

## On the Division of Nuclei in the Mycetozoa.

By ARTHUR LISTER, F.L.S.

[Read 2nd February, 1893.]

(PLATES XXXV. &amp; XXXVI.)

AT a meeting of the Society on Dec. 1st, 1892, I exhibited a series of preparations showing the changes which took place in the nuclei of Mycetozoa previous to the formation of spores in the sporangium. I gave an account of my investigations on the subject, which I now offer in greater detail, together with observations on nuclear division in the swarm-cells, a point which I referred to at the meeting as requiring further elucidation.

In De Bary's work, dated 1884\*, he states:—"Nuclei were not at first observed in the plasmodia; Cienkowski even stated expressly that the nuclei present in the swarm-cells disappeared when they coalesce; Schmitz and Strasburger have recently established the presence of nuclei in the plasmodium, and it may be presumed that they are the persistent nuclei of the swarm-cells and the products of their division."

Strasburger published in 'Botanische Zeitung,' May 1884, his account of the development of the sporangia of *Trichia fallax*. He gathered a large number of sporangia in different stages of growth, and cut sections after hardening them by different processes and embedding in elder-pith. In some of these sections he discovered division of nuclei taking place with the now well-known figures of karyokinesis; and he states, "Very possibly this mode of nuclear division takes place only at the stage immediately preceding spore-formation; previously the number of nuclei did not appear to increase, whereas final division would be necessary to provide the smaller nuclei we find in the spores."

I exhibited at the meeting a photograph taken from a thin network of creeping plasmodium of *Badhamia utricularis*, killed with alcohol while the streaming was in full activity over a thin cover-glass, and stained with magenta,  $\times 450$ . It showed the nuclei in large numbers. I also showed several of such films killed with Flemming's fluid stained with picrocarmine and mounted in balsam; these exhibited the vast abundance of the nuclei more clearly.

For several years past I have endeavoured to discover by what

\* 'Comparative Morphology and Biology of the Fungi, Mycetozoa, and Bacteria.' Oxford ed. 1887.

process the multiplication of these nuclei came about. During this time I have made many stainings of the plasmodium of a large number of species, and though I have often seen appearances which implied that simple division took place, I was never able to detect the smallest indication of karyokinesis.

From the cultivation of plasmodia it was obvious that there was a continual increase in the number of the nuclei. *Badhamia utricularis* affords especial facilities for such cultivations, as it grows rapidly when supplied with such fungi as *Stereum hirsutum* and *Polyporus versicolor*.

In January 1887, I gathered the plasmodium of *Badhamia* from a stump in my garden, and from that time to the present I have had an almost continuous series of cultivations from that one source. I have sometimes had as many as 17 colonies growing at once; some of these would change to sporangia and some I allowed to dry into sclerotium, which at any time I could revive to replace those that had produced their spores\*.

Stainings of the plasmodium made at any time of the year, and either immediately previous to the change to sporangia or weeks before, always showed the nuclei of the same character: they are about  $3\ \mu$  in diameter, varying from  $2.5\ \mu$  to  $5\ \mu$  (Pl. XXXV. fig. 1); they contain usually a single nucleolus but sometimes two or three of smaller dimensions.

Considering that the plasmodium with which our stainings were made was suddenly killed when in full vitality and preserved so perfectly that the minute vacuoles in the thinnest parts of the film were distinctly defined, it is perhaps remarkable that we do not more frequently meet with unmistakable instances showing the nuclei in the actual process of division, but such is the fact. Although we know that rapid multiplication of nuclei must go on in quickly-growing plasmodium, we may search a mounting for a long time without meeting with the dividing stage; at the same time it is met with frequently enough, I think, to justify the conclusion that the nuclei multiply by simple division. Fig. 2, *a*, *b* (Pl. XXXV.), represent some of the forms in which such division appears to be going on. In almost every field of the

\* The encysting of the plasmodium when it passes into the condition of sclerotium appears to be analogous to the formation of microcysts in the swarm-cells: a number of nuclei with the accompanying plasma are inclosed by a cyst-wall, which is either dissolved or remains persistent according to the species, when the sclerotium is revived; but there is nothing to lead us to suppose that nuclear division is involved in the process.

microscope we notice pairs in close proximity, often connected by a bridge of nuclear matter, if we may judge from its taking an equal depth of stain; this appearance is so frequently seen that it is scarcely possible to be an accidental arrangement, but suggests that division has occurred and that the two halves are not yet entirely free. Fig. 2 *a* (Pl. XXXV.) gives an earlier stage in which two nuclei seem to be clearly united. It is in the thin parts of the film, where the nuclei are flattened and the plasmodic granules widely scattered, that this condition can best be seen; in deeper parts it is very difficult to ascertain whether such appearances are not occasioned by two nuclei overlapping at their edges. See "Additional Note" at end of paper.

In a paper recently published in Cohn's 'Beiträge zur Biologie der Pflanzen,'\* speaking of the plasmodium of Mycetozoa, Rosen states: "We may look in vain for nuclei caught in division. One may, indeed, without great error, put down the number of nuclei in a plasmodium as equal to the number of united single amoebæ which the plasmodium contains."

From my cultivations, in which the nuclei increase in number a thousandfold, it is evident that this view cannot be sustained; but Rosen's experience is interesting as showing the difficulty there is in detecting the mode of increase.

My figures are drawn from stainings made at Lyme Regis in Sept. 1892, in the preparation of which my son, Mr. J. J. Lister, assisted. On his return to Cambridge he made a series of experiments with two species of Mycetozoa with the view of finding the karyokinetic appearances described by Strasburger, following his method but extending his observations by noting the times when the nuclear changes took place in the gradual development of the sporangia ending in the formation of spores.

I give his account in his own words:—

"On Oct. 15, 1892, I collected a number of young sporangia which had emerged from an elm-stump in the Backs of St. John's College. Some of these were hardened in Flemming's fluid and others were kept to ripen. When mature they were identified as belonging to the species *Trichia varia*, Pers.

"On Oct. 18th a fresh crop of sporangia of this species appeared on the same stump, and from that day till the 23rd I made a daily gathering of sporangia and preserved them in the same manner.

\* Cohn, Beitr. z. Biol. Pflanzen, Bd. vi. Hft. 2. Breslau, 1892.

"I cut sections of each batch, staining with borax carmine before embedding in paraffin.

"The sporangia gathered on Oct. 15 show the nuclei in various stages of division by karyokinesis.

"In the sporangia of the later gatherings the karyokinetic division of the nuclei occurred between the morning of Oct. 20 and that of Oct. 21. On Oct. 20 nuclei were scattered through the undivided protoplasm, while twenty-four hours later each nucleus was the centre of a young spore, distinctly separated from its neighbours, although no spore-wall could yet be seen.

"On Oct. 28th I found a crop of young sporangia on a rotten elm log, and brought it to the Laboratory to treat in the same manner as the *Trichia*. Part of it was allowed to ripen and was identified as *Arcyria incarnata*, Pers.

"At 2.40 P.M. development was progressing so rapidly that from this time until 5.40 a small group of sporangia was preserved every hour, namely, at 2.40, 3.35, 4.40, and 5.40. On cutting sections of this series, the division of the nuclei by karyokinesis was found to be in full progress at 5.40, while the sporangia preserved an hour earlier showed no sign of it.

"On Nov. 5 the process was repeated with a fresh crop of *Arcyria incarnata* from the same stump. Sporangia were preserved at 9.18, 9.55, 10.45, 11.25, and 12 noon.

"In this series karyokinesis was in progress at 10.45. The nuclei at the 9.55 stage show some indication of change, but none of them have reached the *metaphasis* of the process of division, while at 11.25 the *metaphasis* was finished and the protoplasm already aggregated round the daughter nuclei."

At Lyme Regis similar experiments were then made with *Trichia fragilis*, *Trichia fallax*, *Comatricha Friesiana*, and *Phy-sarum leucophæum*; but instead of hardening the sporangia and cutting sections, the contents of the young sporangia were smeared over thin cover-glass and then treated with Flemming's fluid, stained with picrocarmine, and mounted in balsam.

The following records show the period when karyokinesis appeared:—

Nov. 13. A large growth of *Comatricha Friesiana*, just rising from rotten wood, was brought indoors and placed under a bell-jar.

12.45 P.M. The ovoid sporangia were nearly sessile, the dark stalks just appearing at the base. The staining showed the nuclei containing one to three nucleoli lying in a loose reticulum.

3 P.M., 3.45, 4.45, 5.45, 6.10. Nuclei with the same appearance as at 12.45.

6.45. The black stalk had elongated to four times the diameter of the globose sporangia, in which the dense net of capillitium had developed; nuclei unaltered.

6.55. The nuclei showed a changed aspect. Instead of containing one to three distinct nucleoli, they had a more uniform appearance. Although the objects are so minute that the structure is difficult to make out, they may be presumed to have reached the stage immediately antecedent to that described by Strasburger as the coil. A more definite coil stage was obtained in other series.

7.15. Nuclear spindles perfectly developed.

7.35. The two halves of the nuclear plate separating.

8 P.M. In the greater part of the preparation the spores had formed, each containing a small disk-shaped nucleus; in one part a less advanced stage appeared; the plasma was aggregated round the dividing nuclei in masses of two spores' capacity, the daughter-nuclei being widely separated; and elsewhere these masses were constricting to form the spores (Pl. XXXVI. fig. 9). A later staining showed the spore-wall forming, with the nucleus globose and increased in size. Next day the remaining sporangia of the gathering had perfectly developed the ripe spores.

Nov. 28. A cluster of *Physarum leucophæum* was rising in white knobs on a rotten stump, the stalks not having begun to form; they were placed under a bell-jar as in the last case, and smears were taken in the same manner. The first preparation was made at the time of gathering.

10.55 A.M. The nuclei had a loosely reticulated structure and contained one to four irregular nucleoli: the plasmodium was charged with refuse matter.

4.30 P.M. The refuse matter had been discharged from the sporangia and was stored in the short stalks which had now formed.

5.15, 7.30, 8.30. No marked alteration in the appearance of the nuclei.

9.10. The nuclei had a uniform granular appearance as if the network had broken up into numerous short rods. The preparation corresponded with that of *Comatricha* at 6.55. Numerous darkly-coloured small nuclear bodies were present in the staining. I will refer to these later.

9.50. The nuclear plate had divided, corresponding with the

*Comatricha* of 7.35. The stage showing the nuclear spindles, matching with the 7.15 staining of *Comatricha*, was missed in this series; but one of a less carefully timed preparation of another gathering of *Physarum leucophæum* supplied the gap. The small nuclear bodies (mostly contained in a vacuole) were numerous in this slide.

10.25. Young spores formed containing a small discoid nucleus, corresponding with the 8 P.M. staining of *Comatricha*.

10.40. Nuclei in the young spores spherical.

On Dec. 5 a piece of rotten wood bearing a crop of young *Trichia fallax* was placed under a bell-jar, and stainings were taken which showed karyokinesis about an hour before spore-formation; but it was not a healthy development, probably owing to their having been exposed to frost when the plasmodium was just emerging. However, on Dec. 12 a growth of upwards of 200 sporangia came up in rosy globules on the same piece of rotten wood, which had remained all the time under the bell-jar. This gave an opportunity for preparations being made under the most favourable conditions with no danger of the disturbance to which plasmodium is liable when removed from its natural surroundings. In addition to the interest attaching to *Trichia fallax* as having been examined by Strasburger, it is a species representing a group which are slower in maturing than *Comatricha* or *Physarum*; for whereas in the latter genera the spores begin to form within 12 hours from the time when the sporangia first make their appearance, in *Trichia* this process takes nearly four times as long and the spores are not fully ripe under about four days.

The following record gives the times when the sporangia were examined. It will be noticed that those taken at 10.20 and 10.40 on Dec. 13 do not follow in regular sequence, which is probably due to the fact that in this species the sporangia do not rise from the matrix quite simultaneously, so that some are rather more backward than others.

Dec. 12, 10 A.M. Sporangia of *Trichia fallax* rising in pink globes. Nuclei showing a reticulated structure and containing one or two nucleoli.

4 P.M. Sporangia ovate, stalked; nuclei as at 10 A.M.

9 P.M. No elaters formed, but vacuolar cavities showing in the protoplasm.

Dec. 13, 8.30 A.M. Elaters formed with rounded ends, no spiral markings; nuclei unaltered

1 P.M. Elaters pointed and showing faint spirals.

7 P.M. Elaters with well-formed spirals; nuclei unaltered; many small deeply-stained nuclear bodies distributed through the preparation (Pl. XXXV. fig. 3).

7.20, 8, 8.30, 9 P.M. No apparent change from 7 P.M.

9.30 P.M. The spindle had formed with a thick nuclear plate appearing as if composed of numerous short rods in confused aggregation; spindle-fibres distinct (fig. 5).

9.55. The two halves of the nuclear plate in different degrees of separation.

10.20. No spindles; a reticulum suggesting the "coil" stage, —a less advanced development.

10.40. The nuclear plate widely divided, a few retaining the spindle-form (fig. 6).

11.5. A remarkably beautiful and uniform preparation; all the nuclei of hexagonal figure, the two halves of the nuclear plate separated to the distance of their diameter; the spindle-fibres sharply defined.

11.27. Daughter nuclei separated, discoid (fig. 7).

11.47. No apparent difference from the last.

Dec. 14, 12.5 A.M. An appearance of spore-formation beginning.

12.20. A few spores seen of normal size.

1.15. Spores increased in number.

1.50. do. do.

2.15. Some spores showing a delicate spore-wall (fig. 8).

A large growth of *Trichia fragilis*, which emerged from rotten wood in October, gave sharp karyokinetic figures corresponding with those of *T. fallax* as far as stainings were taken, but they were not carefully timed.

Until quite recently I had not seen karyokinesis in *Badhamia utricularis*. I was particularly anxious to observe it in this species, because, as I have said before, I have cultivated the plasmodium for six years from one source and have made a large number of stainings of the creeping stage with the object, if possible, of detecting the dividing nuclei.

On Jan. 28 one of the cultivations, which had been fed on *Auricularia mesenterica* for some months, showed signs of changing to fruit, and at 10 o'clock at night it was seen to be concentrating into sporangia. At 4 o'clock the next morning between one and two thousand well-formed sporangia were hanging in clusters, like golden grapes, from the fungus on which the plasmodium had been feeding. From this time to 11.37 A.M.

the contents of a sporangium was smeared on a thin cover-glass every half-hour, and after 6 o'clock every quarter of an hour, and placed in Flemming's fluid. A check staining with acetic gentian-violet was made on each occasion to note any change that might occur. At 4.15 in the morning there was an indication of the formation of capillitium by the appearance of vacuolar spaces in the plasma, in which granules of lime, which abound in the plasmodium, were seen to be collecting. The nuclei had the same character as in the streaming plasmodium and, with the exception of the further development of the capillitium, there was no apparent difference in the successive stainings until 11.37. At this period the gentian-violet stain showed a changed appearance in the nuclei, suggesting that the "coil" stage had been reached. From 11.45 to 12.45, when spores had formed, smears were taken every two minutes. I have 37 stainings taken during this hour, and, with the exception of two which show only spores, we have in these mountings many thousands of nuclei in every stage of karyokinetic change. Four hours later the sporangia under the bell-jar were black from the dark-walled spores.

Rosen, in the paper before alluded to, describes his investigations relating to *Fuligo* and *Lycogala*, in which he observes that shortly before the formation of the spores a nuclear plate is formed, and that this plate divides and the two halves separate; but he did not succeed in making out the presence of the spindle-fibres. He agrees with Strasburger in supposing that there is a simplification in the karyokinetic process in Mycetozoa as compared with that observed in higher forms. He thinks the coil stage may be absent in this group, and further states that the smaller the nucleus the more simple is the process. I cannot think that this view is borne out by the preparations of the species I have examined. We have, in the first place, the change from the condition of the nucleus with distinct nucleoli lying in a close reticulum to that of a more uniform structure in which no nucleolus can be detected; we then see the chromatin matter withdrawing from the nuclear wall, and presenting much the appearance of a continuous coil (Pl. XXXV. fig. 4); this is followed by the formation of the nuclear plate, in which about eight segments may be counted when seen in profile; from this plate the spindle-fibres can be clearly seen converging towards a point at the opposite poles: then we have the stage when the nuclear plate has divided, the spindle-fibres are seen to connect the separated



halves, and also to extend to the now widely-diverging poles (figs. 6 and 9), while the nuclear wall has vanished; after this the spindle-fibres disappear, and each daughter nucleus becomes enclosed in a spore. The chromatin elements composing the nuclear plate, when seen under the highest powers, appear as elongated curved bodies, though their exact shape cannot be made out, nor can any indication of longitudinal splitting be detected.

From the appearances above described, there seems to be strong evidence that the process followed in these minute nuclei is of the same character as that observed in those of larger size.

I wish to call attention to a remarkable change that took place in the behaviour of the plasma of *Badhamia* at the time of the appearance of the spindle stage. Until the condition which I take to be the coil was reached, the plasma, when spread on the cover-glasses, was viscid and smeared with some difficulty, forming lumpy aggregations, but as soon as the spindle had formed it spread like cream in an even layer. The stainings showed, as had before been observed to some extent in *Comatricha* and *Physarum*, that at the time when the viscid condition ceased, the plasma broke up in irregular masses enclosing numerous nuclei with the nuclear plate in various stages of division; as the daughter nuclei separated, a further breaking up took place, until each dividing nucleus was surrounded by a definite amount of plasma of the capacity of two spores; this again constricted to form the spores. The process in these species is very rapid. In *Trichia fallax*, on the other hand, the plasma does not break up until the final spore-formation takes place and the daughter nuclei have separated\*.

The small nuclear bodies before referred to seen in the preparations of *Physarum leucophæum* and *Trichia fallax* may, perhaps, be abortive nuclei. In *Comatricha Friesiana* they could not be found either before or after the formation of the spores, though more than 30 stainings of the critical stages were examined. In *Trichia fallax* they were not present in the earlier conditions,

\* Note.—Since writing the above, we have made a successful series of stainings of the sporangia of *Didymium squamulosum*, *Craterium vulgare*, and *Badhamia panicea*. In all of these the breaking up of the spore-plasma took place in the same manner as in *Badhamia utricularis*, the dividing nucleus always being surrounded by a mass of two spores' capacity before the final division into spores.

but showed in considerable abundance when the elaters were forming (Pl. XXXV. fig. 3). They were, for the most part, more deeply stained than the normal nuclei and resembled oil-globules enclosed in vacuoles; intermediate forms showing more or less of reticulated structure were found between these and the well-formed nuclei. At the stage when the spindle occurred they were more faintly stained, and when the spores appeared they were often difficult to detect, although most of the spores contained one or two of them (Pl. XXXV. fig. 8). In *Physarum leucophæum* they were more striking than in the last-named species. In the first stage, when the sporangia were just rising, they were conspicuous by their dark staining, the absence of reticulated structure, and by appearing in pairs, often apparently adhering, the couples being surrounded by a hyaline envelope. Four hours later they were still more numerous, of the same character as in the former staining, and with the couples in great numbers. Two hours later again, when the nuclear halves had divided, they were no longer in couples, but so numerous that 30 could be counted in one field of the  $\frac{1}{10}$  ob. gl. In another hour the spores had formed and a large number of them contained one or more of these nuclear bodies enclosed in a vacuole as deeply stained as the true nucleus.

These observations may not be of much value, but the objects are so striking that they can hardly be passed over without notice.

The experiments above described were made with three species of *Trichia*, one of *Comatricha*, one of *Physarum*, one of *Badhamia*, and one of *Arcyria*, representing genera of widely differing characters. They give essentially the same results and afford a definite confirmation to Strasburger's surmise that division by karyokinesis in the sporangia of Mycetozoa is only found immediately before the formation of spores. They also show that in the cases in which the stainings have been carefully timed this division occurs but once, and within an hour from the period when the young spores make their appearance.

We have now to consider the change which takes place in the nuclei of the swarm-cells when division occurs in those bodies.

I had by me a specimen of *Reticularia Lycoperdon* gathered in May 1890. Experience had shown that it is a species whose spores germinate rapidly and with great regularity, but as the specimen had been preserved in a dry cupboard in which naphthalin had been freely scattered from time to time, it seemed doubtful

whether the spores would have retained their vitality for so long a period. That they had done so was soon proved. On Dec. 3 I shook some of the spores into a watch-glass, giving one drop of methylated spirit to expel the air from among them, and adding about 10 minims of filtered rain-water; in a few hours the swarm-cells were hatched in great numbers; on the following day a large proportion of the spores were empty, perhaps nine-tenths of their number, and the water was milky with the multitude of swarm-cells in active motion. A drop of this water was placed on a glass slide and dilute acetic gentian-violet was added; the swarm-cells were instantly killed, retaining their natural form with the flagellum extended; in these the nuclei were faintly stained, but here and there a few cells could be noticed with the flagellum withdrawn and in process of dividing; some were of globular form, others had become oblong, and others again were constricted or were about to separate. In all these stages the nuclei were deeply stained, so that they could at once be distinguished among the host of flagellated swarm-cells by which they were surrounded, and each darkly-stained nucleus was seen to be in one or another state of karyokinetic change.

In some of the spherical cells the nuclear plate had formed, and was seen, in profile, to consist of about six segments (Pl. XXXVI. fig. 10); in others division of the plate had taken place and the nuclear halves had just separated (fig. 10, *c*); in favourable instances the spindle-fibres could be detected converging to the poles of the spindle. In the oblong forms the two nuclear halves had retreated to the distance of about four times their diameter, and where the stain was of the right intensity the spindle-fibres could be distinctly seen connecting the daughter nuclei (fig. 10, *d*). Where separation of the cells had nearly occurred the still deeply-stained daughter nuclei had a discoid or crescentic outline, and took an excentric position in the daughter cells at the most distant point of divergence from one another (fig. 10, *g*).

After the swarm-cells have completely divided, the nuclei soon lose the property of retaining the deep stain; it is only while the cells remain attached to each other, though ever so slightly, that their nuclei are conspicuously darker than those of the neighbouring cells.

On the third day after wetting the spores, the swarm-cells were in vast abundance in the watch-glass, and mostly smaller in size

than on the first day, possibly from the absence of nutritive matter; dividing-cells were observed, showing, as before, darkly-stained karyokinetic figures. There could be little doubt from their size that some at least of the dividing-cells were the offspring of a former division.

Spores from another gathering of *Reticularia*, collected nearly two years ago, germinated as quickly and showed the same karyokinetic process in the nuclei as in the former case.

*Amaurochate atra*, collected last summer, produced swarm-cells a few hours after the spores had been placed in water; division began sooner in these than in *Reticularia*, with the same appearance of karyokinesis, the dividing nuclei taking a deep stain as before described. Although the swarm-cells of this species are much larger than those of *Reticularia*, they are not so favourable for observation, because the greater depth of granular protoplasm obscures the definition in gentian-violet stainings, at the same time the spindle-threads connecting the separated halves of the nuclear plate were clearly indicated in some instances.

Numerous experiments were made with the spores of *Chondrioderma difforme*; those cultivated in a hanging drop and supplied with a thin section of the testa of a cress seed gave the best result. Three days after having been wetted and when the swarm-cells were very numerous and chiefly in the amoeboid form, they were killed with osmic vapour and stained with acetic gentian-violet. The nucleoli alone in the active cells took a deep stain, the rest of the nucleus being scarcely more coloured than the body of the swarm-cell; but when karyokinesis took place the stages were strongly marked, as in the other species.

Stainings were made with acetic methyl green, picrocarmine, and mauvine, but none of them answered so well as acetic gentian-violet; for, although it is unsuitable for permanent mountings, the deep colour which it immediately gives to the dividing nucleus, in strong contrast with those of the active cells, allows several hours of favourable observation before the preparation becomes obscured by the concentration of the stain.

In making smears of the sporangia of *Comatricha* for preparation in Flemming's solution, the stage of development was ascertained by staining the remainder with dilute gentian-violet, and it was interesting to observe how precisely the deep colouring of the dividing nucleus corresponded with the same stage of karyokinesis in the swarm-cells. Although the definition was not so

sharp as in the mountings in balsam stained with picrocarmine, it was abundantly sufficient to indicate the period at which it was desirable to make frequent preparations\*.

In reviewing the observations recorded in this paper we find:—

1. Karyokinesis takes place in the nucleus at the time when the swarm-cell divides. From former observations we know that when the swarm-cells unite to form the plasmodium, their nuclei remain distinct and do not coalesce.

2. The examination of over a hundred stainings of streaming plasmodium in which we know that the nuclei have multiplied in vast numbers, and in which no indication of karyokinesis occurs, leads to the conclusion that they increase by simple division. Although from the nature of the case such division must be difficult to detect, we frequently meet with appearances which support this conclusion from actual observation.

3. Within one hour before the formation of spores in the sporangium, in other words, when division of the protoplasm into true cells takes place, we have again the phenomenon of karyokinesis.

Or, leaving aside the question of the sclerotium, we may further generalize thus:—

Whenever cell-formation occurs in the life-history of the Mycetozoa, the nuclei divide by karyokinesis.

ADDITIONAL NOTE.—Since writing this paper, convincing proof has been obtained that, under certain conditions, the nuclei in the streaming plasmodium divide by karyokinesis.

Mr. J. J. Lister, of Cambridge, has just repeated experiments with the plasmodium of *Badhamia utricularis*. A portion of sclerotium of this species was revived, and the plasmodium spread itself in large fans over a thin cover-glass. Four of these films connected by veins of plasmodium were taken at the same time and killed with Flemming's fluid and stained. Two of the

\* I have lately succeeded in making permanent mountings of the swarm-cells of *Amaurochate* and *Reticularia* by the following method:—A drop of water containing the swarm-cells is mixed with a small quantity of Flemming's fluid on a square cover-glass; it is allowed to evaporate almost to dryness, sufficiently to permit the objects to adhere to the glass, which is then floated on a watery solution of Erlich's hæmatoxylin and stained for twelve hours, washed for two minutes, passed through alcohol from 30 per cent. to absolute, then through oil of cloves, and mounted in balsam.

The karyokinetic figures in these preparations show the spindle-fibres more clearly than in the best of the fleeting stainings with acetic gentian-violet.

mountings show the nuclei in the stage described in my paper, with no sign of karyokinetic change; in the other two mountings every nucleus is dividing by karyokinesis, some are in the spindle stage, in other parts of the preparation the nuclear plate has divided and the daughter nuclei are in different degrees of separation.

This throws important light on the subject, and modifies the conclusion expressed at the end of my paper. Whether we can accept this observation as explaining the entire process of nuclear multiplication in the plasmodium, or whether, as seems probable, there is also increase by direct division, is a point which requires further investigation.

#### EXPLANATION OF THE PLATES.

##### PLATE XXXV.

Fig. 1. Resting nuclei from plasmodium (*a*) of *Badhamia utricularis*, (*b*) of *Trichia fragilis*.

Fig. 2. Nuclei apparently in process of direct division, from plasmodium of (*a*) *Badhamia utricularis* and (*b*) *Trichia fragilis*.

Figs. 3-8 represent successive stages of division of nuclei by karyokinesis in the young sporangium of *Trichia fallax*.

Fig. 3. Nuclei and "small nuclear bodies" an hour before nuclear division.

Fig. 4. Nuclei in process of indirect division: *a*, "coil" stage; *b*, between the "coil" stage and the "nuclear spindle"; *d*, *e*, "nuclear spindle," the nuclear wall still persistent; *c*, probably the foreshortened aspect of the spindle "*d*." (The chromatin elements in this fig. are represented as too globular and sharply defined.)

Fig. 5. Division rather further advanced, the nuclear wall having disappeared: *b*, the spindle *a* seen from the pole.

Fig. 6. The "nuclear plate" is widely divided into two parts by the separation of the daughter segments.

Fig. 7. The daughter nuclei have completely separated from one another, but are still disk-shaped.

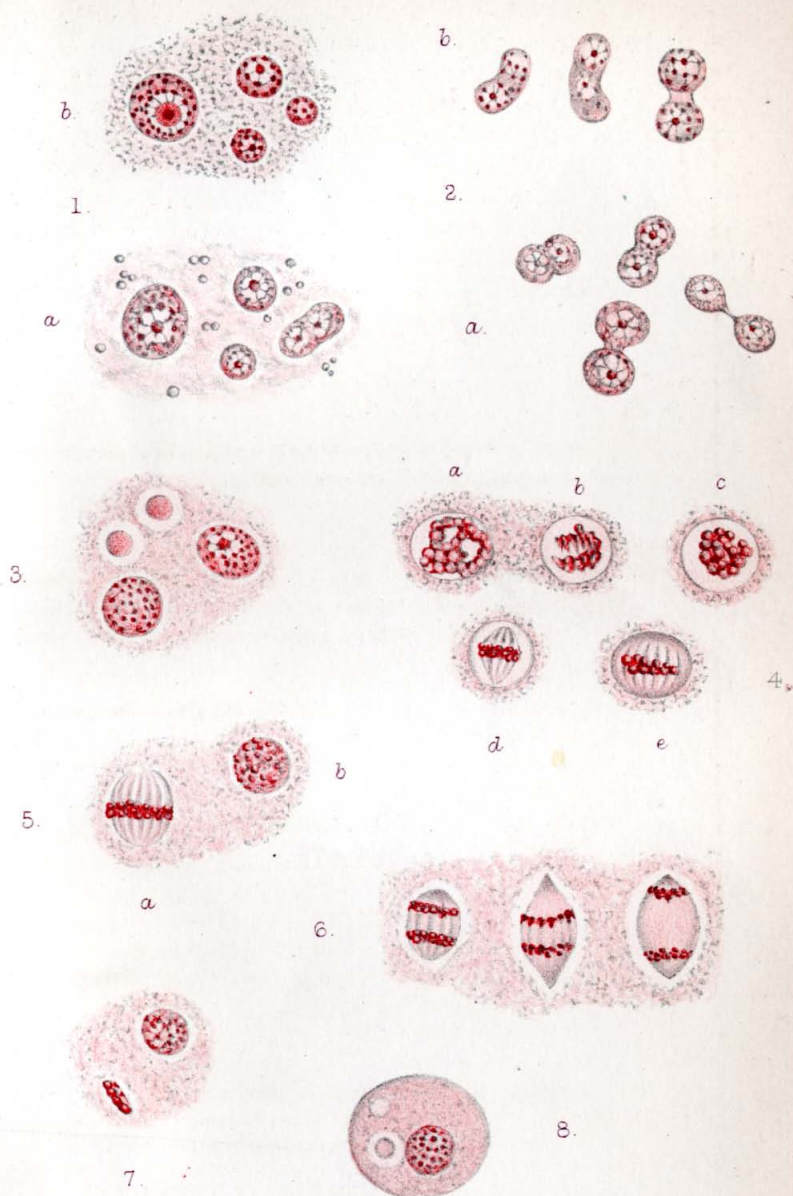
Fig. 8. A young spore containing a spherical nucleus and one small "nuclear body."

##### PLATE XXXVI.

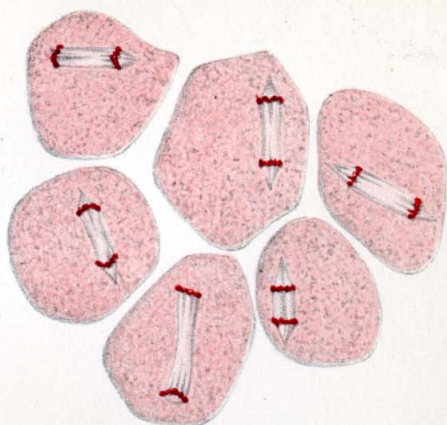
Fig. 9. Dividing nuclei, each contained in a mass of protoplasm of two spores' capacity, from the young sporangium of *Comatricha Friesiana*.

Fig. 10. Swarm-cells of *Reticularia Lycoperdon*: *a*, active flagellate swarm-cell; *b-g*, successive stages in division of swarm-cell accompanied by division of nucleus by karyokinesis.

*Note*.—Figs. 1-9 are drawn from preparations killed with Flemming's fluid, stained with picrocarmine, and mounted in Canada balsam. Fig. 10 is drawn from preparations killed and stained with acetic gentian-violet. All the figures  $\times 1600$ .



9.



a.



b.



c.



10.

d.



e.



f.



g.

