle. × 1875.

Fig. 36. Telophase; sixteen bent chromosomes. × 2808.

THIRD DIVISION.

Fig. 37. Early prophase, showing delicate spireme. × 1875.

Fig. 38. Spireme stage; two centrosome-like bodies. × 1875.

Fig. 39. Later spireme; centrosome-like bodies. × 1875.

Fig. 40. Chromosome formation in four nuclei. × 1875.

Fig. 41. Nucleus showing sixteen curved chromosomes. × 2808.

Fig. 42. Metaphase of third division. × 1875.

Fig. 43. Passage of curved chromosomes to poles. × 1875.

Fig. 44. Telophase; lateral view, and polar view in which the sixteen ends of eight curved chromosomes can be counted. × 2808.

Fig. 45. Another polar view showing the eight chromosomes. × 2808.

Fig. 46. Telophase in lateral view. × 1875.

Fig. 47. Later stage, formation of nuclear beaks has begun. × 1875.

Fig. 48. Spore formation. × 1875.

Fig. 49. Young spore. × 1875.

Fig. 50. Trinucleate ascus. × 1250.

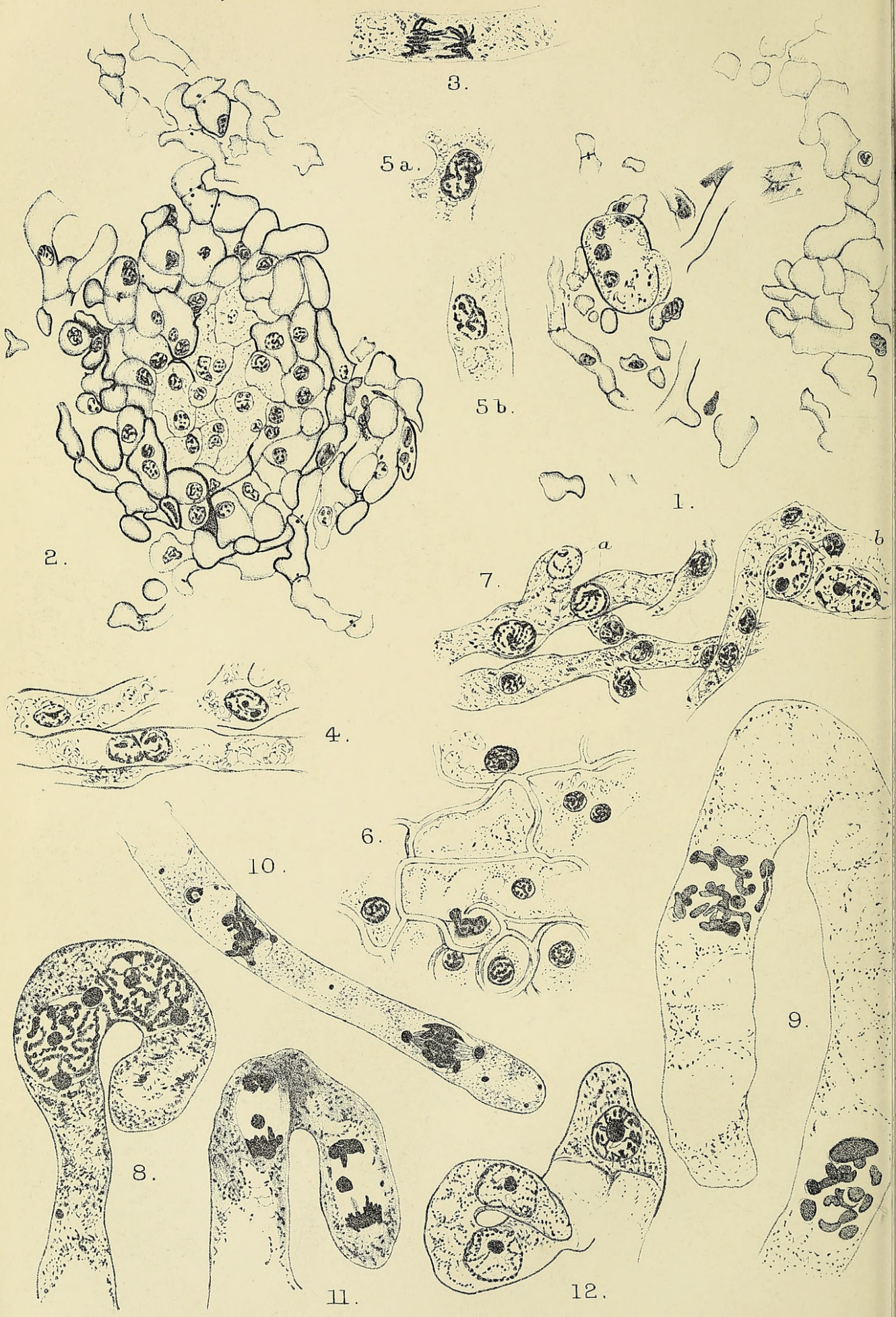
Fig. 51. Tetranucleate ascus. × 1250.

Fig. 52. Ascus containing two nuclei in synapsis. × 1875.

Fig. 53. Ascus containing a single nucleus in which the chromatin is aggregated into two masses. × 1875.

Fig. 54. Fusion between two nuclei in the ascus after the second division. × 1250.

Fig. 55. Ascus containing three normal spores and one with five nuclei. × 1250.



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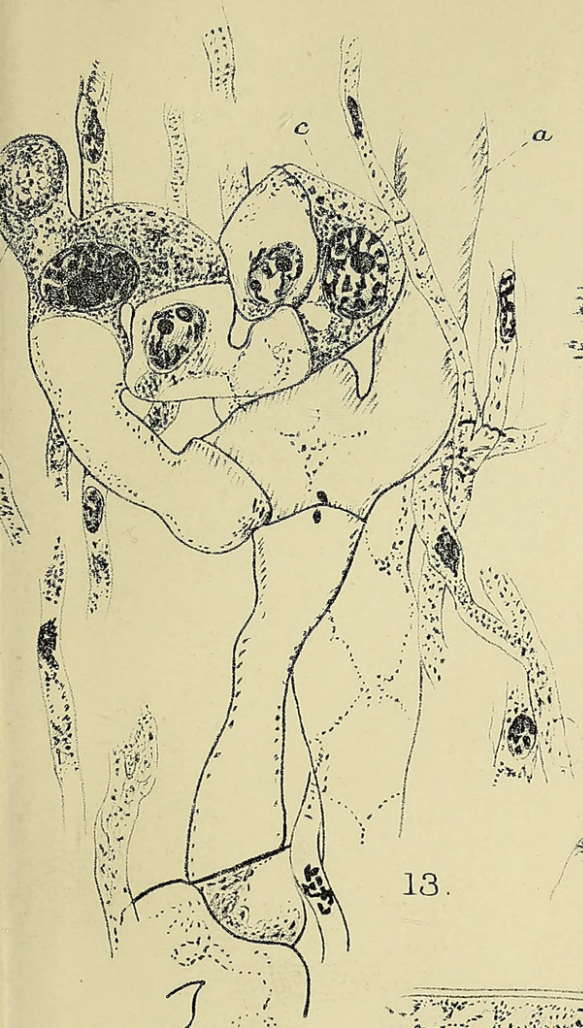
FRASER — HUMARIA RUTILANS.



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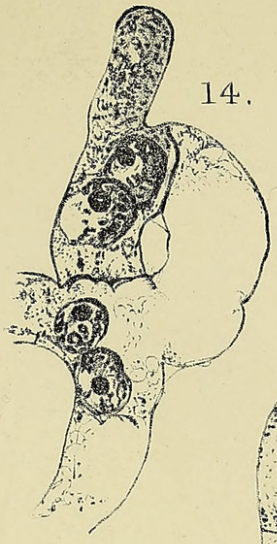

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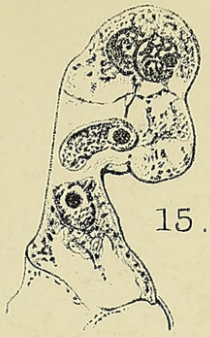

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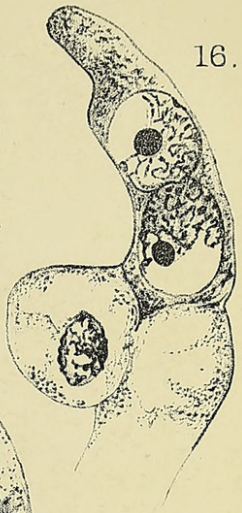

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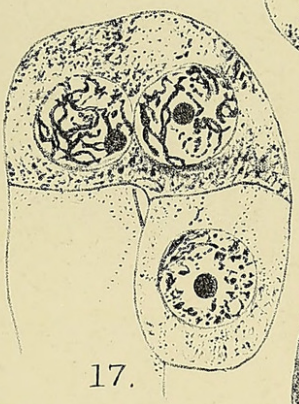

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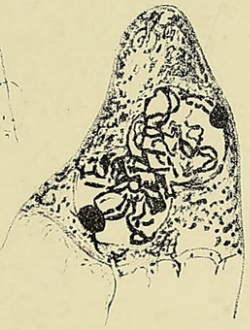

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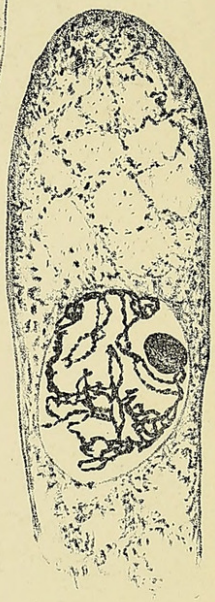

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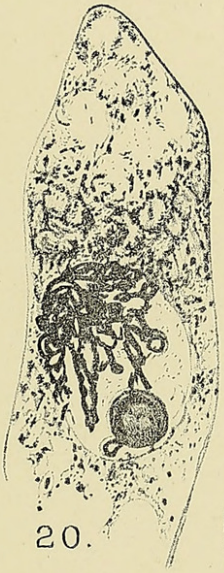

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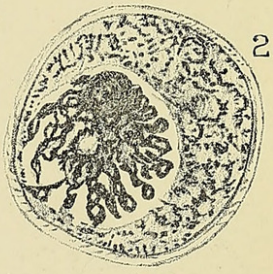

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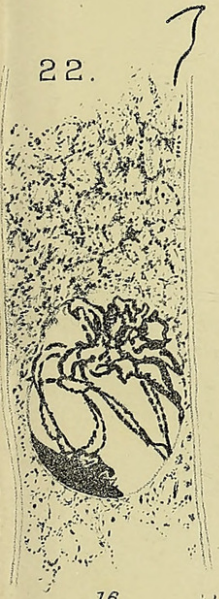

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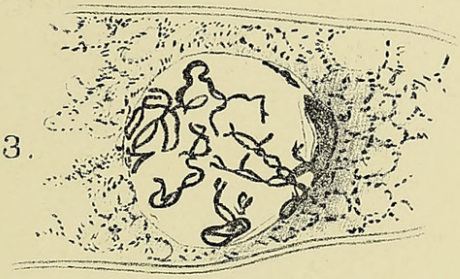

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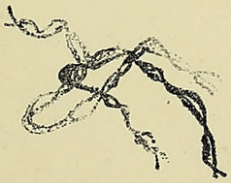

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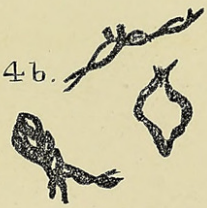

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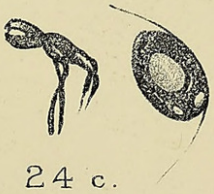

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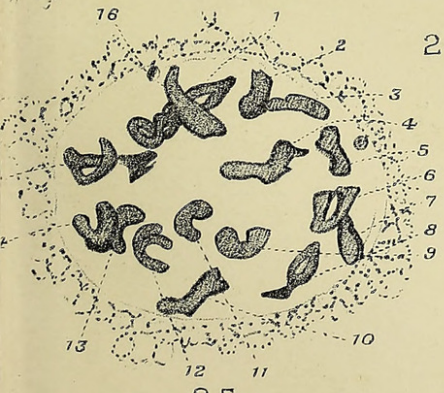

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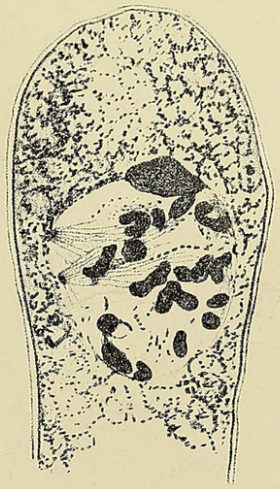

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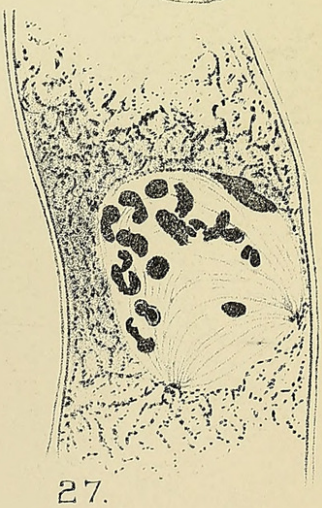

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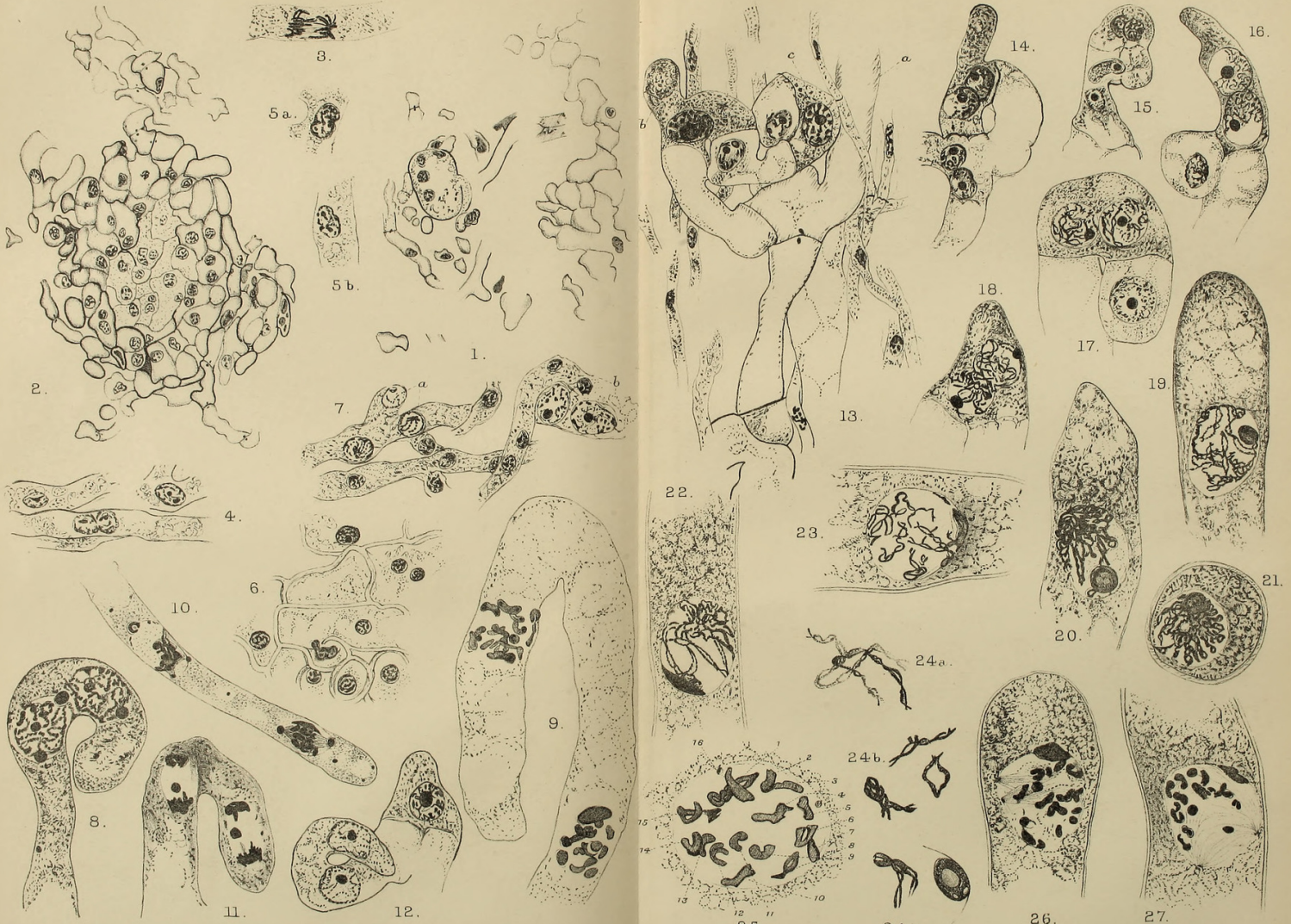

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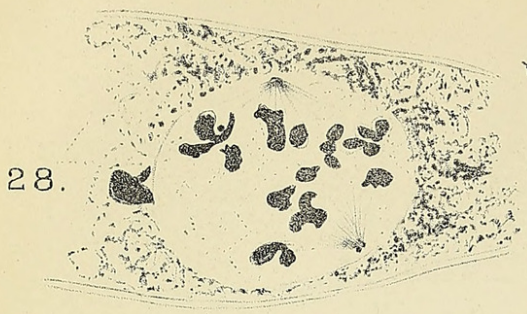
Huth lith. et imp.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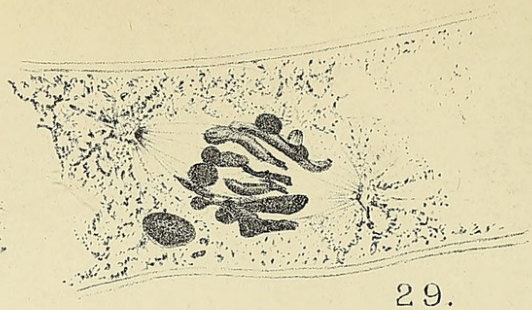
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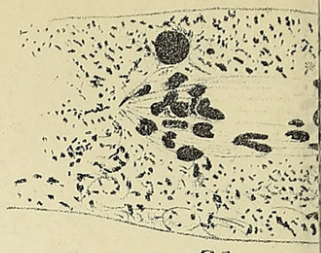
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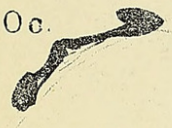
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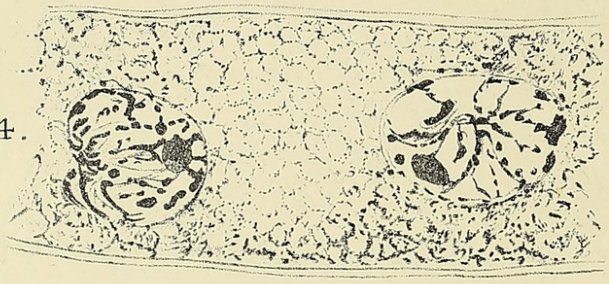
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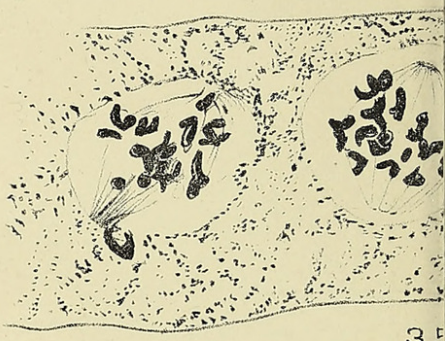
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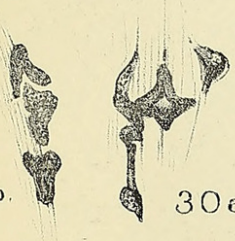
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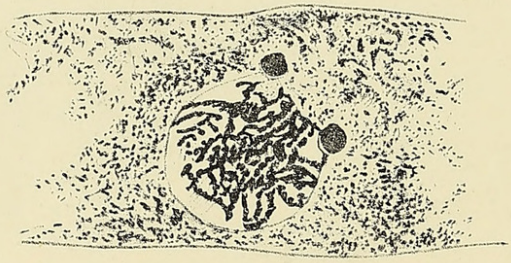
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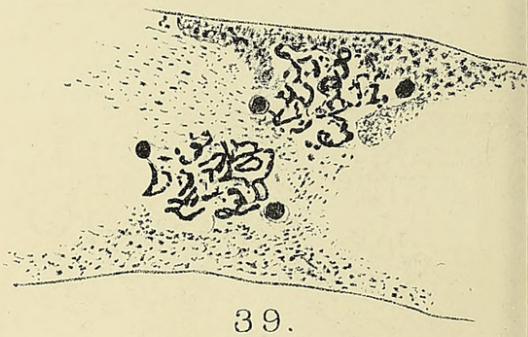
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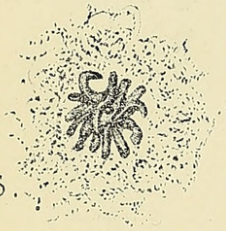
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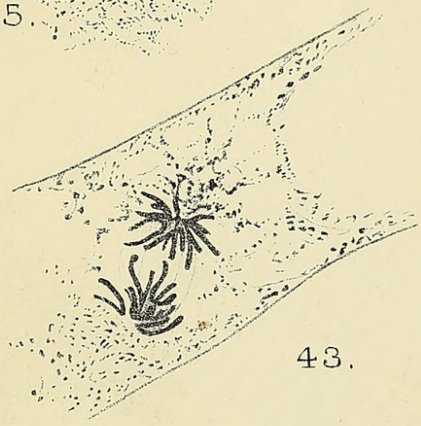
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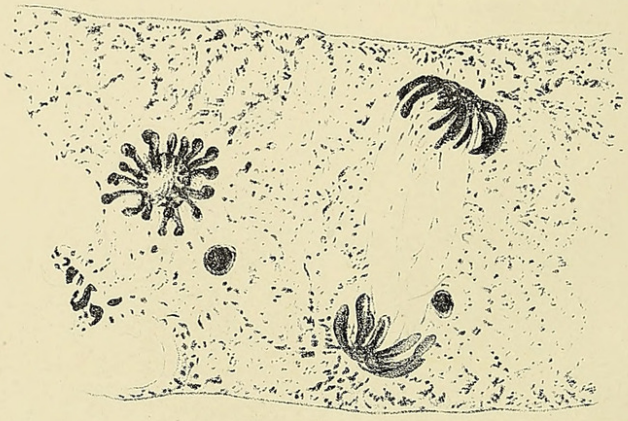
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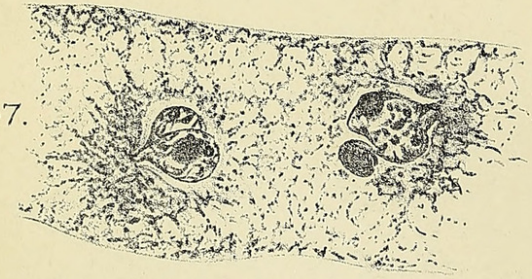
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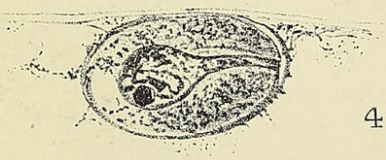
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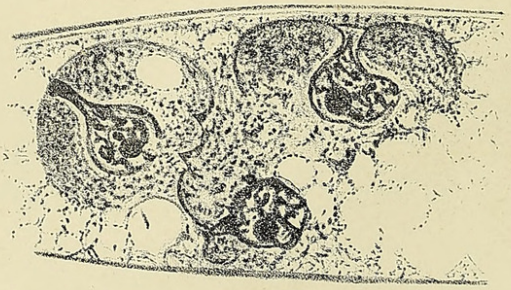
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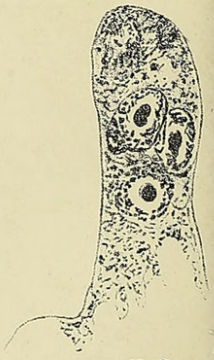
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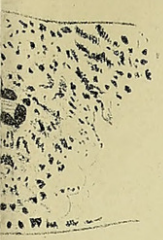

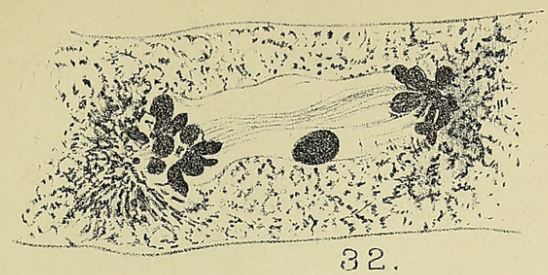

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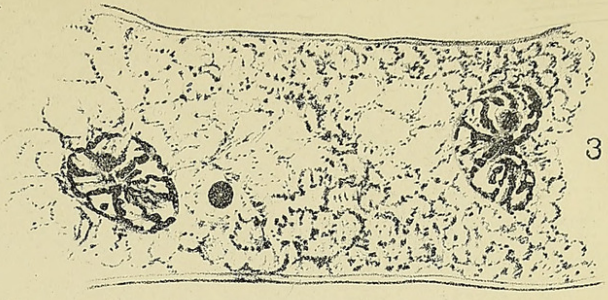

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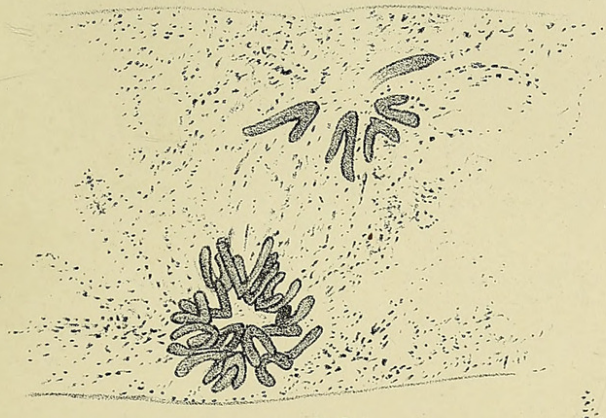

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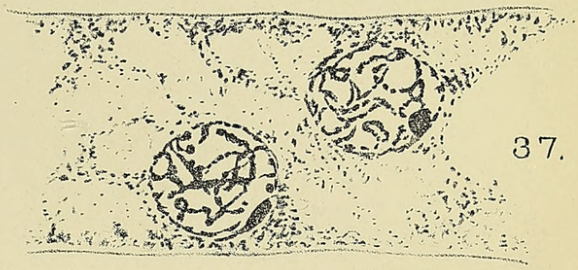

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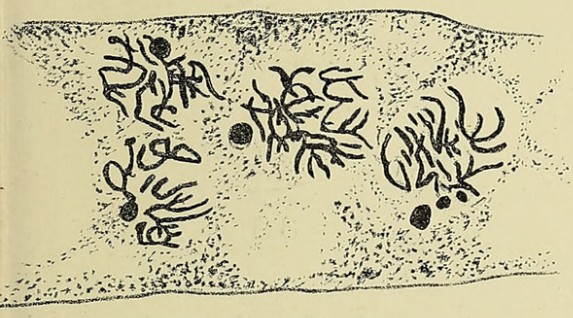

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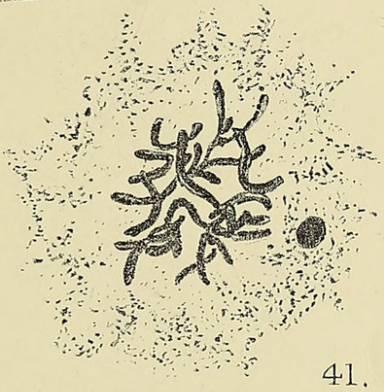

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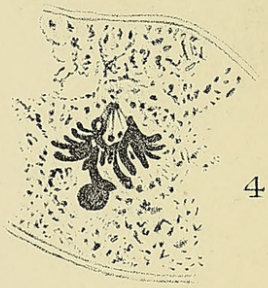

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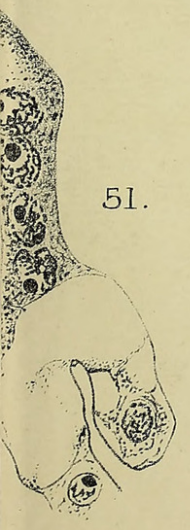

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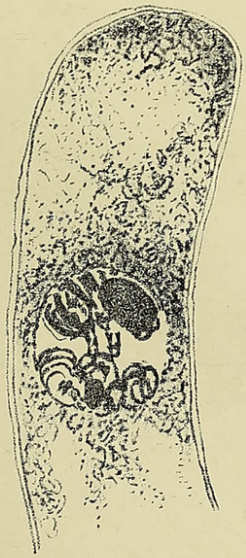

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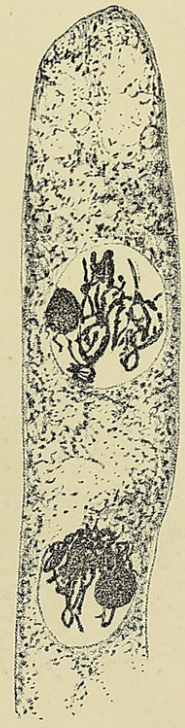

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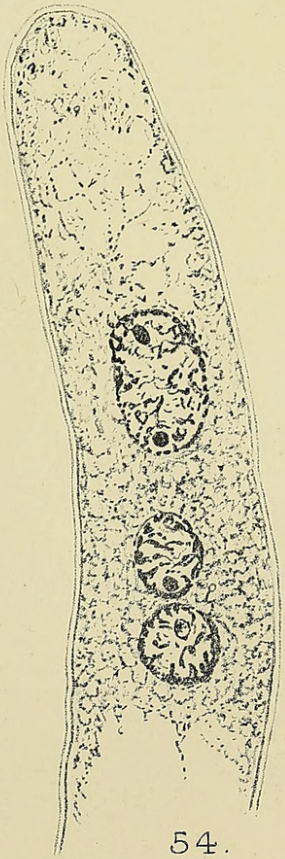

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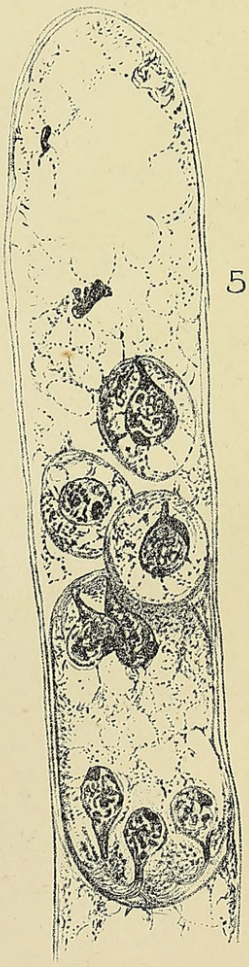

55.

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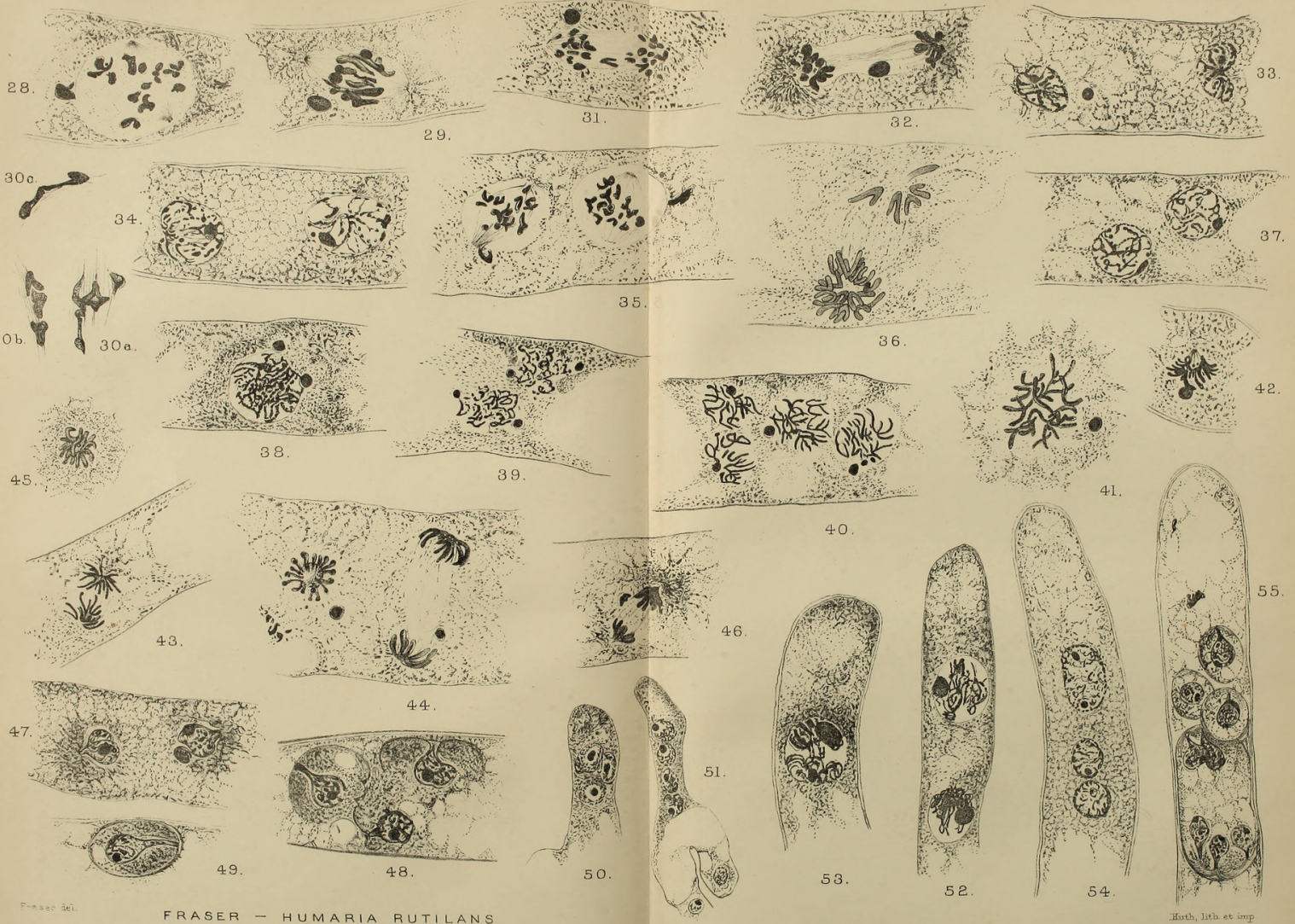

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