

Studies of Reproductive Elements.

II. *Noctiluca miliaris*, Sur.; its Division and Spore-formation.*

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With Plates XI-XIV.

A. DESCRIPTIVE PART.

a. Division.

The individuals of the genus *Noctiluca* which are preparing to divide can easily be distinguished from the others by their spherical form, caused by the absence of the peristome and of teeth, and by the diminished size of the tentacle (Figs. 1 and 2); but the *Staborgan* is always to be seen as a narrow line of cytoplasm, extending backwards from the nucleus and marking the line of division. The tentacle appears to be drawn into the body of the animal, as *Cienkowsky* (8, p. 50) first observed, and not thrown off, as *Robin* (6, p. 1064) is

* The materials upon which the following observations were carried out were in part collected by myself from different parts of the sea coast near Tokyo, for instance, in Numadsu, Zushi, Tateyama-in-Boshiu, etc., and in part from the Misaki Marine Biological Station of the Science College of the University, where I worked for a fortnight during the summer of 1890, for a week during the winter of 1892-93 and again for a week at the end of August this year. For all these opportunities and for the free use of the library of the Zoological Laboratory of the Science College, I express my warmest thanks to the authorities of the College, and especially to Professor Mitsukuri.

inclined to think. My figures (1 and 2) show the stages of its withdrawal.

The central protoplasm, which in ordinary conditions takes a more or less elongated form, then becomes concentrated into a star-shaped mass around the nucleus. Close to the nucleus, part of it differentiates itself from the rest by coarser granulation. It is usually spherical in shape (Figs. 1 and 2), but sometimes elliptical (Fig. 31). This cytoplasmic mass represents the kinetic centre of division, as will be later shown, and corresponds to the *sphère attractive* of *van Beneden*, or *Archoplasma* of *Boveri*. In many cases this archoplasmic mass is formed before the complete disappearance of the peristome, teeth, etc., as is shown in Fig. 31. The relative position of the archoplasm and the nucleus does not seem to be always the same; but both bodies lie in the direction of the *Staborgan*, as will be seen in Figs. 1, 2, and 31. In Figs. 1 and 31 the archoplasm lies behind the nucleus, whereas in Figs. 2 and 3 it lies in front of it. In living animals the nucleus at this stage appears as a transparent vesicle, and the archoplasm is very distinct, owing to coarse granulation.

The archoplasm now stretches itself in a direction more or less in the vertical plane passing through the *Staborgan*, and forms a relatively large spindle (Fig. 3). It undoubtedly corresponds to the central spindle of *Hermann* (20, p. 580). This spindle gradually elongates and assumes a position longitudinal to the line of division, and divides the nucleus (Fig. 4). The central mass becomes at the same time separated into two portions, each concentrating around its archoplasm. A narrow line of cytoplasm now appears also in front of the central mass in the direction of the *Staborgan*. The spindle parts at the middle, dividing the nucleus into two daughter nuclei. These (the daughter nuclei) at first lie in the line of division, but soon change their positions and come to lie right and left of it

(Figs. 5-9). Which of the two nuclei goes into the one half and which into the other, is not easy to ascertain, as a narrow line of cytoplasm, as above stated, now stretches also in front of the nucleus nearly in the same manner as does the *Staborgan* behind, so that the fore and hind ends are difficult to distinguish. But in all ascertained cases, the front nucleus goes into the right side and the hinder one into the left. All the while the archoplasm remains close to the nucleus. The line of division gradually extends both ways towards opposite surfaces of the body, and the animal assumes the well-known biscuit-shape. Meanwhile, the two protoplasmic masses with their respective nucleus and archoplasm shift their positions and come to lie on each side of the line of division, nearly opposite to each other (Fig. 9); so that division of the animal now appears to take place in the longitudinal plane. The relative position of the nucleus and the archoplasm does not seem to remain the same during the earlier stages of the division, (Figs. 5-9), but at later stages the former lies farther away from the dividing line (Figs. 10-12).

The time during which all these changes take place, from the stage shown in Fig. 3 to the stage of Fig. 9, varies greatly in different individuals; but from observations upon fourteen animals kept in a moist chamber at the Misaki station, during the latter part of August 1893, the time required varies from six to eight hours.

The division of the body proceeds, until only a small connecting bridge of cytoplasm is left (Figs. 10 and 11). During this stage, tentacles usually grow on both halves, which swim together like the Siamese twins. Such a couple, with rather short tentacles, is shown in Fig. 11. That shown in Fig. 12 has tentacles much more developed. This figure is drawn from a living specimen, so that the nuclei (*n*) appear quite transparent, while both the archoplasms (*a*) are seen as

dark granular masses close to the nuclei. The protoplasmic connection (*p. c.*) is reduced to a narrow bridge.

The changes which take place in the nucleus as well as details of the nuclear division will be spoken of in connection with the process of spore-formation.

b. Spore-formation.

Spore-formation is always preceded by the concentration of the central plasm into a rather small area, and by the disappearance of all other structures, such as the tentacle, flagellum, teeth, etc. The individual appears, therefore, as about to divide, except in not possessing mouth and *Staborgan*. Its central plasm is also raised a little above the general surface of the body, and forms a low knob-like elevation, as has been observed both by *Cienkowski* (89, p. 54) and, by *Robin* (6, p. 1069). Just as in division, we find here a spherical concentration of the granular cytoplasm about the nucleus, which is also quite transparent in living specimens. Thus, Fig. 13, which is drawn from an individual in this stage, shows the nucleus (*n*) as a spherical vesicle, while the archoplasm (*a*) appears as a granular mass. The division of the nucleus is also preceded here by that of the archoplasm, and we thus obtain a stage, represented in Fig. 14, which exactly corresponds to the stage of division represented by Fig. 4. In what direction the spindle lies in this case, it is not, however, possible to tell, as the external signs of the bilateral nature of the animal can not all be recognised. The division proceeds still further, till the stage is reached represented in Fig. 15 or 16, the latter of which is drawn from a living specimen. We sometimes meet with a sort of partial segmentation of the entire body at this stage, the plane of division cutting the connecting axis of the two nuclei, as has been observed by *Cienkowski* (7, p. 134). Fig. 17 represents a stage with four nuclei, on the side of each of which is seen a large archoplasmic

spindle, showing thus their further division. The cytoplasm surrounding each of the nuclei and archoplasms is connected with the others by a fine network, like the protoplasm in the segmentation of *Leptodora* eggs observed by *Weismann* and by myself (37, Taf. II, Fig. 30). Fig. 18 gives a side view of a four-nucleated stage, in which the nucleus on the right side is already divided into two daughter nuclei, while that on the left is in the spindle form. Fig. 19 represents a stage immediately succeeding this. Here, in this individual, is seen a partial line of division of the entire body, marking its surface into two hemispheres. A further stage is represented by Fig. 20, where we see six nuclei preparing to divide. Of these two belong to one half and four to the other, showing thus that the division of the buds does not occur quite regularly even at this early stage. The first line of division, although now very faint, is still clearly to be recognised. In the living condition the body of the *Noctiluca* had been imperfectly divided into two portions at the part where the nuclei lie. This is shown more distinctly in Fig. 21, where ten nuclei are to be found. Of these six belong to one half and four belong to the other. The partial line of division of the body is also plainly to be seen in this specimen. Fig. 22 represents a further stage of budding, viewed laterally. All the nuclei visible are represented in this figure, and their number is fifty-one, showing again the irregularity of division. A further stage of sporulation is seen in Figs. 23-30. Figs. 23-26 are drawn from sections of nearly ripe spores, while those represented by Figs. 27-30 are from preparations of entire spores, drawn under different magnifying powers. In Figs. 25,26 (the spore on the right hand side of the figure), and 27-30, the spore cells are quite separated from each other, and they have all got a long flagellum springing out from the basal third of the cell, where the archoplasm lies.

c. Nuclear Division.

In a short paper¹ sent to the *Naturforschenden Gesellschaft zu Freiburg im Breisgau*, I treated at some length of the structure of the nucleus in the resting stage, as well as of the peculiar manner in which the division of the *Noctiluca*-nucleus takes place. As the present account covers the same ground, some of the facts described in that paper are necessarily repeated in this one. Nevertheless I do not hesitate to go again over the same subject here, for two reasons, namely: (*a*) many new facts have become known, and the nature of some of the doubtful phenomena been ascertained since then; (*b*) the papers appeal to two different sets of readers.

1. *Nucleus in resting stage*:—The living nucleus appears quite transparent and homogeneous, as all other observers of *Noctiluca* have long ago described. It is covered by a rather thick membrane, whose contour is often seen as double. When treated with reagents, such as acetic acid, osmic acid, chromic acid, etc., a number of chromatic bodies can easily be distinguished in it. These elements appear as strings of deeply staining bodies which often take an **S**-form. Each string consists, in well prepared specimens, of a number of disc-shaped microsomes arranged one after the other like the chains of mammalian red blood-corpuscles. These strings lie either singly or, more often, two or four united together. In the paper named above, I supposed the single string to be primary, and the compounds of two or four to be secondary, probably produced by division of the single string. In accordance with this supposition, I designated the number of chromosomes as ten. In many cases this number seems to hold true, as Figs. 33, 34, 40-47 show; but in other cases, such

¹ This paper was written for the *Festschriften* commemorating Prof. Weismann's 60th birthday which occurs on the 17th January, 1894.

as Figs. 35 or 36, the number of chromosomes counts greatly above this, and I am not, at present, able to tell exactly their number.

2. *Prophasis*:—Figs. 31, 33, and 34 are taken from preparations of nuclei which are just preparing to divide. In Fig. 31 the chromosomes are not very distinctly to be seen, owing to the deep staining with hæmatoxylin. We see, however, on the left hand side a curved double line undoubtedly representing the segmentation of one of the chromosomes. This is very beautifully seen in preparations represented by Figs. 33 and 34, both of which are stained with a combination of acid-fuchsin and methylen-blue. In both nuclei the chromosomes are seen to be composed of a double row of minute microsomes, the number of which in a single chromosome is not with certainty to be determined, since the entire length of a chromosome is very rarely to be made out. In a few cases, however, I counted them to be ten or twelve in a single row, as will be seen in the V-shaped chromosome, lying a little to the left, in the nucleus represented by Fig. 33. A nearly equal number will be counted, though not so clearly, in one of the two chromosomes at the right hand of the same nucleus. Similar states of chromosomes are seen in Figs. 44-47, drawn from preparations of spore-forming individuals in about the same stage as that represented by Fig. 13. In Fig. 44 the chromosomes, except a few, do not seem to lie in any definite order, but to be scattered more or less irregularly in the nucleus; but in Figs. 33, 34 and 45, they lie more or less parallel with each other, their long axis being in the direction of the archoplasm found just external to the nucleus. In Fig. 46 the above position of the chromosomes is not so clearly expressed as in the figures just mentioned, although the archoplasm can be distinctly seen on one side of the nucleus. This, however, is only an optical illusion caused by the position of the archoplasm which does

not lie in the plane of the paper, but a little beneath it. By focussing the tube, however, we can distinctly observe the chromosomes as radiating from the pole where the archoplasm lies. A close examination of the chromosomes, represented by Figs. 44-47, of the spore-forming individuals, and those, Figs. 33 and 34, of the nuclei just before division, seems to show that there is some marked difference between the two. While the chromosomes of the nuclei of the dividing individuals are represented by a double row of microsomes, those of the nuclei of the spore-forming individuals appear to consist of four rows (comp. Figs. 47 and 33). This difference is undoubtedly due to the division and the spore-formation. *In division the nucleus has to divide only once, and hence the chromosomes require only once to divide, while in the spore-formation divisions of the nuclei take place rapidly one after the other, and two divisions take place almost simultaneously.* The way in which two divisions take place simultaneously can be made out from Figs. 44 and 47, where some of the chromosomes are seen at their ends. The microsomes become thicker at their periphery, and form a row of rings. Each chromatin-ring or, perhaps more correctly, the microsome-ring becomes thickened at four places and the interspaces between these thickenings break up and thus form four daughter microsomes. Sometimes the thickening does not take place simultaneously at four places and the ring does not break up into four at once, as will be seen in a ring at the right hand side of Fig. 47, where it is thickened only at one point. Another ring at the left hand side is broken at one place, and appears C-shaped. A ring represented on the right hand side of the nucleus Fig. 44, shows a very interesting stage. This ring consists of two thick curves connected together by narrow cementing substance—the *linin*-thread. The ends of these curves are again thickened, showing that the division of the ring into four daughter microsomes does

not in reality take place simultaneously, but by a succession of two divisions rapidly following one after the other.

3. *Spindle stage*:—In my paper for Prof. Weismann's birth-day celebration I have given a figure of a nucleus and an archoplasm in the latter of which could be observed a clear space next the nucleus. This space was in apparent communication with the interior of the nucleus. Since then I have been enabled to find a number of fibres in this space (Fig. 46), but the existence of any communication between these fibres and the interior of the nucleus can not with certainty be made out. The archoplasm becomes elongated and forms a very large spindle. This corresponds undoubtedly with the central spindle of *Hermann*, as stated above, but will be called the ARCHOPLASMIC SPINDLE, in distinction from other spindles already described in karyokinetic divisions of other animals and whose origin is still under discussion; while the fibres of this spindle will be called the CENTRAL FIBRES, for reasons stated below. At first the archoplasmic spindle lies tangentially on the surface of the nucleus, but soon assumes a curved figure, depressing at the same time the nuclear wall. In consequence of this the nucleus takes a broad C-shaped figure, in the concavity of which the archoplasmic spindle lies. A notion of these stages may be gathered from Figs. 3, 21, and 24. The chromosomes which, at the stages of Figs. 45, 46, 48, &c., are seen radiating from a single point, become with the division of the archoplasm separated into two masses, one attached to each end of the spindle. The archoplasmic spindle elongates and the nucleus assumes the form represented by Figs. 20, 35, 36, 49, and 52. Fig. 35, prepared from specimens killed with *Flemming's* fluid and stained with *Böhmer's* hæmatoxylin, represents the division of the nucleus in a dividing individual. The nucleus assumes more or less the shape of a dumbbell, and the archoplasms at both ends

of it are irregular in outline. The chromosomes have now distinctly divided into two portions radiating from the pole of the nucleus where the archoplasms lie. Each chromosome consisting of a number of microsomes arranged like beads, is bent upon itself, as is so usual in this stage of karyokinesis in other animals. The number of these chromosomes, as stated above, is very difficult to ascertain. The most interesting phenomenon in this figure (35) is the spindle fibres, of which there are two kinds; those, which I call CENTRAL FIBRES (Figs. 35, 36, 37, 48, *cf.*), lying imbedded in the concavity found in the wall of the nucleus, and standing in no direct relation with the nucleoplasm; and those (Figs. 35, 36, 37, 48, *rf.*) which are seen at the side of the central fibres, running from the centre of an archoplasm to the bent ends of the chromosomes, and which I will call the RADIATING FIBRES. Where they join the nuclear wall, the latter appears quite indistinct, but whether they pass through the wall of the nucleus at these points and are directly attached to the chromosomes can not be made out. In Fig. 37, which represents a section of a dividing nucleus at about the same stage as Figs. 35, 36, and 37, a homogeneous mass of nucleoplasm is found at the poles of the nucleus, external to the bent ends of the chromosomes. In this mass of nucleoplasm, which is easily distinguished from the more granular archoplasm lying outside, are seen many parallel fibres running in the same direction as the radiating fibres of the archoplasm represented in Figs. 35, 36, and 48, but unfortunately not to be seen in the series of sections represented by Fig. 37. We shall come to this again in the part devoted to general considerations, as further discussion of the subject is beyond the region of observation.

When the division has proceeded as far as Fig. 35 or 36, the median portion of the archoplasmic spindle is swollen up a little, as

will be seen in these two figures and also in Fig. 4. This part of the spindle is left behind after the complete separation of the nuclei in the form of a small diagonal figure (Fig. 39 *x*). What becomes of this figure is not ascertained. Fig. 35 is drawn from preparations of a specimen killed with picro-acetic acid and coloured with fuchsin-methylen blue. The archoplasm, and the central as well as the radiating fibres are seen as in Fig. 36, but some of the chromosomes are here more or less swollen up, owing perhaps to the action of the acetic acid. Fig. 35 *A*. is drawn from the same nucleus as that of Fig. 35 but at a level lower than that of the archoplasmic spindle. It should be mentioned by the way that the spindle lies always nearer the surface of the cell than to the nucleus. In both these figures will be seen a number of narrow lines passing between the free ends of the separating chromosomes, and quite distinct from the central fibres. These are the *Verbindungsäden* of German authors, whose origin is undoubtedly to be found in the *linin*-thread of the nucleus.

The difference we observed, in the chromosomes in the case of prophase, between the nuclei of the dividing individuals and those of the spore-forming ones, is also here discernible. Fig. 48 represents an individual in which the first two buds are just dividing; these are seen more magnified in Fig. 48 *A* and *B*. In both of them the nucleus is much elongated and dumbbell-shaped, with a large archoplasm at each end, represented as usual by rough granulations staining very deeply with aniline dyes. Scattered within the swollen ends of the nuclei are seen many chromosomes, each of which is distinctly discernible as a double row of minute microsome; whereas in the nuclei of dividing individuals at about the same stage, the chromosomes consist of only a single row (compare this figure with Figs. 35 and 36). In Fig. 48, *A*. in which the ob-

ject is looked at a little from one side, the central fibres (*c. f.*) lying in the furrow of the nucleus, and the radiating fibres (*r. f.*) proceeding from the upper archoplasm, are distinctly visible. It thus seems that segmentation of the chromosomes in spore-formation takes place before the previous division of the nucleus is yet at its end. This is also to be looked upon certainly as the result of the rapid progress of the bud-formation.

4. *Anaphasis*:—The details of anaphasis can only be observed in individuals which divide, since in those which form spores the nucleus divides successively without the intervention of any resting stages, as mentioned above; and at the end of the spore-formation the diminished size of the nucleus makes it very difficult to observe the individual chromosomes with any accuracy. I will therefore only give my observations of the changes, at this stage, in the nuclein substance of the dividing individuals. Fig. 38 represents one of the nuclei soon after division. The parallel arrangement of the chromosomes, their relative position in regard to the archoplasm (*a*), and the general shape of the nucleus are all displayed very plainly. The positions of some of the chromosomes at its right lower corner, however, become more or less irregular in their relations. Fig. 40, which represents one of the nuclei nearly at the stage of Fig. 7, shows that the chromosomes have lost their parallel arrangement. The microsomes are, however, still very small, represented only by small dark-coloured dots. In Figs. 39-43 the microsomes have become somewhat swollen up, assuming nearly the characters of those in the resting nuclei. The microsomes in Fig. 42, however, appear to be a little too large, owing perhaps to the action of the acetic acid with which the specimen was treated.

5. *The fate of the archoplasm*:—The archoplasm, as we have stated, comes to be seen first at the stage a little before the division or the

spore-formation. During the whole process of the division of the nucleus it remains always closely attached to the outside of the latter, and in the dividing individual is still to be recognised just before the separation of the individuals, as represented by Fig. 12, and even for some time after the complete separation, as one of my preparations of the animal twelve hours after has shown very plainly. After the formation of the peristome, teeth, etc. it becomes indistinct. In the spore-buds the archoplasm remains till the final stage when the spore is ready to swim off from the body of the mother animal, as Figs. 23-30, 49-51 show very plainly. In nearly ripe spores, Figs. 27-30, the archoplasm lies on that side of the body which is attached to the mother cell, and is therefore at the head end of the spore when it detaches itself from the body of the mother. This is more clearly to be seen from sections of nearly ripe spores, represented by Figs. 23-26. I sought after this body in ripe spores treated with acetic-acid-methyl-green, but could not get any satisfactory knowledge of its existence. From the position of the archoplasm, and from the manner of division in the foregoing stages, it is clear that the free end of the spore corresponds to the equatorial part of the archoplasmic spindle, while the end attached to the surface of the mother animal is the pole of the spindle.

6. *The centrosome*:—In many cases in the centre of the archoplasm is seen a small round body, which often colours very deeply with aniline dyes, especially with such colouring matters as rubin, iodine-green-methylen-blue, etc., but also with hæmatoxylin, when this is used in conjunction with iron ammonium alum, as given by *Heidenhein* (15, p. 118-119). In a short note on the conjugation of *Noctiluca* (26, p. 3) I stated that at the poles of the two conjugating nuclei, deeply-staining round-bodies are found, similar to those seen in the centre of the archoplasm, and suggested that such bodies

are the centrosomes so commonly observed at the poles of the spindle of the dividing nuclei of other animals. The body now in question is seen in Figs. 35, 39, 45, 49, 50, 51, (*c*). In Fig. 39 it is very plainly visible in the centre of the upper archoplasm as a small round body surrounded by a clear space. In the lower archoplasm of the same figure, it can not be seen so well, owing to its position beneath the nucleus, but is discernible by focussing the tube. In Fig. 44 two centrosomes are seen close together in the centre of the archoplasm. The nucleus to which this archoplasm belongs is, as stated above, in the condition just before division, while in Fig. 45 it is again seen as a single body. It is also seen in four cells in Fig. 49, as small dark-staining dots. This is also the case in Fig. 50, and in the archoplasm on the left hand side of Fig. 52. It is not visible in other figures, while in the archoplasm represented by Fig. 42 and more clearly by Fig. 48, there is seen a number of small bodies in place of the centrosomes. These bodies are not always quite spherical like the ordinary centrosomes, many of them being more or less elongated, and often presenting curved rods like those, described by *Hermann* (20, p. 585), in the spermatocyte of *Proteus*; they are perhaps to be looked upon as a group of centrosomes like those, described by *M. Heidenhein* (16, p. 54-68), in the lymphocytes of rabbits. In my previous paper I have given a case where the probable origin of the centrosome from the nucleus is shown. Since then I have met with no case similar to that one, but in another, I saw, at the side of a nucleus which had just divided, a small deeply stained body (*c*), close to the nuclear wall, (Fig. 38) probably representing the last stage of the disappearance of the centrosome within the nucleus.

B. GENERAL CONSIDERATIONS.

a. *Division.*

Cienkowsky, in his second paper on *Noctiluca* (8, p. 55), speaks of its reproduction by means of division and says:—"Sie wurde am vollständigsten von *Brightwell* verfolgt, dessen Beobachtungen ich so wohl an normal gebauten wie auch an eingekugelten Individuen bestätigen konnte." *Cienkowsky* thus believes with *Brightwell* (5, p. 10) that the division can take place not only in normal individuals but also in animals in which the tentacle, mouth, etc. have become lost. So far as I have observed, I can only confirm *Robin's* observations (6, p. 1064) that the division takes place only in individuals in which the tentacles have disappeared. The plane of division is longitudinal to the body of the animal, as *Robin* also states; but the first spindle lies in the direction of the longitudinal plane, cutting the animal, therefore, transversely. The resulting nuclei separate from each other and finally come to lie opposite, as is also pointed out by *Robin*. In the dividing individual, the plane of division of the nucleus appears to lie in the longitudinal axis of the body from the first.

The primary dividing furrow makes its appearance along the dorsal median line of the continuation of the mouth and the *Staborgan*, and extends till the body has taken the form of a biscuit; but the complete separation of the body into two parts proceeds, according to my observations, from the periphery towards the central protoplasmic mass, which for a long time remains continuous, as may be seen in Figs. 10, 11, and 12. *Robin*, who studied very thoroughly the division of *Noctiluca*, has arrived, so I learn from *Bütschli* (6, p. 1063-1067), at conclusions which in the main are in accordance with mine; the only difference being in the manner of the final division of the

individuals, which, according to him, goes either from the periphery towards the central mass or *vice versa*.

b. Spore-formation.

The general phases of spore-formation have been worked over by previous writers, such as *Cienkowsky* (7, 8), *Robin* (31), and others. The spore-forming individuals appear to possess a less quantity of cell-substance than ordinary individuals, owing to the flowing together of the protoplasmic network with the central mass, as *Cienkowsky* (7, 8) rightly observed. The observations of the same naturalist of the partial division of the entire body in the earlier phases of budding, are, as stated above, in accordance with my own, although the lines of division are not so deep as *Cienkowsky* shows them in his Fig. 14 (7); but then division of the body into four parts is more seldom met with than that into two.

The two nuclei resulting from the division of the first nucleus do not usually remain stationary, but soon change their relative positions, and thus the second division does not generally take place at right angles to the first, as will be seen in Figs. 15, 16, and 48. In consequence of this, the first four nuclei very seldom lie regularly on the surface of the cell. The time of the division of the first four nuclei is also not exactly the same in all of them, considerable fluctuations being observed in this and also in all the succeeding stages. Accordingly, the number of the nuclei, as well as that of the spores, is not uniformly a multiple of two, as counted by *Robin* (6, p. 1069), but quite irregular. *Cienkowsky* (7, p. 135) and also *Robin* (6, p. 1070) admit, in fact, that the division becomes tolerably irregular when the number of the nuclei becomes large. The number of ripe spores also varies much according to the size of the individual, being less in smaller individuals than in larger ones. I have counted in one case more

than five-hundred spores in a very large animal, while in other cases the number was little over three-hundred. But this also is not always the case, for the area covered by the spores varies in different individuals, though in usual cases it is nearly one-half to one-third of the half surface of the cell.

We will now speak about some points of more or less interest in the phenomena displayed in the karyokinetic division of the cells of other animals compared with those presented in the above described cell-division of *Noctiluca*. These points concern the ARCHOPLASM, the CENTROSOME, and the SPINDLE FIBRES.

The ARCHOPLASM is, as will have been seen from the preceding description, first generally found quite near the nucleus when this is in preparation to divide, (see Figs. 1, 2, 13, 31 etc.). At this time the nuclear wall is always distinctly to be seen, and the size of the archoplasm as compared with that of the nucleus is such as to make its origin from the nucleoplasm very improbable. The nucleus at this stage and in the living condition, appears more or less homogeneous and transparent, while the archoplasm, consisting of coarse granules, is so conspicuous as to be easily mistaken for it. This fact perhaps explains the statements of *Cienkowsky* (18, p. 54), who supposes the nucleus to disappear at the beginning of the spore-formation in the animal. *Robin* (6, p. 1070) has observed the archoplasm and the changes it passes through in nuclear division, but seems to have mistaken it for the nucleus. In dividing individuals, the archoplasm remains closely attached to the nucleus and for a long time until the complete separation of the daughter individuals, as will be seen in Fig. 12; and in spore-forming animals up to the time when the spore is ready to swim away from the surface of the

body, the most remarkable thing here being the flagellum which takes its origin from the centre of the archoplasm, as already stated. *Strasburger* (**35**, p. 65) gives many similar cases in the swarm spores of many plants, where the cilia spring out of the *kinoplasm* (*archoplasm* of Boveri), or at least from the point where the last trace of the *kinoplasm* was found. We shall come back to this again soon.

Looking over the literature of the nuclear division of Protozoons, there is, so far as I know, as yet no case mentioned of the existence of the archoplasm and the centrosome, except in a short notice of mine (**26**, p. 3). The gathering of the cytoplasm and the conical bodies at the poles of the dividing nucleus was observed by *Schewiakoff* (**32**, p. 221) in *Euglypha alveolata*. But these appear at the opposite poles of the nucleus from the beginning, as he very explicitly says:—"Bevor noch die Abplattung des kugeligen Kerns stattfindet, sah man das Cytoplasma an zwei beliebigen entgegengesetzten Stellen, den zukünftigen Polen, sich anhäufen. Kurze Zeit darauf beginnt die Abplattung und man bemerkt gleichzeitig, dass die Kernwandung an diesen beiden Stellen in den Kern sich etwas einstülpt, wodurch zwei kleine Dellen gebildet werden. Auf dem Grund diese Dellen gewahrt man einen kleinen homogenen Höcker, dessen Umrisse, dank der starken Lichtbrechung, deutlich hervortreten. Besonders scharf treten sie bei abgetödteten Thieren hervor, und erscheinen als mattglänzende, gut begrenzte, ellipsoidale Körper, die von Farbmitteln nicht im mindesten tingiert werden." He justly compares this body with the polar corpuscles of *Ed. van Beneden*, and considers the hyaline part of the *Spirochona* nucleus, observed by *R. Hertwig*, to be in the same category. According to *R. Hertwig* (**23**, p. 156), the nucleus of *Spirochona gemmipara* consists of two parts, a larger granular part and a smaller hyaline part,

separated from each other by a transverse line. Stained with *Beale's* carmine, the former colours much more quickly than the latter. The same state of things was observed by this author in *Leptodiscus medusoides* (24, p. 311), the only representative of *Cystoflagellata*, other than *Noctiluca*, found by *Hertwig* at Messina. The nucleus consists here also of two parts, "einem feinkörnigen und einem homogenen." "In dem einen Falle war die homogene Kernsubstanz unverändert, dagegen fanden sich in der feinkörnigen grössere und kleinere Körperchen, die sich in Carmin dunkler färbten und offenbar eine bedeutendere Dichtigkeit besaßen. In anderen Fall liess sich die Differenzierung in zwei Substanzen nicht nachweisen" (24, p. 312). The first change that occurs in the homogeneous part of the *Spirochona* nucleus before its division is the appearance of a small central corn (23, p. 161). This he calls a "nucleolus," which is very probably the free area around the centrosome, such as is represented in my Fig. 13. Some changes occur in the granular part also, and the nucleus soon assumes an elongated form with homogeneous masses placed at its poles. In this way the nucleus assumes a form in which five parts are distinctly to be made out: viz., two homogeneous terminal plates, two striated portions, and a granular median part, considered by *Hertwig* to correspond with the nuclear plate. There is thus a remarkable resemblance between the homogeneous part of the *Spirochona* nucleus and the archoplasm of *Noctiluca*, although the aspects of the two are different, the former looking transparent and the latter granular. But no one will doubt of this similarity, who compares my Fig. 14 or 52 with *Hertwig's* Fig. 17, *a*. What transformations the homogeneous part of the *Spirochona* nucleus undergoes during the division is not quite clear from *Hertwig's* descriptions. From what we get from the *Noctiluca* nucleus, it seems very probable that

the division commences in this homogeneous part. The median granular part of the spindle of the *Spirochona* nucleus, as is given in *Hertwig's* Fig. 17, *b* and *c*, is not to be seen in the *Noctiluca* spindle. It is, however, very probable that here the uniform length of the chromosomes causes the appearance of a distinct median portion. One great difference to be observed between the two, is that in *Spirochona* and *Leptodiscus* the archoplasin is so closely united with the nucleus that it appears as if it formed a part of it. Whether we have to consider the two nuclear parts as a single nucleus, or whether the granular part alone is to be taken as a nucleus is not quite clear, since no membrane is to be seen around the nucleus, although *Hertwig* believes in the existence of one from analogy with nuclei of other Protozoons. In *Leptodiscus*, however, *Hertwig* speaks of the existence of a distinct membrane around the two portions of the nucleus taken together. In this connection *Hertwig* says (24, p. 311-312):—"Der Kern des *Leptodiscus* stimmt somit vollkommen mit dem der *Spirochona gemnipara* überein. Wie ich in einer früheren Arbeit gezeigt habe, besteht der Nucleus dieses Infusors ebenfalls aus einer feinkörnigen und einer homogenen Substanz, die sich beide gegen einander mit einer glatten Contour absetzen und gemeinsam von einer zarten Kernmembran umhüllt werden." Although division of the *Leptodiscus* nucleus was not observed by *Hertwig*, the perfect similarity of its structure with that of *Spirochona*, makes it very probable that here also the homogeneous portion plays an active part in the cell-division and corresponds with the archoplasm of the *Noctiluca* nucleus.

The occurrence of the kinetic centres—the archoplasms—apart from the nucleus in *Noctiluca*, deserves special attention. *Richard Hertwig* (25, p. 106) in his very interesting lecture on fertilization and conjugation delivered in Berlin, speaking of the constant occur-

rence of the centrosome in Metazoa, makes the following remarks on it as regards the Protozoa:—

“Mit diesen für die Metazoen geltenden Verallgemeinerungen sind die Beobachtungen an Protozoen zunächst nicht in Einklang zu bringen. Meines Wissens ist nur für *Noctiluca* die Anwesenheit eines vom Kern unabhängigen Centrosomas von *Ishikawa* angegeben worden, und auch hier handelt es sich nur um eine Vermuthung. Wo sonst Protozoenkerne genauer auf ihre Theilung hin geprüft worden sind, hat sich herausgestellt, dass die activen Substanzen, welche die Kerntheilung veranlassen, in Inneren des Kernes liegen, und als Bestandtheile desselben angesehen werden müssen. Ich habe das Gesagte für *Actinosphærium* nachweisen können; bei *Euglypha*, welche nach *Schewiakoff's* Untersuchungen in der Kerntheilung mit *Actinosphærium* sehr übereinstimmt, scheint ein gleiches Verhalten zu herrschen. Am beweiskräftigsten sind aber die Nebenkerne der Infusorien, deren Theilung am auffälligsten unter den Protozoen an die Sporenbildung der Metazoen erinnert.”

In *Actinosphærium eichhorni* and in *Spirochona* it is true that the kinetic substance of the nuclear division—the archoplasm—lies within the nucleus. In the micronuclei of all the Infusorians, as far as they have been studied by many eminent investigators, there is as yet no case known where extranuclear kinetic centres are proved to exist. The state of things appears, however, to be a little different in *Euglypha alveolata*, where the *Polkörperchen* lie in small concavities of the nuclear membrane at two opposite poles of the nucleus. Of the origin of the *Polkörperchen*, *Schewiakoff* (32, p. 222) says:—

“Verfolgt man aufmerksam seine Entstehung, so kann man die Vermuthung aussprechen, dass es, wenn auch theilweise, aus dem sich differenzirenden Cytoplasma gebildet sind.” It, therefore, appears

that the kinetic centre—the *Polkörperchen*—of *Euglypha* is derived from the cytoplasm and not from the nuclear substance, just as in the case of *Noctiluca*. But while in *Noctiluca* the archoplasm appears to remain in the cytoplasm, in *Euglypha* it becomes intermixed with the nucleus, when the division of the latter is completed, as we see from the following words of *Schewiakoff* (32, p. 238):—“Vor allen Dingen wird das ehemalige Polkörperchen vollkommen in die Kernsubstanz eingezogen; man gewahrt nichts mehr von den linsenartigen Hervorstülpung; wie es noch in den kurz vorhergehenden Stadien der Fall war, sondern der Kern erscheint einheitlich und besitzt eine regelmässige glatte Oberfläche.” It thus appears, that the *Polkörperchen* here are formed from the cytoplasm at the beginning of the nuclear division, and become drawn into the body of the nucleus at the end of it. The *Polkörperchen* is therefore not a permanent body, but has always to be formed anew from a part of the cytoplasm at the beginning of each nuclear division. As I have no ground to doubt the accuracy of the very beautiful observations of *Schewiakoff*, I have only to conclude that the fate of his *Polkörperchen* is not exactly the same with that of the archoplasm of *Noctiluca*, nor do I doubt the observations of *Hertwig* and others of the kinetic centres lying within the nucleus. From all this it appears that the above statement of *R. Hertwig* as regards the position of the kinetic substance in Protozoa and Metazoa, holds true; that in Protozoa this substance lies within the nucleus and that in Metazoa it lies outside it. But it appears also that there is considerable fluctuation within the Protozoa. Of special interest in this connection is the position of the archoplasm in *Noctiluca* and in *Leptodiscus*—the two relatives of *Cystoflagellata*: for while in *Leptodiscus* this substance lies within the nucleus, in *Noctiluca* it lies outside it. *Cystoflagellata* thus forms, in this res-

pect, an intermediate stage, so to speak, between the Metazoa and other Protozoa. If in *Euglypha alveolata* the *Polkörperchen* is formed from the nuclear substance and becomes connected with the cytoplasm during the nuclear division, to be again withdrawn into the nucleus, it forms in that case an interesting connecting link between *Leptodiscus* and *Noctiluca*! But we must confine ourselves to facts only.

Another interesting thing about the *Noctiluca* archoplasm is its extreme similarity with the *Neben Kern* of *von la Valette St. George* (13, 14), and *Platner* (29). Just like the archoplasm of *Noctiluca*, the *Neben Kern* of these authors is formed by the consolidation of a part of the cytoplasm at one side of the nucleus, and when this becomes visible the "Schleifenbündel des Kerns ist stets mit der Spitze gegen jene Verdichtung hingerrichtet" (*la Valette* 13, p. 7). The part played by the *Neben Kern* appears, however, not quite clear, but *von la Valette St. George* considers it very probable that the spindle fibres are formed from its elements in *Blatta*, since these transform again to form the *Neben Kern* (13, p. 10). When this fact is proved, then the parallelism between the *Noctiluca* archoplasm and the *Neben Kern* of *von la Valette St. George* will be proved beyond all doubt. It will not be, however, a rash conclusion to consider these two bodies as identical, especially when we consider the fate of the *Neben Kern* in the formation of the spermatozoa, as will be seen later on. The close similarity between the two will also be seen by comparing *von la Valette's* Fig. 6 on Plate III of "*Kölliker's Festschrift, 1887*" with my Figs. 1, 2, 6, or 7.

Of not less interest than the archoplasm is the origin of the spindle-fibres. Two different opinions are here also to be met with as in the case of the origin of the kinetic centre. *Strasburger* in his earlier works (33, 34) as well as in his newest (35, p. 59-62), has shown in

many vegetable cells the cytoplasmic origin of the spindle fibres. In *Spirogyra polytaniata* a part of the cytoplasm—the ktoplasm—differentiates itself in the form of parallel fibres on both sides of the flattened nucleus, even when the nuclear wall is very plainly to be seen. Meanwhile the nucleus becomes flatter than before, and the chromosomes arrange themselves in the equatorial plane. The parallel fibres come now to be seen distinctly within the nucleus on both sides of the equatorial row of chromosomes. The fibres are, according to *Strasburger*, the same fibres as were seen outside the nucleus and have pushed into the nucleus through the sieve-like pores formed in the nuclear wall, and attached themselves to the chromosomes. The nuclear membrane disappears after this, and a perfect spindle with the equatorial row of chromosomes is formed (34, p. 11). In the nuclear division of the protoplasmic *Wandbeleg* of the embryo-sac of *Leucjum æstivum*, the same author describes the formation of the spindle out of the cytoplasm surrounding the nucleus at the time when the nuclear wall still exists. In the pollen-mother-cells of *Lilium bulbiferum* (36, p. 182-183), *Strasburger* describes the formation of the spindle as like that in *Spirogyra*. Here twelve fibres are thrown out from both the *Centrospheres* towards the centre of the nucleus and push into its interior, and then, the nuclear wall becoming indistinct, a perfect spindle is formed by the union of the free ends of the fibres at the equator. In his latest paper (36) *Strasburger* still holds his views as to the cytoplasmic origin of the spindle-fibres for plant cells. On the other hand, *Pfitzner* (28, p. 655), *Rabl* (30, p. 269), *Zacharias* (39, p. 852; 40, p. 334), *O. Hertwig* (22, p. 163), and others, believe in the formation of the spindle fibres out of the nuclear substance. *O. Hertwig* on this point says (22, p. 163):—“Bei einzelnen Mollusken (Pterotrachea, Phyllirhoë) haben Fol und ich beobachtet, dass die Polspindel im

Innern des Keimbläschens, welches hier übrigens von geringer Grösse ist, angelegt wird, so lange noch die Kernmembran vorhanden ist. Die Annahme, dass in diesem Fall Protoplasma von aussen in den Kernraum hineingedrungen sei, will mir wenigstens als eine gezwungene erscheinen." While thus the views of *Strasburger* and *Hertwig* are quite different as to the origin of the spindle fibres, the former deriving them from the cytoplasm and the latter from the nucleoplasm, the opinions of *Flemming* (12, p. 75), *Platner* (29, p. 70), and others stand midway between, since according to these authors the equatorial part of the spindle is formed from the nucleoplasm, and the polar parts from the cytoplasm. The late investigation by *August Brauer* (4) of the spermatogenesis of *Ascaris megalocephala* is very interesting on this point, inasmuch as the origin of the spindle fibres is different in two varieties of the same species. In *Ascaris megalocephala univalens* the centrosome is found in the nucleus, and there forms a small spindle which this author compares with the central spindle of *Hermann*, although the origin of that is different, as will be shown later on. In *Ascaris megalocephala bivalens*, on the other hand, the centrosome is first found in the cytoplasm, closely attached to the nuclear wall. By the division of this is also formed a small spindle lying first tangentially to the surface of the nucleus, but the two centrosomes gradually separate from each other until they come to lie diametrically opposite to each other. What becomes of the central spindle fibres is not known, but the author is inclined to think that these are divided in the separation of the centrosome (4, p. 181).

Of no less interest than the above are the observations of *Hermann*, the result of whose investigation on the spermatocytes of *Salamandra maculata*, specially directed to the study of the origin of the spindle fibres, remarkably accords with that of mine upon the

Noctiluca spindle. In the cytoplasm of the spermatocyte at some distance from the nucleus, there is formed a small spindle, from the poles of which, when it has attained a certain size, a number of fibres is seen passing to the chromosomes. By growth and change of position, it gradually attains the ordinary form of the spindle with equatorial chromosomes. In this full grown spindle there are thus two sets of fibres: the one continuous between the two centrosomes and lying axially; and the other passing from the centrosomes to the chromosomes. The latter set of fibres therefore lies more or less like a mantle above the other set, which he calls the *central spindle* (20, p. 580). When division of the cell is completed the fibres of the central spindle return to the protoplasm (?), while the other fibres collect together to form the archoplasms of the daughter cells. This description of *Hermann's* stands thus in beautiful correspondence with that of the formation of the spindle in *Noctiluca*, given above; but with the difference that in *Noctiluca*, from the persistence of the nuclear wall, the central spindle does not lie, strictly speaking, in the axis of the whole system, but in the groove formed on one side of the nucleus by the depression of its wall, and therefore the mantle-like fibres as well as the chromosomes do not lie quite around the axial spindle. This otherwise exact correspondence of the spindle fibres in these two widely different animals is, beyond all doubt, a very interesting phenomenon, and renders desirable, in my opinion, further investigations into the structure and formation of different kinds of cells in other animals and perhaps also in plants.

In this connection I must not leave unnoticed the question of the *Verbindungsfäden*, of whose origin there are also as many interpretations as there are questions on other matters. Many investigators, such as *O. Hertwig* (21, 22), *Boveri* (3), *Ed. van Beneden* and *Neyt* (1), and

Brauer (4), consider the *Verbindungsfasern* to be formed from the *linin*-substance by the separation of the chromosomes towards the poles of the spindle. According to *Strasburger* (36, p. 183), these are formed from the same substance as the kinetic fibres, which enter from the poles of the spindle and meet in the equatorial plane. This view accords well with that of *Hermann*, in so far that the latter author considers the central spindle as forming the *Verbindungsfasern* of other investigators. In spite of the great similarity between the structures of the spindle in *Noctiluca* and *Salamandra* this view of *Hermann's* concerning the *Verbindungsfasern* can not be applied in explanation of the same structure in the *Noctiluca* spindle, since, as we see from the above description, the persistence of the nuclear wall in *Noctiluca* naturally shuts off all possibility of confounding the fibres of the central spindle with those extending between the free ends of the separating chromosomes. Moreover, the two sets of fibres optically appear quite different from each other, as will be seen in Fig. 37, (*c.f.*) and (*r.f.*).

Of the origin of the radial fibres in *Noctiluca*, I can say but a few words, since the whole problem still remains very obscure and requires a thorough study with better methods and optical instruments.¹ In sections given above, these fibres, which are found within the nucleus and probably attached to the chromosomes, appear to come into close juxtaposition, but not to be continuous with those without, *i.e.*, those seen within and without the nucleus appear to be different from each other, the former originating from the nucleoplasm and the latter from the cytoplasm, just as *Brauer* thinks concerning the formation of the spindle fibres of *Ascaris megalocephala bivalens* (4, p. 182-183).

1. It may be here remarked that our climate is not fitted for the use of Zeiss apochromatic systems, the dampness of the air in the warm season soon bringing considerable alterations in the lenses.

Last of all, the part played by the archoplasm in the formation of the cilia in *Noctiluca* deserves some attention. *Strasburger* in his *Histologische Beiträge*, Heft IV., gives many similar cases of the formation of the cilia from the archoplasm (35, p. 62-132; 36, p. 184). The observations of *Henking* (17, 18), *Platner* (29), and above all of *von la Valette St. George* (13, 14), of the part played by the *Nebenkern* in the formation of the spermatozoons are very interesting in this connection, since the *Nebenkern* of these authors (especially of *von la Valette St. George*) corresponds, as said above, in all particulars with my 'archoplasm'; and it is, therefore, very reasonable *a priori* to consider that that part of the cytoplasm which is especially differentiated for kinetic functions, is transformed to form a part of the tail of a spermatozoon or the flagellum of a *Noctiluca* spore.

C. Summary

1) The division of the animal is preceded by the loss of the peristome, teeth, and the tentacle, the last of which is not thrown off, as *Robin* is inclined to think, but is withdrawn into the body of the animal. The mouth and the *Staborgan* are, however, always present (*Robin*).

2) The spore-forming individuals differ from the dividing ones in not possessing the mouth and the *Staborgan* in addition to the organs above mentioned, and by the excessively empty appearance of the cell interior (*Cienkowsky*).

3) The division of the nucleus is always preceded by the concentration of part of the cytoplasm in the form of a spherical or oval granular body, mostly close to the nucleus. This is the archoplasm or the kinetic centre of division, and corresponds most probably with the *Nebenkern* of *von la Valette St. George*.

4) In living animals at the stage of (3), the nucleus appears more

or less homogeneous and transparent, and is not so distinctly to be seen as the archoplasm. But treated with reagents, the chromosomes come into view distinctly.

5) Each chromosome consists of a row of disc-shaped microsomes irregularly scattered in the nucleoplasm. The number of the chromosomes is not clear, but in most cases has been counted to be ten.

6) The chromatin substance of each of the microsome discs collects at the periphery and forms a microsome-ring.

7) In the nucleus of a dividing animal, each microsome-ring splits into half-rings thus dividing a chromosome in halves, while in that of the spore-forming animal two successive divisions of a microsome-ring take place, so that a single chromosome is directly divided into four daughter ones.

8) The chromosomes collect on that side of the nucleus which is nearest to the archoplasm, and spread out towards the other pole. The pole where the archoplasm lies thus corresponds to *Rabl's Polfeld* and the other pole to his *Gegenpol*.

9) The archoplasm divides and forms a very large spindle which first lies tangential to the surface of the nucleus. This division of the archoplasm is succeeded by the separation of the chromosomes into two groups each attracted (?) by its respective archoplasm.

10) The archoplasmic spindle thus formed pushes-in the nuclear wall on which it lies, and the nucleus assumes in consequence a half-ring form.

11) By the separation of the archoplasms, a spindle is produced which in all essential characters appears like the form known as the "disaster stage," with a large archoplasmic mass at each end of the spindle.

12) The fibres of this spindle are therefore continuous from one pole to the other and lying outside the nuclear wall become in no way

connected with the chromosomes. But there is seen at this stage another set of fibres running from the centre of the archoplasm to the polar ends of the chromosomes. This structure of the spindle corresponds exactly with that of the spermatocyte of *Salamandra maculata*, as investigated by *Hermann*, with only the difference of the persistence of the nuclear wall in *Noctiluca*, and the necessary modification in consequence of this fact. The optical appearance of these two kinds of fibres is different, just as in *Salamandra*.

13) Besides these two sets of fibres, the *Verbindungsfäden* are clearly to be recognised extending between the separating chromosomes.

14) The central spindle fibres originate from the archoplasm, the radial fibres probably from both the cyto- and nucleo-plasms, and the *Verbindungsfäden* from the *linin*-substance.

15) In the spore-buds the archoplasm is to be seen lying close to the nucleus up to the time of the full development of the spore just before its detachment from the mother animal, and a part of it becomes transformed into the flagellum, just as in many vegetable swarm-spores, as *Strasburger* shows.

16) In the centre of the archoplasm is generally seen a centrosome, which often shows a dumb-bell form. Sometimes, however, two centrosomes are found in the archoplasm of the spore-forming cells. In many cases, again, there is found in the centre of the archoplasm a number of small oval, rod-shaped, or curved bodies, staining exactly like centrosomes, instead of one or two centrosomes. These may represent the group of centrosomes of *Heidenheim*.

17) The origin and the fate of the centrosome are not known. In a few instances it appears to be formed from the nucleus.

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Explanation of Plates, XI-XIV.

<p><i>a</i> — archoplasm. <i>a sp</i>—archoplasmic spindle. <i>cf</i> — central fibres. <i>n</i> — nucleus. <i>rf</i> — radial fibres.</p>		<p><i>t</i> — tentacle. <i>x</i> — median part of the archoplasmic spindle left after the division of the nucleus.</p>
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$\frac{2}{B} \times Z$ = Drawn by means of camera lucida under Zeiss' objective B with eye-piece 2 : magnified 60 diameters.

$\frac{I}{III} \times S$ = Drawn by means of camera lucida under Seibert's objective III with eye-piece 1 : magnified 120 diameters.

$\frac{2}{1/12} \times Z$ = Drawn by means of camera lucida under Zeiss' homogeneous immersion $1/12$ with eye-piece 1 : magnified 350 diameters.

$\frac{4}{1/12} \times Z$ = Drawn by means of camera lucida under Zeiss' homogeneous immersion $1/12$ with eye-piece 4 : magnified 650 diameters.

Plate XI.

(Division of the animal.)

Fig. 1. Individual preparing to divide; tentacle (*t*) much reduced in size; archoplasm (*a*) very plainly visible as a granular spherical body lying close to the nucleus (*n*). Killed with Flemming's stronger fluid, and stained with Böhmer's hæmatoxylin.

Fig. 2. Another individual of about the same stage as that of fig. 1; in centre of archoplasm a centrosome surrounded by clear space. Similarly treated as above.

Fig. 3. Central plasm of a dividing individual with archoplasmic spindle (*a sp*) lying close to the nucleus; chromosomes gathered towards pole of nucleus

where spindle touches it. Acetic acid methyl-green preparation.

Fig. 4. More advanced stage of division; nucleus now dumbbell-shaped with archoplasms at its poles; narrow line of protoplasm marking line of division of animal seen in front of and behind central protoplasmic mass; the latter also constricted into two portions. Acetic acid methyl-green preparation.

Figs. 5-9. Different stages of division, showing various positions of archoplasm in regard to those of nucleus. All acetic acid methyl-green preparations.

Plate XII.

(Figs. 10, 11, & 12 represent *division*; Figs. 13-19, *spore-formation*.)

Figs. 10 and 11. Further stages of division; tentacles already at stage of fig. 11. Killed with Flemming's stronger fluid, and stained with Böhmer's hæmatoxylin.

Fig. 12. Portion of dividing individual with narrow protoplasmic connection (*p c*) still visible; tentacles grown much longer. Drawn from a living animal.

Fig. 13. First stage of spore-formation; archoplasm (*a*) as a granulated mass lying close to nucleus (*n*); dumbbell-shaped clear space within archoplasm, which represents clear zone around a dividing centrosome. Drawn from living animal.

Fig. 14. Further stage of spore-formation about the same as that of fig. 4. Acetic acid methyl-green preparation.

Fig. 15. Still further stage of spore-formation; division of nucleus now nearly at an end, parts still connected by narrow bridge. Acetic acid methyl-green preparation.

Fig. 16. Central plasm and dividing nucleus at stage similar to that of last figure; drawn from living animal.

Fig. 17. Four-nucleated stage of spore-formation; archoplasms belonging to nuclei in state of division, each forming large spindle. Drawn from acetic acid methyl-green preparation.

Fig. 18. Side view of stage slightly later than that of fig. 17. One of the four nuclei, on right-hand of figure already divided into two daughter nuclei. (The two dumbbell-shaped nuclei, not represented in the figure, are also in a similar

stage of division to that represented on the left of the figure). Acetic acid methyl-green preparation.

Fig. 19. Stage similar to that of fig. 18, showing line of partial division of animal. Picro-acetic acid preparation without staining.

Plate XIII.

(Figs. 20-30, represent *spore-formation*; Figs. 31-37, *division*.)

Fig. 20. Stage of sporulation more advanced than that of fig. 19, showing also line of partial division of animal. (It will be observed that the number of the nuclei in each half is not equal, four lying on one side and two on the other). Acetic acid methyl-green preparation.

Fig. 21. Stage of spore-formation still later than above, showing also line of partial division, six nuclei lying on one side and four on the other. Acetic acid methyl-green preparation.

Fig. 22. Stage with fifty-one nuclei seen laterally; spore-cells on the other side represented by fainter shading. Acetic acid methyl-green preparation.

Figs. 23-26. Sections of spore-buds in last stage of division. Killed with Flemming's stronger fluid and stained with combination of Böhmer's hæmatoxylin and rubin.

Figs. 27-30. Ripe spores just before detachment from the mother-body. Killed with Flemming's stronger solution and stained with carbolic acid fuchsin.

Fig. 31. Part of an individual just preparing to divide; very large archoplasm close to nucleus; tentacle and teeth still visible. Killed with Flemming's stronger solution and stained with Böhmer's hæmatoxylin.

Fig. 32. Nucleus of individual preparing to divide. Killed with Boveri's picro-acetic acid, and stained with acid-fuchsin and methylen-blue.

Figs. 33 & 34. Nuclei of two dividing individuals, in which the longitudinal division of chromosomes are beautifully to be seen. Killed with Flemming's stronger fluid, and stained with acid-fuchsin and methylen-blue.

Figs. 35 & 35. A. Nucleus in process of division as seen at two different levels: fig. 35 at level of archoplasmic spindle, and fig. 35. A. at a lower level; centrosome within the archoplasm on the lower side. Killed with Flemming's stronger fluid and stained with Böhmer's hæmatoxylin.

Fig. 36. Stage like the last drawn at the level of archoplasmic spindle. CENTRAL- and RADIAL-FIBRES beautifully seen; owing to accumulation of dirty matters over archoplasms, centrosomes not to be recognised. Killed with Flemming's stronger fluid and stained with Böhmer's hæmatoxylin.

Fig. 37. Section of nucleus in division at about same stage as that represented by fig. 35 or 36; RADIAL-FIBRES in the homogeneous mass of nucleoplasm at lower pole of nucleus, different appearance from CENTRAL-FIBRES. Killed with Flemming's stronger fluid and stained with Böhmer's hæmatoxylin.

Plate XIV.

(Figs. 38-43, *division*; Figs. 44-52, *spore-formation*.)

Fig. 38. One of the nuclei of a dividing individual just after division; chromosomes still radiating from pole where archoplasm lies; small centrosome lying close to nucleus. Killed with Flemming's stronger fluid and stained with Böhmer's hæmatoxylin.

Fig. 39. Stage same as fig. 38. Centrosome surrounded by clear space at centre of upper archoplasm; in lower archoplasm hidden below nucleus and therefore not well seen; part of CENTRAL-SPINDLE in shape of a diagonal figure (*x*) between the separating nuclei. Killed with Boveri's picro-acetic acid solution and stained with Böhmer's hæmatoxylin.

Fig. 40. Another nucleus in anaphasis; microsomes plainly visible. Killed with Flemming's stronger fluid and stained with Böhmer's hæmatoxylin.

Fig. 41. Nucleus at stage slightly later than that of fig. 40. Microsomes no longer in rows, but more or less irregular and thus assuming the position in the resting nuclei. Killed with Flemming's stronger fluid, and stained with acid-fuchsin and methylen-blue.

Fig. 42. Similar nucleus treated with Boveri's picro-acetic acid and stained with acid-fuchsin and methylen-blue; microsomes very much swollen by action of acetic acid.

Fig. 43. Nuclei of a dividing individual at about the stage fig. 7 or 8. Killed with Flemming's stronger fluid and stained with acid-fuchsin and methylen-blue.

Fig. 44. Archoplasm and nucleus of spore-forming individual at about same stage as that represented by fig. 13; the two centrosomes plainly seen at side of nucleus within archoplasm. Killed with Flemming's stronger solution and stained with acid-fuchsin and methylen-blue.

Fig. 45. Similar stage to above. Single centrosome surrounded by a clear space and many fine fibres seen in the archoplasm next to nucleus; chromosomes gathered up at pole of nucleus facing archoplasm. Killed with Flemming's stronger fluid, and stained with acid-fuchsin and methylen-blue.

Fig. 46. Similar stage to fig. 45. Here the archoplasm lies at a lower level than nucleus. Treated as above.

Fig. 47. Nucleus of another individual at about same stage as above. Many of the chromosomes showing very plainly longitudinal divisions into four rows of microsomes. Treated as above.

Fig. 48. Two spore-buds in division; represented more highly magnified in fig. 48 A and B. Chromosomes within poles of nuclei longitudinally divided already at this stage; many darkly-staining bodies within archoplasms, probably corresponding to group of centrosomes observed by Heidenhein. Killed with Flemming's stronger fluid, and stained with acid-fuchsin and methylen-blue.

Fig. 49-51. Divisions of spore-buds; centrosomes in many of them. Killed with Flemming's stronger fluid, and stained with acid-fuchsin and methylen-blue.

Fig. 52. Abnormal division of spore-bud into three daughter cells. Treated as above.



