Mitotic division of the nuclei of the Cyanophyceae.

By

Edgar W. Olive.

With plates I—II.

One of the most eagerly investigated as well as one of the most confused problems connected with recent cytological researches is that concerning the cell organization of the *Cyanophyceae*. Is there a chromatophore; and if so, what is its nature? Does the cell possess a nucleus, or is it a non-nucleated organism? If the so-called „central body" is a nucleus, then is its division direct or indirect? These constitute the main disputed questions. Even after the exhaustive studies of Fischer (97), Hegler (01), Kohl (03), Wager (03), and others, the real nature of the central body has been regarded by the majority as still open to question.

In attempting a comparison of the relations of the chromatin in the nuclei of many of the lower plants, the writer found that thin sections of various *Cyanophyceae* showed with comparative clearness the internal structure of the cell, and also made quite evident the nuclear nature of the central body. Furthermore, the most modern methods of fixation and staining, as one would expect, have proved entirely successful, contrary to the statements of Hegler, who asserts that the usual methods were not successful in procuring a sharp differentiation. In fact, it has been demonstrated to the writer's complete satisfaction that the Hegler method of fixation itself gave, on the other hand, extremely poor results; and the conclusion has been reached, after much trial, that it is only by means of thin sections, properly stained, that certain important details of the cytology of these minute organisms can be made out.

The writer naturally hopes that in the present paper this difficult question has been brought somewhat nearer solution. As will be seen, however, the subject is regarded as far from closed, especially from a physiological point of view. Two papers have just appeared, the very exhaustive one by Professor Kohl and the long promised one by Mr. Wager, and it has therefore been thought advisable to publish some of the results of this investigation at once, before it is fully completed. Some

Author.	Cyanophycin granules	Slime globules	Coloring matter	Central body
Schmitz (79)	In Gloeocapsa, „slime globules“?			In Gloeocapsa, probably a nucleus.
„ (80)				In Gloeocapsa, microsomes, not a nucleus.
„ (83)				Cyanophyceae without nuclei.
Wille (83)		Nucleoli.		In Tolypthrix, a nucleus.
Reinhardt (85)		Nucleoli.		Nucleus.
Scott (87)				Nucleus, with mitotic division.
Zacharias (87)				Nucleus, with chromatin.
„ (90)	Kind of carbohydrate. „Körner“.	„Centralkörner.“		Probably not a nucleus; „Centralsubstanz“.
Ernst (89)		Nuclei.		Many nuclei.
Borzi	„Cyanophycin granules“. Gelatinous, and starch-like.			
Hansgirg (85)	Paramylum.			
Bütschli (90)		Chromatin: „rote Körnchen“.		Nucleus, with amitotic division.
Deinega (91)	Isomer of starch.		Peripheral chromatophore.	Undetermined.
Fischer (91)				Bütschli's nucleus not a nucleus.
Zukal (92)		Nuclei, forming naked cells.	Peripheral chromatophore.	Many nuclei, with amitotic division.
Dangeard (92)				Nucleus.
Hieronymus (92)	Part of nucleus; chromatin?	Chromatin?	In minute granules.	„Open cell nucleus“.
Zacharias (92)				
Marx (92)	Fatty substance?	Fatty substance?	In minute granules.	Not a nucleus.

Author	Cyanophycin granules	Slime globules	Coloring matter	Central body
Palla (93)	Carbohydrate; first visible product of assimilation.	„Schleimkugeln"; undetermined.	Peripheral chromatophore.	Not a nucleus; homogeneous.
Chodat et Malinesco (93)	Distinguished one kind of granule.			Not a nucleus.
Chodat (94)	Distinguished 3 kinds of granules — cyanophycin, slime globules, soluble starch.			Not a nucleus. Central body is a vacuole, containing these granular substances.
Zukal (94)	Cut off from central body. Can change into slime globules.	Nuclei, out off from the central body.		Formed by fusion of cyanophycin granules and slime globules.
Stockmayer (94)				Not a nucleus; shows „Wabenstructur".
Hegler (95)				Nucleus, with mitotic division.
Nadson (95)	Reserve food granules.	Chromatin granules.	In walls of peripheral protoplasm.	Similar to nucleus.
Fischer (97)	Reserve assimilation products.		Peripheral chromatophore.	Not a nucleus; cytoplasm only.
Macallum (99)				Contains chromatin, but is not a nucleus.
Hegler (01)	Albuminous crystals. Reserve food.	„Schleimvacuolen"; Albuminous-like slime stuff.	In minute granules, „Cyanoplasten".	Nucleus, with mitotic division.
Massart (02)				Not a nucleus.
Bütschli (02)				Nucleus, with mitotic division.
Kohl (03)	Reserve albumen. Crystalloids.	Albuminous slime. „Zentralkörner".	In minute granules.	Nucleus, with mitotic division.
Wager (03)			In minute granules.	Nucleus, with amitotic division.
Lawson (03)				Primitive nucleus.

of the conclusions have already been given in December in a brief résumé before the botanical section of the American Association for the advancement of Science.

I take pleasure in acknowledging my great obligations to the Carnegie Institution of Washington for a grant, by means of which I have been enabled to pursue these investigations in the laboratory of Professor Strasburger. To Professor Strasburger particularly, for his many kindnesses and for his unfailing interest and helpful advice; and to his assistant, Dr. Max Koernike, and to others in the Botanical Institute of the University of Bonn, I am also deeply indebted.

Historical review.

Among the thirty or more who have written on the cell structure of these organisms, it is impossible to find any two writers who agree in all details. Indeed, in seeking to disentangle the literature relating to this subject, one finds that several authors even disagree with their own earlier views. It is, moreover, at times almost impossible to gain from the text an author's exact meaning. For example, it is difficult in the extreme to make certain, when Hieronymus used the expression „the cyanophycin granules represent the nucleus of higher plants", whether he really had in mind the cyanophycin granules or the slime globules, or „red granules" of Bütschli. It is highly probable, however, that he meant the latter. And it is, moreover, not at all easy to follow understandingly Zukal's researches, remarkable for their opacity, and interpret whether his many nuclei in the cells of the *Cyanophyceae* were slime globules or cyanophycin granules; yet these two kinds of granular inclusions are readily distinguished from each other in their staining and chemical reactions as well as in their location in the cell.

In several of the more recent articles on this group, notably in those of Fischer (97), Hegler (01), and Kohl (03), are given excellent reviews of the literature relating to the subject. It has been thought better, therefore, for the purposes of brevity, to present in this paper a table (pp. 2, 3), condensed mostly from Hegler's admirable review, showing very concisely the more important conclusions which have been reached with reference to the principal topics concerning the cell, viz., the cyanophycin granules, the slime globules, the manner of distribution of blue and green coloring matters, and lastly, the nature of that portion with which the writer is in this paper more directly concerned, the so-called „central body". In no other way, it seems to me, can the astounding confusion which prevails be so graphically presented. Where great doubt exists as to the meaning of the author, I have followed my interpretation with a question (?). Later, the views of several writers will be more fully discussed.

Material and methods.

The material for the present research was collected for the most part in green houses, the large *Oscillatoria princeps* from

the Botanical Garden of Harvard University, and the other species from the Botanical Garden of the University of Bonn; or from ponds in the neighborhood of Bonn. Five species of *Oscillatoria*, and one species each of *Phormidium*, *Calothrix*, *Nostoc*, *Gloeocapsa*, and *Cylindrospermum*, have been studied.

But two methods of fixation have been generally employed; viz., Flemming's weaker solution, and Strasburger's modification of Flemming's mixture, sometimes called the „middle solution". The SO_2-alcohol mixture, recommended by Hegler, gives, as he asserts, a sharply marked central body, but I have found that the nuclei so treated are unnaturally shrunken, and that the whole cell is frequently plasmolized (see figs. 2, 3), hence I early abandoned this method. After fixation, the masses of filaments were washed for a few hours in water, then dehydrated by passing through the usual grades of alcohols. They were then carried through chloroform or xylol, successively infiltrated with paraffine melting at 52^0 and 60^0, and finally sectioned 1—4 μ thick. The much employed stains, Flemming's safranin, gentian violet, with or without orange G, and Heidenhain's iron haematoxylin, sometimes followed by eosin or orange, gave most excellent results. For certain purposes, a mixture of methyl blue and eosin, or of methylene blue and eosin, was used; and a few other stains were tried, but none were so satisfactory for the nuclear elements as the two standard stains mentioned above.

In studying the preparations, a glass globe filter and condenser, filled with a light blue solution of ammoniated copper sulphate, was used, in connection with a Welsbach gas lamp. So far as the writer has been able to discover, only three investigators have so far attempted to cut sections of these plants — Hegler, Fischer, and Wager. A thorough examination of the Hegler preparations, loaned me through the kindness of Professor G. Karsten, have convinced me that his sections of *Anabaena* were entirely too thick to enable him to discover from them much that was new. His preparations show, as do also the photogravures illustrating his exhaustive article, a central body which is so deeply stained that chromatic and achromatic substances can not be distinguished from each other. Judging, however, from the text, Hegler must have seen clearly the chromatin granules lying in the achromatic portion of the central body, as his description of them shows.

Fischer's drawings show that his cross sections of *Oscillatoria* must have been excellent and some, at least, well stained. He gives but two drawings of longitudinal sections of *Oscillatoria*, both stained with Delafield's haematoxylin, figure 42 showing the slime globules, or „red granules" of Bütschli, lying imbedded in the central body, and figure 49, showing simply the deeply stained central body alone. It may assist us in finding a reason for Fischer's decision against the nuclear nature of the central body by comparing his figure 36 with figure 18 of

the present paper. Both are cross sections of *Oscillatoria princeps,* drawn with camera to about the same scale. Both are stained with iron haematoxylin and show much the same features. It will be noticed, however, that the vacuoles in Fischer's figure are drawn perfectly round instead of angular and the chromatin granules are relatively too small, facts which I have supposed might be attributable to the carelessness of the lithographer to whom the making of the drawings was intrusted. It is truly remarkable, however, that Fischer did not at least attempt to give an explanation of the minute black granules shown in this figure as well as in figure 37, other than simply to call them „Granulationen". Even a casual glance will show that they are not the same as the relatively much larger granules of his figure 41 b, e. g., or, indeed, of any of his figures of granular inclusions. How the fact that the minute granules shown in figure 36 stain exactly as do the chromatin granules and that they have in addition other characteristics which belong to the chromatin of nuclei, could have escaped so noted an observer as Fischer, is to the writer inexplicable.

Wager, in his recent preliminary paper, shows apparently only one figure which is drawn from a section, and, in the opinion of the writer, this cross section was probably strongly overstained. At any rate, in my own iron haematoxylin preparations, I can find many similar appear, ances, resulting from a failure to wash out sufficiently. In fact I have seen, in my own preparations, satisfactory explanations for such misleading appearances as are shown by Wager in his figure 1, which lead him to the conclusion that the division of the nucleus is direct. And, moreover, in thick, deeply stained sections, one may find similar figures to those given by Kohl — figures 10—12, and 16, of Plate e; 14 and 15 of Plate f; and the most of the figures of Plates i and k, — to prove the opposite conclusion that the division of the nucleus is indirect. It is easy to find, in overstained or badly fixed mounts, such long streaks of blended chromatic and fibrous achromatic elements (see figs. 2, 3) as are shown by the figures of both Wager and Kohl, and which are interpreted by the latter as the chromosomes of a mitotic figure, and by Wager as the chromatin granules of a simple amitotic division. Overstained or poorly fixed preparations and attempts to fathom from without instead of examining from thin sections the internal structure of a cell which contains at least three different kinds of granular inclusions and a protoplasmic structure showing considerable amount of differentiation, must be held in the main responsible for the extreme confusion and conflicting results with which we are confronted.

The coloring matters.

As was first pointed out by Schmitz, in 1879, a close examination of one of the blue green algae reveals the fact that, even in the living condition, we may distinguish two

portions, an outer colored part and an inner colorless part, the latter constituting the so called „central body". Granular inclusions may also frequently be seen, particularly in certain species of *Oscillatoria*, in which they are often arranged on both sides of and parallel to the cross walls. Occasionally, granules of varying size may be observed, which appear to lie within the central portion (fig. 1).

As will be seen from the table on pages 2 and 3, the majority of writers maintain that the blue and green coloring matters are contained both together in minute granules, or plastids, which occur in large numbers scattered through the peripheral portion of the cytoplasm. Fischer and a few others assert, on the other hand, that the color is diffused through the dense peripheral portion of the protoplasm. This hollow cylindrical or spherical part, he calls the chromatophore. The writer agrees perfectly with the statements of Fischer in regard to the color-bearing portion.

One can see, it is true, granules in the living cells; but all the granulations which I have ever seen have proved to be the colorless cell inclusions. In the thinnest and most favorably stained sections, furthermore, both longitudinal as well as cross, I have never been able to detect in the peripheral chromatophore any granulation whatever. (See, e. g., figs. 8, 10, 16. 18. 32). If minute plastids were present, they should certainly be visible in the permanent preparations as well as in the living.

The absolute failure to find „cyanoplastids" in thin sections of any of the six genera studied does not, by the way, preclude the possibility that the chlorophyll and phycocyanin may possibly be in the form of minute globules, which may disappear on treatment with certain reagents. But I have never been able to bring myself to see such colored globules, after many attempts under the most favorable conditions.

In addition to the absence of plastids in stained preparations, the following observations still further strengthen Fischer's conclusions with reference to the peripheral chromatophore. If *Oscillatoria Froehlichia*, e. g., or any other large species, be placed in chloroform water[1]) for one or two days, the blue coloring matter is extracted, and may then be seen dissolved in the water. The chlorophyll alone remains in the cells. A distinct granulation may now be seen, especially in the central portion, as shown in fig. 29. If light pressure be then applied to the cover glass, the cells of the filament may frequently be broken apart and the isolated, flattened cells be turned on end. Fig. 28 represents such a disc-shaped cell, which consists of the somewhat shrunken protoplasm only, the wall having been torn off. The bright green color will be seen in this end view to

[1]) Made according to Hegler's directions by shaking up a small quantity of chloroform in water, allowing it to settle. then decanting the water. which is then used in the experiments.

be confined to the peripheral denser portion, and only in the central colorless (or at times slightly greenish) region will be observed the granules, now distinctly colorless, which we had previously remarked in side view. The color is in this instance not caused by minute green granules, but it appears rather to be due to a uniformly diffused substance, confined to the peripheral, sometimes distinctly fibrous, region which Fischer calls the chromatophore. Such a chromatophore probably finds its parallel in many of the lower algae, in *Ulothrix*, for example, in *Hydrodictyon*, and others.

The granulation in *Gloeocapsa* is clearly visible, both in the living condition as well as in the cell treated with chloroform water. Fig. 36 represents a cell so treated, with much of the phycocyanin still confined in the space between the shrunken cell and the thick, gelatinous membrane. In this condition, it is impossible to say whether the granules which we can see so clearly are green or not. But when the chloroform water is allowed to act for several days, sometimes the wall of the dead cell is broken down (fig. 35), leaving imbedded in the firm gelatinous membrane nothing but the multitude of colorless granules. These granules, which Schmitz called slime globules, are probably, therefore, merely granules of reserve food matter, although the writer is not yet prepared to say that they are cyanophycin.

The central body.

Schmitz was the first, in 1879, to call the central body in the cell of *Gloeocapsa* a nucleus. The very next year, however, (80) he came to the conclusion, after further study, that the minute granules of this central portion were microsomes and that they did not represent a nucleus. This opinion he repeated three years later (83), after studying many *Cyanophyceae*, and he made the general statement that these plants possessed cells without nuclei. Zacharias (87) after his first studies on *Tolypothrix* and *Oscillatoria*, also held the opinion that the central body contains chromatin and that it represents a nucleus, but, later (90), he saw reasons to modify his views and concluded that, although the body in question contains chromatin, it is probably not a nucleus. To this opinion, Zacharias evidently still clings.

Macallum (99) also holds a similar view, that, although he has demonstrated that chromatin is present in the cells of the *Cyanophyceae* and the *Bacteria*, they are nevertheless non-nucleated organisms. On the contrary, Lawson (03) contends that the chromatin granules represent the nucleus, „since every highly organized nucleus passes through a stage in its development when it consists of nothing but chromatin."

Others who believe that the central body is not a nucleus are Marx (92), who, indeed, could see a central body „nur äußerst selten", Palla (93), Chodat (94), Stockmayer (94), Fischer

(97) and Massart (02). Among those who have asserted that it is a nucleus, which, moreover, divides mitotically, are Scott (87), Bütschli (02), Hegler (01) and Kohl (03). Wager (03) believes that the central body divides by direct division; while Ernst (89) and Zukal (94) think that each of the many slime globules represents a nucleus, which divides by simple fragmentation.

The theories of Hieronymus (92), Zukal (94) and Chodat (94) are historically interesting and deserve special notice. Chodat thought that the central portion of the protoplasm of the cyanophyceous cell became vacuolated, or emulsified, and that this appearance, together with the granular contents of the vacuoles — the cyanophycin granules, the slime globules, and the „soluble starch“ — caused the differentation known as the central body. Zukal and Hieronymus have theories which present one point of resemblance to each other. Zukal regarded the slime globules as the true nuclei, which, according to him, divide, form membranes about themselves, and thus represent many „naked cells“ within the one cyanophyceous cell. These nuclei, he says, may be formed in two ways: they are either cut off from the central body or else they are produced from cyanophycin granules, which may be slowly changed into nuclei. And most curious theory of all-the central body is itself formed from the fusion of the cyanophycin granules and slime globules! Thus the central body may on the one hand cut off portions of itself to form cyanophycin granules and slime globules, and on the other hand, it may be itself reformed by fusion of these two kinds of granular substances!

Hieronymus calls the central body an „open cell nucleus“, as distinguished from the „closed“ nucleus of higher organisms. This really means nothing more, in my interpretation, than that the central body is devoid of the nuclear membrane which is characteristic of the resting nuclei of higher plants. Hieronymus says, moreover, that the cyanophycin granules (he probably means here rather the slime globules, or „red granules“) are pushed out from the nucleus, and that they represent the chromatin granules. Herein his theory bears some resemblance to that of Zukal.

We are now prepared to examine more closely the central body, which has occasioned so much confusion and difference of opinion. One of the main arguments of Fischer against the nuclear nature of this body is the fact that it occupies such a large proportion of the space in the cell. It indeed strikes one at first examination that the central body is comparatively large and that the cytoplasmic portion of the cell is relatively small. as is well shown by longitudinal sections of *Oscillatoria* (figs. 7 to 10, 14, 17).

In filaments examined as a whole as well as in sections too deeply colored, the central portion usually stains as is shown in

figs. 2, 3, 11, 13, 77—79. It will be seen by contrasting figs. 8 and 10, e. g., with figs. 11—13, that the central body in the former drawings is made up both of deeply staining chromatin substance, and of an achromatic portion; whereas, in the latter preparations, no such differentiation is clearly visible. Especially in figs. 11, 12, and 13, which are from slides colored with methylene blue, iron haematoxylin, and Flemming's triple stain respectively, the dense central body appears to be homogeneous, as is claimed by Palla. These preparations were simply overstained and not sufficiently washed out. The same is true of figs. 2 and 3, with the difference that the washing out of the stain has been carried on a step further, so that a portion only of the achromatic substance remains deeply stained. This results in dark streaks of chromatin and achromatin, which may sometimes give the appearance of long chromosomes, such as are figured by Kohl and Wager.

In all the forms studied by the writer, including species of *Oscillatoria*, *Phormidium*, *Calothrix*, *Nostoc*, *Gloeocapsa* and *Cylindrospermum*, both chromatin and achromatin could be made out in the central body, in properly differentiated preparations. Two striking peculiarities were at once noted. First, that the achromatic portion appeared to be often made up of an unusually dense substance; and, secondly, that the chromatin granules seemed relatively very minute. Particularly in *Cylindrospermum* were these peculiarities noticable, for it could not be determined, even with the highest available magnification, that the achromatic portion was made up of fibrous protoplasm, as could be demonstrated in nearly all the other cases; and further, the chromatin granules were so minute that they long escaped detection (figs. 80, 82—85, 89, 90).

The extreme density of this kinoplasmic, fibrous mass (called by Palla „Füllsubstanz"), which makes up the bulk of the central body, is probably mainly responsible for the inability of the majority of investigators to detect the chromatin granules enclosed within it. Particularly in cross-sections of the actively dividing nuclei of *Oscillatoria princeps* and *O. Froelichia* can the fibrous nature as well as the density of the achromatin, after careful examination, be made out (note, e. g., the mass in the middle of fig. 18). It will be further seen that, in the longitudinal sections (figs. 10 and 11), the density of the kinoplasm varies, although, in this view, its fibrous nature is not so easily demonstrable.

In all the forms studied, with the one exception of *Cylindrospermum*, the writer has discovered that the number of the minute chromatin granules is constant for the same species. The fact that, in *Cylindrospermum*, the cells are comparatively long may have prevented me finding a cross-section in which the chromosomes, as I have called them, were grouped favorably for counting. For example, in some cross sections, as few as four can be counted, in others, six, or even ten by focussing up and

down (figs. 82 and 83). In *Glococapsa polydermatica* Kützing (figs. 62, 64, 76), and *Nostoc commune* Vaucher, eight chromosomes could usually be counted. In *Oscillatoria tenuis* Agardh fig. 4), *Oscillatoria* sp. (figs. 15, 26, 27), *Phormidium* sp. (figs. 32, 33), *Calothrix thermalis* Hansgirg[1]) (fig. 43), there are sixteen chromosomes.

The cells in *Oscillatoria Froelichia* Kützing and *O. princeps* Vaucher are so short that the central body takes on the form of a flattened disc, appearing in section as shown in the figs. 7—13. The shallowness of the nucleus undoubtedly accounts for the difficulty in determining definitely the number of the chromosomes, since those which belong to the lower group may easily be counted with those in the upper focus. There are, however, in all probability, thirty two in the cells of these two species, although for a long time I thought there were about twice that number (figs. 18, 24).

In those cells in which there are sixteen chromosomes, one may usually count in median longitudinal view three or four (figs. 2, 6, 14, 17, 34, 37); while in the two large *Oscillatorias*, we may frequently see as many as eight in side view (figs. 7, 10). This fact leads one to the conclusion that *Tolypothrix* also has sixteen chromosomes, since both Kohl and Wager show in their drawings about four or five in median section; this opinion is, however, at variance with that of Kohl, who holds that the cells in *Tolypothrix* contain but four to six.

It will be noticed, in most of the drawings of longitudinal sections of the various filamentous forms (figs. 8—13, 17, 34, 37) that there is shown, in some cases much more clearly than in others, minute fibrillar projections from the central body. These are seen in one drawing only (fig. 12) to run from the central body completely to the cross walls. In certain instances in which the bleaching of the stain has been carried a step too far, these fibrils are not at all visible (figs. 6, 7). In fig. 12, the combination of iron haematoxylin with eosin and the failure to wash the stains out sufficiently have resulted in a black, undifferentiated central portion, and a reddish cytoplasm. The fibrillar projections, in this instance, and the thin, delicate cytoplasmic layer lining the cross walls, as well as the peripheral chromatophore, are all stained red with the eosin. We can now observe that the projections are connected at their outer extremities with the lining layer of protoplasm along the cross partition walls, and further, that at the central body end, each is joined with a chromosome (figs. 8, 10, 17, 34, 37). In the spaces between the fibers, as will be explained later, are granules of reserve food, the cyanophycin granules (see fig. 21). There remains no doubt in the mind of the writer that these fibrillar projections represent the mantle fibers, or „Zugfäsern“ of the mitotic figure. They are attached to the wall at the one end

[1]) Kindly determined by Prof. O. Kirchner.

and at the other, they join with a chromosome. The extension of the cell in length by osmotic forces, possibly combined with the actual shortening of the fibers themselves is the probable cause of pulling of the divided chromosomes apart.

It remains, then, to apply to the fibrous portion between the separating chromosomes in figs. 6, 8, 10, 14, 17, 37, the term „connecting fibers", or „central spindle" in order to complete our conception of the achromatic figure, in which we have, especially in the short-celled species of *Oscillatoria*, a „spindle" which is not at all spindle-shaped, but is rather in the form of a more or less thick disc. This disc-shaped central body, as is seen, e. g., in fig. 10, is finally cut in two equatorially by the ring formed wall which grows in from the outer wall; this process will be fully discussed later. It is obvious that a single centrosome would not suffice for such a peculiar, broad-poled figure. As a matter of fact, however, no structures resembling centrosomes have been observed in any of the species examined.

As we should expect in such a long-celled species as *Cylindrospermum*, we find the nucleus also greatly elongated; and here, furthermore, the whole karyokinetic figure has usually the spindle shape seen in the higher plants, instead of the flattened-disc shape of the short celled *Oscillatorias* (figs. 77, 80, 84, 85, 89, 90). Hegler shows in *Anabaena* also central bodies which are similar in form to those of *Cylindrospermum*.

Proof that the process of division is mitotic.

In the foregoing discussion, we have spoken of the dividing central body as a „mitotic figure", and it has been pointed out that this figure possesses both chromatin granules and an achromatic, fibrous substance. Simply showing that chromatin and achromatin are present far from proves, however, that the process of division is one of mitosis; although it would seem that merely the fact that the number of chromatin granules in every cell is constant, should furnish sufficient proof. But if one should judge solely from such appearances as are shown in figs. 2 and 3, and from many drawings given by Kohl and others, we may, in fact, with equal right, decide with Wager that the division is direct.

The nucleus of the *Cyanophyceae* must certainly divide by one of the two methods—either by mitosis or by amitosis. If amitotic, as claimed by Wager and others, then the whole mass of the central body must undergo a simple constriction, and there should be no spindle, and no spireme arrangement of the chromatin. The most essential act accompanying mitotic division, on the other hand, is that the chromatin granules are each split in two, so that the „daughter nuclei receive precisely equivalent portions of chromatin from the mother-nucleus" (Wilson, 1900, p. 70). Usually, moreover, during the preliminary stages of mitosis, the chromatin granules are arranged along a more or less convoluted thread, which, whether continuous or discontinuous,

splits throughout its entire length into two exactly equivalent halves" (Wilson, p. 70). A third attribute of a typical mitosis is the presence of a fibrous, achromatic mass known as a spindle.

It remains, therefore, to be proved, first, that, in the division of the central body of the *Cyanophyceae*, a spindle is present; second, that the chromatin is arranged, at some time during the process, in the form of a spireme thread; and, lastly and most important of all, that the chromatin granules are halved and that an equal number is distributed to each daughter cell.

It is perhaps advisable, at the very outset, to discuss briefly the staining reactions of the chromatic and achromatic elements of the nucleus. While the writer realizes fully that staining reactions should by no means constitute the principal argument in support of the mitotic division of the central body, yet there can be no doubt that a comparison with the well known results already obtained with nuclear stains will be of value. The writer is well aware, further, that staining reactions are frequently misleading; and that the most credible data concerning complex nuclear phenomena are furnished by observing simply the changes which take place. Those most valuable stains, Heidenhain's iron haematoxylin and Flemming's triple stain, when used to check and to supplement each other, in my opinion, assist as perhaps no other stains can, in the interpretation of the complex structures with which we have to deal in the central body. Iron haematoxylin gives generally much the sharper differentiation and is the easier of the two to use; but the objection has been rightly raised that other things than nuclear structures may be stained by it and that, consequently, great care must be employed in drawing conclusions. I have found, moreover, particularly in the case of these algae, that Flemming's triple stain is an exceedingly difficult combination to handle so as to obtain the best results; but, on the other hand, it furnishes us with staining reactions which can scarcely be doubted, in the interpretation of chromatic and achromatic elements of the nucleus.

It is sufficient to say here that the most satisfactory details were obtained with well differentiated iron haematoxylin. The minute black chromosomes stood out, sharply defined, in a bluish, or dark, or at times almost invisible achromatic substance (figs. 8, 18, 26, 27, 32, 34 etc.). Flemming's triple stain gave dark reddish, or purplish chromatic structures which were often poorly differentiated, thus giving an appearance which would lead one to the conclusion that chromatin granules and the achromatic substance were fused together (figs. 7, 10, 13, 17, 25 etc.). The dense, achromatic portion of the central body stained with the triple stain dark bluish, or purplish, or even reddish; whereas the fibers which lead from the chromosomes to the cross walls do not readily take any stain sufficiently to bring them out

sharply throughout their entire length. In either of the two standard stains, one can, however, see them, usually very dimly defined, extending only a short distance from the central body. A general cytoplasmic stain sometimes gives better results with these structures; and, in fact, in the experience of the writer, was actually necessary in showing the fibrils in their entire length.

As has been pointed out above, the dense, fibrous, achromatic portion of the dividing central body between the two groups of separating chromosomes (as, for example, in fig. 8), cannot well be interpreted otherwise than as the „central spindle"; and the fibrils that lead from the chromosomes to the walls appear, at least, to function as mantle fibers. There remains, therefore, in presenting proofs of mitotic division, to discuss the more important phenomena accompanying mitosis, viz., the fission of the chromatin granules and their arrangement in a spireme.

A detailed account of the mitotic division of the nucleus in *Gloeocapsa polydermatica* will be reserved till later, since the process in this species involves certain peculiarities which merit a special discussion.

Hegler (01) says that in *Anabaena*, during the division of the nucleus, the minute chromatin granules fuse with one another to form a „grösseren Verbänden, deren Chromosomennatur an günstigem Untersuchungsmaterial nach Fixieren mit schwefliger Säure und Färbung mittels der angeführten Methoden durch ihr weiteres Verhalten beim Teilungsprozess festgestellt werden könnte" (p. 352). I have never seen any such fusion of chromatin granules to form chromosomes. In fact, if normal, such a process as the union of chromatin granules to form chromosomes should take place early in the formation of the spireme thread. I am certain that a fusion of the chromatin granules does not occur in the spireme thread of *Gloeocapsa*, unfortunately the only instance in which I can speak positively on this point.

In the cells of *Oscillatoria*, we can frequently see nuclei which appear to be in a spireme stage (fig. 7, the lowermost cell: fig. 10, the two middle cells; fig. 14, the four cells at the right: fig. 17, the lowermost cell). But particularly in cross sections. do we find appearances which suggest at once a thickened spireme thread, in which the achromatic and chromatic substances seem to be blended (figs. 25, 42). Moreover, such nuclei as are shown in the two middle cells of figs. 8 and 10 probably represent in section a similar condition to the spireme stage of the nucleus of *Gloeocapsa* seen in figs. 68, 70, 74, and 75. It should be kept in mind, however, that, in *Gloeocapsa*, the spireme is in the form of a simple, more or less spiral thread; whereas, that which we see in section in the case of *Oscillatoria*, has its convolutions disposed in a disc-shaped figure. In both instances, we may see the beginnings of the longitudinal fission of the spireme, resulting in the doubling of the number of chromatin granules. In neither case, however, is there evident a subsequent

splitting of this double spireme into segments to form chromosomes: the process appears to be rather merely a concentration, or rounding up into small particles, of chromatin substance, thus resulting in the formation of minute, spherical, or sometimes irregularly shaped granules, which remain imbedded in the achromatic material. The term „segmented spireme" has been applied to such a condition (Wilson 00, p. 67). We are justified in calling the minute chromatin granules themselves, in such a segmented spireme, the chromosomes, by the fact that their number is constant in the cells of plants of the same species.

It can hardly be doubted that a longitudinal splitting of the chromosomes occurs, although this is a point very difficult to determine with absolute certainty. The middle cells of fig. 8 seem to afford proof for such a conclusion. In the lower of the two cells, the chromatin granule at the extreme left appears to be double, while throughout the rest of the nucleus, there is but one row of granules. In the cell above, this doubling has gone on to a farther extent, so that at both sides of the central body, we can see a double row of dark granules. Even more indisputable evidence is furnished by fig. 13, in which only the undifferentiated, deeply stained nuclear figure is shown. In this figure, in each of the two cells, it will be noticed that the nuclear body is deeply divided equatorially by walls which have grown in from the outside walls. Further, it will be seen that a new splitting is beginning at the edges of the daughter central bodies of the two contiguous daughter cells. In the two farthest separated, we see no such splitting at the ends. Before the wall first formed has completely divided the cell, a new division has thus begun and a new wall is growing in to meet this plane of fission. Such an unparalleled example of rapid division occurs, so far as I have observed, only in the two larger species of *Oscillatoria, O. princeps* and *O. Froelichia.* Careful examination, especially in preparations not so deeply overstained, reveals the fact that a chromatin granule lies at each outer extremity of the splitting portion of the central body, whereas only one such dark body is evident in an undivided end. We are therefore justified in the conclusion that each chromatin granule in a mitotic figure must undergo fission in a plane parallel to the subsequent plane of division of the cell, thus agreeing with the corresponding phenomenon of splitting and separation of chromosomes in higher plants.

Kohl's curious scheme for accomplishing the equal division of his chromosomes (03, Taf. k, fig. 12) does not bear much resemblance to the corresponding process occurring in the higher plants. This scheme fails to provide for a longitudinal splitting of the spireme thread, which, instead, is twice transversely segmented, a phenomenon which, so far as I am aware, has been nowhere else observed in the organic kingdom. The first breaking up of his convoluted thread results in a division into six extremely long straight chromosomes, arranged parallel to the

main axis of the cell; while the second division is also transverse, and results in twelve shorter daughter chromosomes. The two lowermost cells of Wager's fig. 1 show nuclear bodies which are quite similar in many respects to some from which Kohl derived his diagrams to illustrate his scheme of mitosis; an observation particularly interesting from the fact that Wager concluded from such appearances that the division of the nucleus is amitotic. It has already been pointed out in this paper that, in the opinion of the writer, such appearances as are figured by both Kohl and Wager are misleading, and that they have probably resulted from too thick or from overstained preparations. Such an opinion is based upon the fact that the writer has also often obtained many similar results in mounts of *Oscillatoria* and other species, in which the cells were either poorly fixed or in which the stain was not well differentiated, owing to insufficient washing out, or to the section being too thick (figs. 2 and 3).

Division of the cell.

It is said that in *Spirogyra* the partition wall which grows across the cell, thus cutting it in two, appears only after the nuclear division is accomplished. In the *Cyanophyceae,* on the other hand, we apparently have the new ring-formed partitions beginning to grow in from the outer walls long before nuclear division is fully completed. The striking example has already been mentioned, how in *Oscillatoria princeps* and *O. Froelichia,* division may take place with such wonderful rapidity that we may have, in one cell at the same time, as many as three ring-formed walls in different stages of growth (figs. 11, 13). In this instance, long before the two daughter nuclei are completely severed from each other, the daughters themselves have begun to divide mitotically. Wager (03) also has noted the fact that several divisions may go on at the same time and he further points out that the division of the cell appears to go on independently of the nuclear division. Figs. 6—9, 14, 17, 34 show the usual condition, in which the cells are completely cut in two before a second division wall begins.

Close examination further reveals the highly interesting fact that in the filamentous forms, the division of the cells takes place with more or less rhythmic regularity. According to Macfarlane (01), a similar wave-like rhythm of division activity has been observed in *Spirogyra.* Fig. 9 is a camera drawing of *Oscillatoria Froelichia,* showing clearly this phenomenon. In this figure, three maxima are indicated, at *a*, *d*, and a third at *bc*, points at which division has progressed the farthest. Two cells below *a* is a central body in which division is least advanced. and half way between *c* and *d* is another point of minimum advancement. At both *b* and *c* are cells which have completed division, the two daughter cells at *b* having apparently finished this act sooner than the two at *c*. In the young nuclei shown

in the four cells at *b* and *c*, although division of the cell has evidently been just accomplished, we can see evidences in the double row of chromatin granules in each that mitotic division has already begun, if not, indeed, already completed. Figs. 7, 8, 10, 13, 14, and 17 also show that division has advanced farther in certain cells than in their immediate neighbors. Fig. 13 is particularly interesting in this connection in that the two contiguous daughter halves have begun to divide, whereas the two nuclear halves uppermost and lowermost in the drawing do not yet show any evidence of fission. It is possible that the distribution of food supply has something to do in causing these rhythmic centers of division.

The nuclear membrane.

It is truly remarkable that, in the actively vegetating filaments of the Cyanophyceae, the nuclei appear to be continually dividing, without entering upon a resting condition. At least, none of the many investigators who have studied the group have been able thus far to find a resting state in which the nucleus forms a membrane and nuclear vacuole as it does in the nuclei of the higher plants. The absence of a nuclear membrane has been claimed by many to be evidence against the nuclear nature of the central body; but, in the opinion of the writer, this membrane does not carry with it such weight, since its absence in this instance is probably due to ã lack of a resting condition prolonged enough in which to produce it. I believe, in fact, that if we could make the cells of *Oscillatoria* rest from their activities sufficiently long, the nuclear membrane and sap cavity, as well as other attributes of the resting nucleus, would be formed. Indeed, as will be explained later, this membrane is actually formed in spores and heterocysts, and possibly even sometimes in active vegetative cells.

Hieronymus (92) thought that the central body is „open", that is, not separated from the cytoplasm by a membrane; hence, he called it an „open cell nucleus". Palla (93), on the other hand, who thought that the whole central portion was homogeneous, says that there is always a colorless membrane between the central body and the peripheral chromatophore. Hegler (01) asserts that such a membrane is not present; while Wager (03) says that, although there is no nuclear membrane, in young cells the central body is often limited towards the cytoplasm by a „vacuolar membrane", which may possibly represent at least a rudimentary nuclear membrane" (p. 407). Kohl (03) remarks that there is wanting in these plants a „deutlich färbbare Kernmembran" (p. 184). Lawson (03), in an article on the nuclear membrane, could find in the Cyanophyceae and Bacteria neither membrane nor karyolymph. It is strange that this writer has overlooked the fact that Strasburger (82), over twenty years ago, anticipated four out of his six conclusions. In giving some of the results of his work with the resting nucleus, Strasburger

says (p. 94) „Dieser Knäuel liegt in einer mit wässerigem Kernsaft erfüllten Kernhöhle. Die Kernhöhle wird durch die Kernwandung abgeschlossen, welche eine Hautschicht des umgebenden Cytoplasma ist". In all the Strasburger text books appears this same idea with respect to the nucleus.

Proceeding on the theory that the lack of the nuclear membrane was due to the continuous vegetative activity of the cells, the writer has tried to produce the membrane by drying up the plants and by starving them. Neither of these trials has proceeded far enough to warrant any definite conclusions as to the success of the experiments. Cultures of *Oscillatoria tenuis* were allowed to lose their moisture slowly, as so often happens to these plants in nature. When thoroughly dry, the filaments were fixed at once, dehydrated, imbedded and sectioned. Fig. 2 shows a section of such a filament in which the central body is somewhat overstained. No nuclear membrane can here be seen. The visible effect of drying appears to be rather a shrinkage of the cytoplasm from the walls, as well as a general contraction of the whole nuclear body. It is well known that the *Cyanophyceae* possess a wonderful power of resistance to dessication and other adverse conditions. It would, indeed, be interesting could it be definitely proved that, in a dried condition, the nuclei of these plants do not themselves enter a special resting state, but that they instead continue to carry on as long as possible their mitotic changes, only ceasing when moisture fails. They could then resume at once their interrupted activities on the return to favorable conditions.

The experiment of starving the filaments of *Oscillatoria* by leaving cultures for a week and more in the darkness was equally unsuccessful in producing a clearly defined nuclear membrane. It is probable that the plants were not left long enough in the dark, for in filaments left there for one week, cyanophycin granules were still abundant, thus showing that stored food was still to be had in plenty. Both Hegler and Kohl say that the cyanophycin disappears after a few weeks in darkness. It is highly probable, then, that starvation and the consequent cessation of mitotic activities would not be evident for some weeks.

Even under normal conditions, the nuclei in the vegetative filaments of *Oscillatoria* sometimes seem to begin, at least, to form a resting nucleus. Such appears to be the case in the lowermost cell in fig. 6, in the most of the cells in fig. 14, and in fig. 16. Such a cross section as is shown in fig. 16 in which the nucleus seems to have a well defined, limiting membrane as well as a sap cavity, was but rarely met with. Usually the cross sections appear as shown in fig. 18. I could not, unfortunately, make certain of nuclear cavity and membrane in figs. 6 and 14, although the resemblance to such structures was indeed very striking.

The absence of a nucleolus in the *Cyanophyceae* is also given by some as proof that the central body is not a nucleus; while

others say that its absence, along with that of the nuclear membrane is only proof of the primitive nature of the cyanophyceous nucleus. Wager, however, says that. under certain conditions, the chromatin substance of the central body is found condensed into a single deeply stained granule suspended by delicate fibers in the center of the cell. I have not seen such a structure as he describes in the forms which I have examined, but, in the resting nuclei of spores and heterocysts, some of the chromatin granules are sometimes larger than others and might readily pass for nucleoli. Many nucleoli among the lower plants and the Protozoa are undoubtedly merely large masses or granules of chromatin; and, if we could produce by experiment resting nuclei in the vegetative cells of the *Cyanophyceae*, it is possible that we would have also in these plants such a concentration of chromatin substance.

The resting nuclei in spores and heterocysts.

The nuclei of spores and heterocysts are of special interest in that they furnish another point of evidence in support of the conclusion that there is no wide and unsurmountable difference between the nuclei of the Cyanophyceae and those of higher plants. It will be seen that the nuclei in figs. 49 and 91, representing young heterocysts of *Calothrix* and *Cylindrospermum* respectively; as well as the nuclei shown in fig. 58, a spore of *Nostoc*; and in 93, a spore of *Cylindrospermum;* and especially those in figs. 100—103, cross sections of young spores of *Cylindrospermum*, resemble closely the resting nucleus with which we are familiar. A cavity, in which we see chromatin granules, and a more or less clearly defined, delicate, nuclear membrane, now contribute to the resemblance, which was lacking in the vegetative stages.

Both Hegler and Kohl come rightly to the conclusion that the cell contents of heterocysts become finally disorganized, and that the nucleus, chromatophore, cyanophycin granules, and slime globules gradually disappear. In fig. 77, which is stained with methylene blue, each of the vegetative cells of the filament of *Cylindrospermum* shows one or more slime globules, the spore one only, while the young heterocyst has two exceedingly minute, reddish slime globules in a bluish background. When older, we find in heterocysts no indication whatever of any granular contents, except the disorganized chromatin of the dead nucleus (note the heterocysts of figs. 37, 38, 50, 53, 80, 88). It is highly important to note, however, that in the young heterocysts of *Calothrix* and *Cylindrospermum*, before disorganization occurs, the nucleus apparently begins to enter a normal resting condition and to form a nuclear vacuole.

The mature spore of *Cylindrospermum* shows one remarkably curious feature, which to the writer remained for a long time an inexplicable puzzle. It will be noted that in fig. 77, a half matured spore of *Cylindrospermum*, the multitude of cyanophycin

granules seem to be in the cytoplasm, and to lie outside the bluish, irregularly defined central body. In fig. 94, which shows a fully matured spore of the same species, similarly stained with methylene blue, the dense outer cytoplasmic zone seems, on the contrary, to be free from granules, which now appear wholly within the central portion. Fig. 93, stained with iron haematoxylin and eosin, is a longitudinal section of a very young spore, in which the cyanophycin granules are stained red, and are seen to be located only in the cytoplasm. Fig. 100 is a cross section of a half matured spore, showing large and abundant red granules, also in the cytoplasm alone. Figs. 101—103 are cross sections of young spores, all stained with Flemming's triple stain, and all showing a well defined resting nucleus; while figs. 96, 97, and 99 are similar sections of old spores. similarly stained. Finally, fig. 95, a median longitudinal section of a mature spore, should be noted. A careful comparison of these figures leads us irresistibly to the conclusion that while. in the young spores, the nucleus appears to begin to enter upon a normal resting state, in the older spores, the abundant cyanophycin granules have encroached so upon the middle, sap-filled, nuclear cavity, that they are finally forced into it and fill the nuclear space. Figs. 96, 97, and 99 all show clearly the unstained, globular spaces in which lie the cyanophycin granules, some of which in the two latter figures are located still in the cytoplasm and others within the nucleus. In figs. 95 and 96, all the food granules appear to lie within the limits of the nucleus. In the preparations from which these latter drawings were made, the chromatin is stained dark reddish or purplish, so that there can be no mistake as to the identity of the minute nuclear granules which are seen in the interstices between the unstained cyanophycin bodies.

A possible explanation of this peculiar phenomenon seen in the spore of *Cylindrospermum* is afforded by the density of the peripheral protoplasm, although this is a point of which I have not yet convinced myself. As the cyanophycin granules accumulate in the cytoplasm in the immediate neighborhood of the nucleus, they finally become so abundant that they are probably forced, on account of their increasing numbers as well as on account of the density of the protoplasm in which they lie, into the nuclear cavity. They thus break down the delicate. forming membrane, push in among the chromatin granules, and in this way, in the mature spore, present the curious appearance of an enormous central body, which is completely filled with reserve food granules. We can see, moreover, in figs. 95—99. that the cyanphycin occupies only the outer portion of the central body, while the middle is filled with a poorly defined. achromatic substance, in which are imbedded chromatin granules.

Such a peculiar encroachment upon nuclear space has certain resemblances to the phenomena seen in the spores of *Nostoc* (figs. 59, 60), in which the nuclei appear to be pressed into

contorted shapes by the surrounding cyanophycin granules: and also to the instance given by Raciborski, in which the nuclei of certain seeds assume irregular shapes, due to the pressure of the granules of food substances.

The writer may here be allowed, before leaving this subject, to give an opinion which has not yet been at all definitely established in his investigations. It is possible that, in the spore of *Cylindrospermum* shown in fig. 80, the nucleus, which is extremely large in comparison with those of the vegetative cells, represents in reality several nuclei. There is some evidence that nuclear division continues in the young, developing spores of these plants, until about four nuclei are formed; no wall, however, separates them. In another species, *Cylindrospermum catenatum*, walls are at once formed, and we have, as a result, several spores borne in a chain, instead of one. The writer hopes to establish these interesting points more definitely by further research. If it be true, however, that there are several nuclei in the spores of this alga, then we can readily understand how such an abundance of chromatin comes to be present.

Mitosis in *Gloeocapsa polydermatica*.

Gloeocapsa presents a peculiar type of cell division which has been, so far as I am aware, nowhere else observed in the organic kingdom. This plant seems to have been employed for study by but few investigators since the time when Schmitz first, in 1879, thought that the granules in the center of the cell represented the nucleus, and, later (80), concluded that he had been mistaken. Sections of young cells are shown in figs. 62, 64, 69 and 76, all stained with iron haematoxylin. Usually about eight dark granules can be counted, which, for reasons which will be explained later, are called chromosomes in the following account, and which are surrounded by an achromatic substance. Minute granules of food material — the „slime globules“ of Schmitz' are abundant in the peripheral protoplasm. The cytoplasm, in the living cell, also contains the diffused pale green coloring matter. Within or close beside the central body may further occur globules of a substance stainable with haematoxylin; these are, in all probability, the „slime globules“ of Palla (figs. 62, 64, 66 etc.). In examining a preparation stained with iron haematoxylin (which gives much better results in this instance than Flemming's triple stain on account of the staining by the latter of the thick, gelatinous wall in which the cells lie), we often find such a spireme-like arrangement of the chromatin as is shown in figs. 63, 68 and 72. It is possible even to distinguish, in fig. 72, the individual granules, bound together by the linin substance; whereas in figs. 63 and 68, the granules of the thread can not be easily made out, perhaps because of overstaining. In figs. 74 and 75, it is plainly evident that the spireme thread has begun to segment at both ends; and in figs. 67 and 70, a complete longitudinal splitting has taken place, for we can now

count approximately twice the original number of chromatin granules. Such a plane of fission, however, leaves this double thread disadvantageously placed with reference to the plane of division of the cell which follows. Comparing with the process as seen in *Oscillatoria* and in the higher plants, we should expect to find the subsequent plane of fission of the cell lengthwise; whereas, in reality, we find it crosswise (figs. 73, 76). For a long time, the writer was at a loss to explain this curious discrepancy, but the finding of such nuclei as drawn in fig. 71 furnished the clue to its solution. Judging from such appearances, it becomes evident that the separation of the chromosomes is accomplished simply by the pulling, or flowing, of the two spireme threads in opposite directions, the one entering the one daughter cell, and the other being drawn into the other cell. In fig. 73, the daughter chromatin masses are completely separated, and we no longer see the elongated thread arrangement; while in fig. 76, a transverse fission plane has cut in two the daughter cells, which have not yet become rounded off at the cut end.

We see, thus, in this species, two differences which separate *Gloeocapsa* widely from other Cyanophyceae, — first, is the fact that the cell is cut in two by simple constriction and not by a ring-formed wall; and, second, that here we have an exceptional phenomenon in that the plane of division of the chromosomes is abnormal. While, ordinarily, the plane of fission of the chromosomes is parallel to the subsequent plane of division of the cell. in *Gloeocapsa*, on the other hand, fission is at right angles to the resulting plane of division.

Summary of mitosis.

It appears obvious to the writer that we have at present a number of indisputable facts which point to the process of the division of the cyanophyceous nucleus as mitotic. And, further, that, although the process may be in some respects rather primitive, the essentials of nuclear division are, in the blue green algae, almost precisely similar to the well known karyokinetic processes seen in the higher plants.

These facts are as follows:

(1) Spindle. First, we have in the dividing central body an achromatic figure, which consists of a central portion, situated between the groups of separating chromosomes, and of a polar portion, corresponding in position to the mantle fibers, which lead from the chromosomes to the cross walls. The mantle fibers apparently have to do with the pulling apart of the divided chromatin granules. The complete achromatic figure evidently corresponds to the spindle, although it does not usually have the common spindle shape. Instead, in the short celled filamentous species, it may have the form of a more or less flattened, broad-poled disc. In the longer celled algae also, the central body assumes somewhat the form of the cell in which

it lies; in some cases, however, e. g., *Cylindrospermum* and *Anabaena*, the poles of the figure are more nearly pointed than in others. In nearly all instances, the pressure of the multitude of cyanophycin granules which lie in the surrounding cytoplasm, as well as the presence of slime globules, which are usually deeply imbedded in one side of the central body, determine largely certain peculiarities and irregularities of its shape. (pp. 18, 19, 20, 22.)

In *Gloeocapsa*, owing to the peculiar method of separation of the two daughter spiremes, the achromatic figure remains throughout the anaphases so inconspicuous (see figs. 70 and 71, e. g.), that the term spindle would hardly be applied in this case. Between the two spiremes of fig. 71, however, there is present an achromatic portion which corresponds obviously in position to the central spindle, while the pulling fibers (if any, indeed, be necessar; ly present), must now be located in an entirely new position, at the ends of the cell, so as to effect the peculiar separation which follows. (p. 29.)

(2) Spireme. We have, further, a spireme thread, particularly evident in the cells of *Gloeocapsa*, in which separate and distinct chromatin granules are usually demonstrable. Such a spireme is most appropriately called a „segmented spireme", and the chromatin granules imbedded in it, since their number is constant for the species, and since there is no further indication of transverse fission, correspond to the chromosomes. Should this be true, then we have the unprecedented phenomenon of a chromosome made up of a single chromatin granule, or chromomere. (pp. 22, 29.)

In the opinion of the writer, the dividing central body in figs. 8 and 10, seen in section, so that the long axis agrees in position with the long axis of the nucleus in *Gloeocapsa* (figs. 74, 75), corresponds to a certain extent to the undoubted spireme in the latter figures. Thus we should have, in *Gloeocapsa*, a simple, more or less spiral spireme, placed crosswise to the subsequent plane of division of the cell, and in *Oscillatoria* and the other forms, a convoluted spireme placed parallel to the subsequent plane of division. It is highly probable, furthermore, that *Oscillatoria* and other filamentous forms also have their chromatin granules arranged, like those of *Gloeocapsa*, in the form of a segmented spireme. If this be true, then the cases of apparent fusion of chromatic and achromatic elements, seen particularly in preparations stained with Flemming's triple stain (as in figs. 7, 10, 17, 25), are misleading, and the granules should all appear instead as sharply defined as is shown in fig. 8. The achromatin, however, undoubtedly varies considerably in density during the nuclear changes. During the times of greatest density, the achromatic portion sometimes stains as deeply as chromatin, so that an appearance of the fusion of the two into a more or less solid thread may be given. This opinion is supported by the fact that it can be readily demonstrated by

proper staining with iron haematoxylin, that the chromosomes in fig. 17, e. g., are globular or nearly so, and that they are quite separate and distinct from the achromatic portion. The achromatin in this instance is apparently most dense in the immediate proximity of the chromosomes. (pp. 21, 22.)

(3) Number of chromosomes constant. One of the strongest evidences that a mitotic division must take place is that the number of chromosomes is constant for the same species. In *Gloeocapsa* and *Nostoc*, there are 8. In two of the species of *Oscillatoria* studied, in *Phormidium* and *Calothrix*, there are 16; while in *Oscillatoria princeps* and in *O. Froelichia*, there are probably 32. In side view, the longitudinal sections of cells with 16 chromosomes show usually 3 or 4; those with 32, as many as 8. (p. 11.)

(4) Longitudinal fission of chromosomes. This constitutes the most necessary accompaniment of mitotic division. In the case of *Gloeocapsa*, it is plainly evident that a longitudinal fission of the spireme thread is taking place, or has already been accomplished, in figs. 67, 70, 71, 74, 75. It is equally evident that an equatorial fission of the central body is occurring in figs. 6, 8, 10, 13, 34, 37, 80, etc. Since we find in such cases as figs. 8 and 13, indisputable evidence of the doubling of the number of chromosomes, one must conclude that each individual chromatin granule is divided longitudinally in a plane parallel to the cross partitions. The fission of all the chromosomes in a single nucleus probably does not take place simultaneously, for it appears to begin at the two extremities or outer edges of the spireme and thence to advance to the middle, in the same manner as the progressive fission of the central body (figs. 8, 13, 74, 75). (pp. 23, 29.)

The fact, observed by many investigators, that the central bodies of the vegetative cells of the Cyanophyceae have no nuclear membrane is due, in the opinion of the writer, to their continuous mitotic activity. It is probable that, if the nuclei could be made to enter a resting condition, they would then form both karyolymph and membrane. This probability is rendered the more credible by certain facts: first, that the nuclei of *Oscillatoria*, even in active filaments, occasionally seem to show nuclear sap and a poorly defined limiting membrane. Secondly, by the fact, observed by Wager, that the nuclei in young cells are often limited toward the cytoplasm by a „vacuolar membrane"; and finally, because in spores and heterocysts we find nuclei in the usual resting condition.

It will be of interest, then, if the vegetative cells of the Cyanophyceae are in a state of continuous mitotic activity, to determine how near to a resting condition their nuclei come. In *Gloeocapsa*, in the very youngest cells (figs. 62, 64, 69, 76), one may see, more or less clearly defined, the eight irregularly disposed chromatin granules, imbedded in an achromatic substance. Probably the next step in the karyokinetic changes is

the arrangement in the elongating cell of the chromatin granules in a spireme thread. In the opinion of the writer, therefore, the nearest approach to a resting state of the nucleus, under the usual conditions, at least, is probably best illustrated by figs. 62 and 64, in which the daughter chromosomes remain separate and distinct, and surrounded by achromatin, until rearranged in a spireme for the next division. A breaking up into smaller granules of chromatin and the formation of a reticulum such as is characteristic of the nuclei of higher organisms is not evident; and no nuclear sap is secreted, consequently there is no limiting membrane. It is possible, however, that fig. 16 illustrates a nearer approach than is usual to a normal resting condition; and that fig. 33 shows the beginnings of the formation of karyolymph (or the spaces may be occupied by slime globules?); and further, that in certain cells of figs. 6 and 14, the nuclei have entered partially into a state of rest.

Cell inclusions.

In the foregoing pages, mention has been often made of cyanophycin granules (a name given by Borzi) and slime globules (Palla), or „Zentralkörner“ (Zacharias) which occur so abundantly in the cells of the Cyanophyceae. It is highly probable, in fact, that the cyanophycin granules are a type of reserve food material peculiar to these plants; while the slime globules have a much wider distribution, having been found by Bütschli in Diatoms, Flagellates, in the epidermal cells of Phanerogams, etc.

Minute plastids — the „Cyanoplasts“, as called by Hegler — are said by several investigators to be present and to contain the green and blue coloring matters. The writer agrees with Fischer, however, that the coloring matters are held, not in plastids, but diffused in a peripheral chromatophore. Hegler, in his article, added another substance — glycogen — to our list of the cell inclusions of the *Cyanophyceae*; and Kohl (03) confirmed his discovery, giving a list of twelve genera in which glycogen occurs. It is the opinion of both Hegler and Kohl that this substance is the first perceptible product of assimilation in the blue green algae. I have not yet been able, however, after many careful tests made both with sections as well as with fresh filaments, successfully to demonstrate glycogen in *Oscillatoria*. Equally unsuccessful have been many tests with sudan and other reagents, made also with *Oscillatoria*, for the purpose of determining the presence of the minute fatty oil globules, said by Zacharias and Kohl to occur in the cytoplasm of *Tolypothrix*.

It is not the purpose of this paper to discuss the composition or the probable function of the two kinds of granular inclusions of the cytoplasm which have been found by the writer in all the forms studied, since the microchemical tests which I have so far made will not warrant definite conclusions. Attention

is again called, however, to the historical table on p. 2, which presents a most interesting variety of conclusions concerning these inclusions. But I wish to record here some differences of opinion as to the position in the cell of the granules. Bütschli (90) and others thought that the slime globules were chromatin granules and naturally believed that they occured within the central body; although, in some higher plants, Bütschli found them scattered through the whole protoplasm. Kohl (03), probably because he had not examined the matter in sections, agrees that they occur only in the nucleus. Hegler says, on the other hand, that the „slime vacuoles", as he calls them, as well as the cyanophycin granules, lie only in the cytoplasm, outside the nucleus. Fig. 27 is a cross section of *Oscillatoria*, showing a large slime globule, obviously imbedded in one side of the nucleus. Fig. 8 represents a preparation, likewise stained with iron haematoxylin, in which the slime globules are stained more or less dark. In this figure, one such body is shown, pressed into one side of each nucleus. On the other hand, certain minute globules in figs. 7, 11 and 13 seem to be actually enclosed within the limits of the central body. Therefore, while it appears possible, since there is no nuclear membrane, that the slime globules may be sometimes, entirely enclosed by the substance of the central body, they lie usually in the cytoplasm in the immediate vicinity of the nucleus.

The cyanophycin granules may sometimes be seen with high powers in living plants of *Oscillatoria* and other forms (fig. 1). Their relative position is much better shown, however, in sections which have been stained with eosin, e. g., together with some other differentiating stain to bring out the nucleus and other parts of the cell. Fig. 21 is such a preparation of an undetermined species of *Oscillatoria* and fig. 22, of the minute species, *O. splendens*. In both cases, the cyanophycin granules lie in the cytoplasm, in close proximity to the cross walls. In one cell in fig. 21 is shown a minute refractive crystal, lying in a vacuole, where, normally, the cyanophycin occurs. In fig. 23, the granules are shown in a cross section of *Oscillatoria*. Fig. 48 represents a preparation of *Calothrix* in which the granules are stained with safranin, and fig. 56, of *Nostoc*, similarly prepared. In these last drawings, as well as in one of *Cylindrospermum* (fig. 77), we observe that the granules occur, scattered irregularly, throughout the cytoplasm.

Experiments with digestion.

Some experiments were undertaken by the writer in order primarily to discover whether the assertions of Fischer (97) are correct in regard to certain conclusions of Zacharias (87) and Bütschli (90). Zacharias claimed that pepsin partly digested the peripheral cytoplasm in *Oscillatoria*, leaving the undigested chromatin of the central part as granular, refractive

bodies lying within a delicate network. He employed this method, in fact, to assist in proving the nuclear nature of the central body. Fischer, on the other hand, contends that such an appearance as that shown, e. g., in fig. 31, is due solely to „enzymatische Kontraktion“, and that no digestion whatever occurs.

In my experiments, filaments of *Oscillatoria Froelichia* and other forms were allowed to remain two or three days, at about 33 ° — 36 ° Cent., in a preparation of Grübler's pepsin; the masses were then washed in water, fixed, carried through the paraffine process, and sectioned. Fig. 20 illustrates a cross section of *Oscillatoria* thus treated, which is drawn to the same scale as the sections of the same species shown in figs. 24 and 25. A comparison of the digested with the undigested sections will at once show that either considerable digestion has taken place, or else a very large amount of „enzymatische Kontraktion“. Fig. 19 is a longitudinal view, also treated with pepsin. In both transverse and longitudinal sections, we can see all the parts of the protoplasm which we have previously noted in the untreated sections — chromatophore and chromatin, as well as the dimly defined achromatic portion of the nucleus. The assertions of Hegler and Kohl that the cyanophycin granules are digested was confirmed; and, further, the statement of Kohl that neither pepsin nor pancreatin will digest the slime globules, is also probably true. It is a much more difficult question as to whether any of the protoplasm itself is so affected. The writer, however, firmly believes that some digestion of the protoplasm does occur, and he bases such a conclusion mainly on the fact, perhaps insufficient in itself, that there is not apparent in the normal cell of *Oscillatoria* enough vacuolar space to account for such an enormous shrinkage of volume, through simple plasmolysis alone.

Summary of results and conclusions.

1. The central body of the *Cyanophyceae* is a nucleus, not essentially different from the nucleus of the higher plants. It consists of a more or less dense, fibrous, achromatic portio, and, enclosed by this, a number of minute, globular, or somewhat irregularly shaped chromatin granules. The chromatic and achromatic substances stain with the standard nuclear stains, e. g., iron haematoxylin and Flemming's triple stain, similarly to the corresponding elements of higher plants (pp. 17—24).

2. In the opinion of the writer, thin, well stained sections, made in both transverse and longitudinal planes, are necessary for the thorough study of the nuclear structure of these organisms.

3. The nucleus of the *Cyanophyceae* usually appears to be in a state of mitotic division. Plants which were subjected to slow desiccation until thoroughly dried showed no perceptible indication of entering a resting condition (p. 26.)

4. Centers of division activity occur with rhythmic regularity in the filamentous forms, a phenomenon already noted in *Spirogyra* (p. 24).

5. The division of the central body is mitotic, since we can find in the changes which it undergoes the usual phenomena which accompany mitosis in the higher organisms (pp. 20—24).

6. The kinoplasmic, achromatic portion of the central body constitutes a „spindle", which has the shape of a flattened disc in the narrow celled species; and in the longer celled forms, of a broad-poled, somewhat cylindrical figure; or, in still others. narrow-poled and spindle-formed. The achromatin consists of a central spindle, which is often very densely fibrous, between the dividing chromosomes; and a portion leading from the chromosomes to the cross walls, which corresponds to the mantle fibers in position and apparently in function (pp. 19—20).

Owing to the peculiar plane of location of the nuclear figure in *Gloeocapsa*, there is little appreciable development of an achromatic spindle (p. 29).

7. A spireme arrangement of the chromatin granules is also evident in the preliminary nuclear changes. The „segmented spireme" in *Gloeocapsa* appears to consist of a simple, more or less spiral thread, having about 8 chromatin granules held by the linin, and situated in the middle of the cell, with its long axis corresponding to the long axis of the cell (p. 29).

In the filamentous species, the spireme apparently consists of a much convoluted thread, and it is further probable that it also is made up of a definite number of distinct chromatin granules, arranged along a linin thread (pp. 22, 31).

8. Finally, the most necessary requirement of mitosis is fulfilled in that a longitudinal fission of the chromosomes occurs. This is plainly evident in the case of *Gloeocapsa*, in which the simple spireme thread divides lengthwise, beginning at the two ends and splitting thence progressively to the middle of the thread. It is highly probable, further, that the splitting of the convoluted spireme of the filamentous species takes place in a somewhat similar manner, since the fission plane begins at the edge of the disc-shaped figure and travels progressively inward to the middle (pp. 22, 23, 31).

9. The number of chromosomes in the cells of the same species is constant. There are 8 chromosomes in *Glococapsa polydermatica* and *Nostoc commune*; 16 in *Oscillatoria tenuis*, in an undet. sp. of *Oscillatoria*, *Calothrix thermalis*, *Phormidium* sp.: and probably 32 in *Oscillatoria princeps* and *O. Froehlichia* (p. 19).

Each chromosome apparently corresponds to a single chromatin granule of the spireme thread. Should this prove true. then this presents the hitherto unrecorded phenomenon of a chromosome which consists of a single chromomere.

10. The division of the cell is usually accomplished by the growing in of a ring-formed wall, which appears to grow independently of and simultaneously with nuclear division (p. 24).

Glococapsa, however, furnishes two peculiarities in its cell division. The cutting in two of the cell is accomplished by simple constriction, instead of by a ring-formed wall; and, secondly, it has an exceptional plane of division which has been, so far as the writer is aware, nowhere else observed. Instead of the division of the cell occurring in a plane parallel to that of nuclear fission, as in normal cases, in *Glococapsa*, the plane of constriction is at right angles to that of the division of the nucleus (p. 30).

11. Although the central body in vegetative filaments seems to be in a state of continuous mitotic activity it appears occasionally to make a beginning toward a resting condition, and to form a delicate membrane and karyolymph. It is probable, however, that the nuclei in the active filaments do not ordinarily approach nearer to a state of rest than the spireme condition or a stage immediately prior to it. This condition is not usually attended by the secretion of karyolymph (p. 32).

12. In spores and heterocysts, on the other hand, the nuclei enter a condition of rest, in which nuclear vacuole and membrane are formed. In heterocysts, the protoplasmic contents soon die, leaving nothing finally evident but disorganized chromatin granules.

In some spores, the nuclear vacuole and membrane may persist; whereas, in the case of *Cylindrospermum* and probably in other forms, the multitude of granules of reserve food encroach so upon the nuclear cavity that, in the mature spores, the membrane appears finally to be broken down and the granules enter the nuclear space. We thus have the peculiar phenomenon of an enormous central body, containing an abundance of cyanophycin bodies, in the interstices of which are the chromatin granules (pp. 27—29).

13. The blue and green coloring matters are held in a diffused state in a peripheral chromatophore, which may have, in some species, the form of a hollow cylinder, or, in others, of a hollow sphere. In the six genera examined, no evidence whatever was found of the presence of minute „cyanoplastids“ (pp. 14—16).

14. The only kinds of granular inclusions which were found were the cyanophycin granules and the slime globules. The cyanophycin granules are evidently a form of stored food; and, in those algae with a cylindrical chromatophore, they lie in the cytoplasm, generally closely packed along both sides of the cross partitions. In those species with a hollow spherical chromatophore, the cyanophycin bodies appear to be located in the chromatophore itself, or, more probably, in the cytoplasm between the chromatophore and the central body (p. 33).

The slime globules are also, at least as a usual thing, located in the cytoplasm. They lie, however, in the immediate vicinity of the nucleus; so close are they, in fact, that they

are usually deeply imbedded in one side of the nuclear body (p. 34).

I have been unable to find any indication of oil or glycogen in *Oscillatoria*.

15. Experiments with peptic digestion resulted in the conclusion that some of the cytoplasm itself is digested, as well as the cyanophycin. Much of the protoplasmic contents, however, appears to remain unaffected, so that one may see clearly in digested sections the shrunken chromatophore and central body, together with the refractive granules of indigestible chromatin (p. 34).

16. For the reasons that the nuclei of the vegetative filaments show occasional indications of the formation of a membrane; and, that in spores and heterocysts, we find at least a beginning of the formation of the usual resting condition; and, finally, that the mitotic processes which take place in these plants are similar to those of higher organisms, we are justified in the conclusion that the central body of the *Cyanophyceae* is not essentially different from the nucleus of the higher plants. It may, however, be called a primitive nucleus from the fact that the chromosomes appear to be made up of a single chromomere, and, secondly, because of the unusual simplicity of the spireme of *Gloeocapsa*.

Madison, Wisconsin, U. S. A.

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Explanation of plates.

The figures were drawn with the aid of a Zeiss camera lucida. All except the nine mentioned below were drawn with a Zeiss apochromatic

immersion objective, 2 mm, 1,30 N. A., and Zeiss compensating ocular 12. Figs. 6, 7, 8, 10, 11, 14, 17, 26, 27 were made with a compens. ocular 18, and the 2 mm objective.

The abreviations Flem. and Heid. stand for Flemming's triple stain and Heidenhain's iron haematoxylin, respectively.

Plate I.

(Figs. 1—6, *Oscillatoria tenuis.*)

Fig. 1. A living filament, showing the blue green peripheral chromatophore and the colorless central portion. The cyanophycin granules and two slime globules are also shown.

Fig. 2. A section stained with Heid. and eosin. The filament was first dried on the ground before fixing. At the end of several months. the dried material was still living. The central body is overstained and, in some cells, appears to have long chromosomes; in certain cells, the central body seems to be undergoing direct division. The shrunken cytoplasm is stained red.

Fig. 3. From a preparation fixed with the Hegler SO_2-Alcohol mixture, and stained with Heid. and eosin. The central body is overstained and appears to be, in some cases, shrunken and poorly fixed.

Fig. 4. A cross section of a well fixed filament, similar to that shown in fig. 6. The dark chromatin granules are surrounded by bluish kinoplasm.

Fig. 5. A cross section of a filament treated similarly to that shown in fig. 2. The shrunken central body shows in cross section such an one as that represented in the uppermost cell in fig. 2.

Fig. 6. A section stained with Heid. The minute black chromatin granules lie in a bluish achromatin. The lowermost cell appears to be in a spireme condition.

(Fig. 7—9, *Oscillatoria Froehlichia.*)

Fig. 7. Stained with Flem. The mantle portion of the spindle is unstained. Slime globules, also unstained, are shown, imbedded in one side of the nucleus.

Fig. 8. A preparation stained with Heid. and eosin. Each sectioned nucleus shows one slime globule, stained light bluish with the l. c. aematoxylin. The minute chromosomes are black and the achromatic spindle is very light bluish. The chromatophore is reddish in color.

Fig. 9. A longitudinal section, stained with Heid., showing at *a*, *bc* and at *d*, three division centers in which division of the central body is at its maximum.

(Figs. 10—13, *Oscillatoria princeps.*)

Fig. 10. A thin section stained with Flem. The dark purplish chromosomes are more distinct in cells 1 and 4 that in 2 and 3. The central spindle is usually bluish, but is sometimes so densely fibrous that it may be even stained bright red with the safranin. The mantle fibers, leading from the chromosomes, are very dimly stained. Cyanophycin and slime are unstained.

Fig. 11. A section stained with methylene blue and eosin; the central body appears almost homogeneous. A secondary division may also be observed.

Fig. 12. A preparation which was stained four times unsuccessfully in Flem., then in Heid. and eosin. The central body is much overstained. The mantle fibers are shown, leading from the central body completely to the protoplasmic „Schicht" along the partition wall.

Fig. 13. A section overstained with Flem. No differentiation into chromatin and achromatin can be seen. A secondary division may be observed in the two contiguous daughter central bodies.

Fig. 14. Section of an undetermined species of *Oscillatoria*, stained with Flem. The nuclei in the four cells at the right of the figure appear to be in a resting condition.

Fig. 15. A cross section of a small species of *Oscillatoria*, stained with Flem. in which about 12—16 bright red chromatin granules may be counted.

Fig. 16. A cross section of *O. Froehlichia*, stained with Flem., in which the dark purplish chromatin granules and dimly defined achromatin appear to lie in a nuclear vacuole. Such an appearance, in which a nuclear membrane appears to be visible, occurs but rarely in vegetative cells.

Fig. 17. From the same preparation as fig. 14. The chromatin and achromatin appear to be fused together. The lowermost nucleus probably represents a section of a spireme condition, in which the fission plane has begun to divide the disc shaped figure.

Fig. 18. Cross section of *O. princeps*, stained with Heid. The chromosomes number about 32. The coarse meshwork of kinoplasm represents the mantle fiber region; in the middle is a denser portion corresponding to the narrow part of the constricted central spindle in fig. 10, cells 1 and 4. The chromatophore is also distinctly kinoplasmic.

Fig. 19. A section of *O. Froehlichia*, after treatment for three days with pepsin. The cyanophycin granules and portions of the protoplasm have evidently been digested. The indigestible chromatin granules are stained dark blue with Heid., while the cytoplasmic portion which remains undigested is unstained. The slime globules may be seen in other sections to be still undigested.

Fig. 20. A cross section of the same, stained with Heid. and eosin. In the shrunken protoplast may be seen chromatophore, chromatin, and achromatin.

Fig. 21. A section of a species of *Oscillatoria*, stained with anilin blue and eosin. The cyanophycin granules along the partition walls are stained red; the central body a dim blue, sometimes with darker, denser, or granular portions showing; the peripheral chromatophore is dark blue.

Fig. 22. *O. splendens*, similarly stained, showing the large, sometimes irregular, cyanophycin granules.

Fig. 23. A cross section of the same species as in fig. 21, similarly stained, with cyanophycin granules red, lying in a dimly blue, protoplasmic network.

Fig. 24. A cross section of *O. Froehlichia*, stained with Flem. About 32 bright reddish chromosomes may be counted. The peripheral chromatophore is stained somewhat bluish.

Fig. 25. A section from the same preparation as that shown in fig. 24, in which the chromatin and achromatin appear to be somewhat fused together.

Fig. 26. Cross section of *Oscillatoria* sp., stained with Heid., showing about 19 chromatin granules; some possibly belong to the lower group of chromosomes. This and the next figure probably represent spireme stages.

Fig. 27. A similar preparation, showing a large, dark-colored slime globule, imbedded in one side of the nucleus. Here only 13 chromosomes can be seen.

Fig. 28. An end view of a cell from a filament of *O. Froehlichia*, which was left three days in chloroform water, in order to extract the phycocyanin. The bright green color of the chromatophore appears to be uniformly diffused, and no indications of plastids are seen. The granules in the central portion are probably cyanophycin and chromatin.

Fig. 29. A side view of a portion of a filament of the same species, similarly treated. The cells are still enclosed within their walls. The peripheral portion is colored a much darker green than the middle. The distinct granulation appears only in the middle and is obviously caused by the same granules seen in end view in fig. 28.

Fig. 30. A freshly treated filament of *O. Froehlichia*, lying in a $20^0{}_0$ solution of KNO_3. Compare the plasmolized cells here with the digested filament in fig. 31.

Fig. 31. A filament of *Oscillatoria* digested for several days in pepsin, at about 35^0 Cent.; then stained with Heid. The granules are dark blue, and they lie in a light bluish central body. The outer portion of the protoplasm is unstained.

(Figs. 32—34, *Phormidium* sp., all stained with Heid.)

Fig. 32. 16 chromosomes show very distinctly in this cross section.

Fig. 33. Two vacuolar formations are seen in the central body. It is possible that these are slime globules. The achromatin is here more distinctly visible than in fig. 32.

Fig. 34. A longitudinal section of a filament, still lying in the thick. gelatinous membrane. The lowermost nucleus is completely divided; but the central spindle may still be dimly seen. The mantle fibers in the latter cell are more sharply defined.

Fig. 35. *Gloeocapsa*, left about two days in chloroform water. Nothing appears to remain in the dead, colorless cells but the colorless cyanophycin granules.

Fig. 36. *Gloeocapsa*, left 24 hours in chloroform water. Some of the extracted phycocyanin may be seen in the space between the cell and the gelatinous wall. The cells so treated are usually bright green; sometimes yellowish. The granulation is very distinct; obviously the granules are the same as those in fig. 35.

Plate II.

(Figs. 37—53, *Calothrix thermalis* Hansgirg.)

Fig. 37. Section of the large end of a filament, in its gelatinous envelope. showing the heterocyst with disorganized nucleus, and three vegetative cells, each with a nucleus in division. In the lowermost cell. division is completed. In the other cells, the stain is not differentiated sufficiently to show clearly chromosomes and central spindle. Mantle fibers are dimly shown. The ring formed walls are not, in this instance, well stained by the Heid.

Fig. 38. A preparation stained with Flem., showing dimly the central body. The cavities in which the cyanophycin granules lie are here seen to surround completely the nuclear body.

Fig. 39. Three cells near the attenuated end of the filament. The central body, poorly stained with Flem., is here greatly elongated.

Fig. 40. A similar section, stained with Heid. Cavities containing cyanophycin are shown in the cytoplasm.

Fig. 41. A broad, vegetative cell, stained with Heid. The achromatic, as well as the chromatic, structures are shown.

Fig. 42. Cross section of a large vegetative cell in which chromatin granules and linin appear somewhat fused together. Flem.

Fig. 43. A cross section in which 16 chromatin granules are seen. Heid.

Fig. 44. A cross section, stained with Flem. At one side of the central body is a slime globule. Many unstained cyanophycin granules are shown in the cytoplasm.

Fig. 45. A similar preparation to that in fig. 44.

Fig. 46. A cross section of an elongated cell. Flem.

Fig. 47. An elongated cell strongly stained with Heid.

Fig. 48. A preparation stained with Flem., in which only cyanophycin granules are stained red with safranin.

Fig. 49. A heterocyst in longitudinal view, stained with Heid. The dark body in the middle is probably a „Verschlußkörper“ (Kohl).

Fig. 50. Cross section of a heterocyst, showing in the middle the „Verschlußkörper“, and surrounding it what appears to be a nuclear vacuole, bounded externally with a ring of disorganized chromatin.

Fig. 51. A similar preparation, with central vacuole.

Fig. 52. A young heterocyst in cross section; the gelatinous sheath is also shown.

Fig. 53. A young heterocyst in cross section; showing a dim achromatic reticulum in the nuclear cavity.

(Figs. 54—60, *Nostoc commune* Vaucher.)

Fig. 54. A preparation stained with Heid. The central body not well differentiated.

Fig. 55. A similar preparation, showing about 8 chromatin granules. Both slime globules and cyanophycin granules are shown.

Fig. 56. Showing cyanophycin granules only, stained with safranin.
Fig. 57. A cross section, showing in the central body both chromatin and achromatin.
Fig. 58. A spore (?) which shows a nucleus in resting condition.
Fig. 59. Another spore, showing about 8 chromatin granules. Flem.
Fig. 60. A similar preparation.

(Figs. 61—76, *Gloeocapsa polydermatica* Kützing, all stained with Heid.

Fig. 61. A dividing cell, with its surrounding gelatinous wall. The central body is too deeply stained.
Fig. 62. A young cell, showing about 8 chromatin granules and the achromatic portion.
Fig. 63. A dividing cell, showing a deeply stained epireme thread. In the vacuolar spaces in the cytoplasm, are the „slime globules“ (of Schmitz).
Fig. 64. A young cell, in which the central body shows clearly 8 chromatin granules and an achromatic portion.
Fig. 65. A cell, apparently in a similar condition to that shown in fig. 64.
Fig. 66. Shows about 8 chromatin granules, and at one side of the central body, a large globule. It is possible that such globular bodies are slime globules; although they do not seem to be stained as the slime in *Oscillatoria* and in other instances with methylene blue.
Fig. 67. An older cell in which about 11 or 12 chromatin granules are shown.
Fig. 68. A spireme stage, in which the simple spireme thread has a distinctly spiral form.
Fig. 69. A young cell, showing only about 7 chromatin granules. Certain fibrous projections from the central body, which extend into the cytoplasm between the food granules, are somewhat stained by the haematoxylin.
Fig. 70. A cell in a state of division in which the spireme thread is double. We can now count about 16 chromatin granules.
Fig. 71. Showing the peculiar manner in which the divided spiremes separate, the one being drawn into the upper daughter cell, the other into the lower. It is possible that there are cytoplasmic fibers, corresponding to the mantle fibers, attaching the spireme to the end of the cell, and exerting a pull as the cell elongates. These are not evident, however. Judging from the figure, there appears to be an actual flowing of the spireme substance into the daughter cell. The central spindle between the separating spiremes is obviously very little developed.
Fig. 72. A spiral spireme thread in which we can count about 7 or 8 chromatin granules.
Fig. 73. A constricting cell in which the two daughter spiremes have completely separated. About 8 chromatin granules can be counted in each daughter cell.
Fig. 74. A spireme which appears to be splitting at the two ends.
Fig. 75. Another instance, in which the splitting of the spireme at the two ends is even more obvious.
Fig. 76. A step further advanced than in fig. 73, in which the constriction plane has completely divided the cells, which have yet become rounded off.

(Figs. 77—103, *Cylindrospermum stagnale* Bornet and Flahault.)

Fig. 77. A preparation freshly stained with methylene blue. The heterocyst show two minute, dark blue slime globules; the spore, one (sometimes several); the vegetative cells each one to several. The granulation in the cytoplasm of the spore is here quite evident.
Fig. 78. A filament, similarly stained, in which the spore cell is no larger than the vegetative cells. The heterocyst bears at its end several bacteria-like bodies.

Fig. 79. Another filament, similarly stained, in which no spore is as yet differentiated. The central body in the young heterocyst is here, unlike in the older conditions, somewhat stained by the methylene blue.
Fig. 80. A longitudinal section, stained with Heid., showing both the chromatic and achromatic elements of the central bodies. Some in the vegetative cells are spindle-shaped; some are apparently undergoing division.
Fig. 81. A vegetative cell, freshly stained with methylene blue, showing a large vacuole in the cytoplasm.
Fig. 82. A cross section of a vegetative cell, stained with Heid. and eosin. One can see about 8—10 chromatin granules lying in a bluish achromatic substance.
Fig. 83. A cross section, showing only about 6 chromatin granules.
Fig. 84. Chromatin and achromatin in longitudinal section. Heid.
Fig. 85. Another similar preparation. Heid.
Fig. 86. A longitudinal section stained with Flem.: the central body is not properly differentiated.
Fig. 87. Cross sections, similarly stained.
Fig. 88. A preparation, showing heterocyst, spore, and one vegetative cell. Heid.
Fig. 89. A longitudinal view, showing a slime globule (sometimes stained dark), imbedded in each nucleus. One cell is being divided by a ring-formed wall. Heid.
Fig. 90. Vegetative cells, stained with Heid. In two cells, the central body is overstained; in the third, the chromatin granules are apparent.
Fig. 91. A young heterocyst, showing a resting nucleus. Heid.
Fig. 92. A longitudinal section, in which the central body is but poorly differentiated. Heid. and eosin.
Fig. 93. A young spore, showing a resting nucleus. The cyanophycin granules in the cytoplasm are stained red. Heid. and eosin.
Fig. 94. A mature spore, freshly stained with methylene blue. The dense peripheral portion of the protoplasm appears to be without granulation, whereas the central body itself contains numberless cyanophycin granules.
Fig. 95. A longitudinal section of a mature spore, stained with Flem., in which the central body is seen to be completely filled with unstained (or sometimes red with safranin) cyanophycin granules. In the interstices between the cyanophycin, are the darkly stained chromatin granules.
Fig. 96. A cross section of a mature spore, stained with Flem. The unstained cyanophycin granules are here seen to occupy only the outer portion of the central body. In the middle is an achromatic substance. The dark granules are chromatin.
Fig. 97. A similar section, in which some cyanophycin granules appear to be in the cytoplasm as well as in the peripheral part of the central body. Flem.
Fig. 98. A cross section stained with anilin blue and eosin. The chromatin is here not differentiated. The cytoplasm and achromatin stain alike blue.
Fig. 99. A cross section of a somewhat younger spore than tha shown in fig. 97; similarly stained. The peripheral chromatophore still contains some cyanophycin.
Fig. 100. A cross section of a young spore, stained with Heid. and eosin. The nucleus possesses a delicate membrane and karyolymph. The cyanophycin is stained red.
Fig. 101. Cross section of a very young spore, showing a nucleus in resting condition. Flem.
Fig. 102. A similar section, similarly stained, of a somewhat older spore.
Fig. 103. A still older spore in cross section. The nuclear membrane has not yet been broken down.

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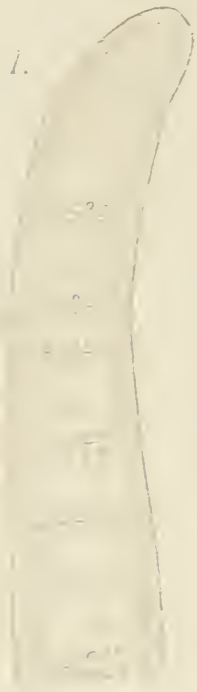

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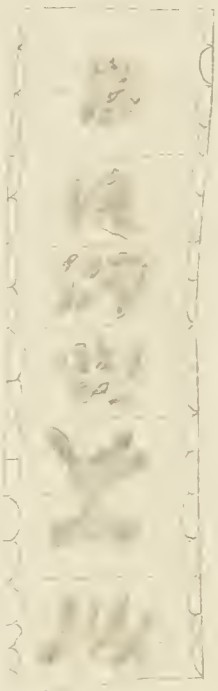

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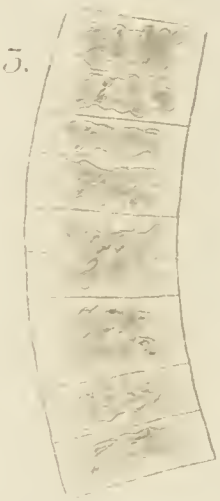

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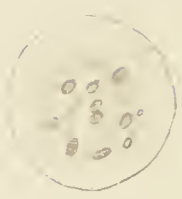

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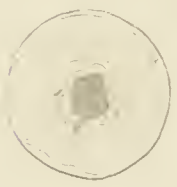

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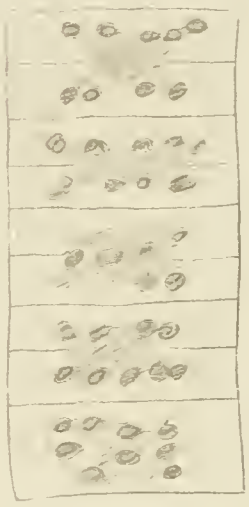

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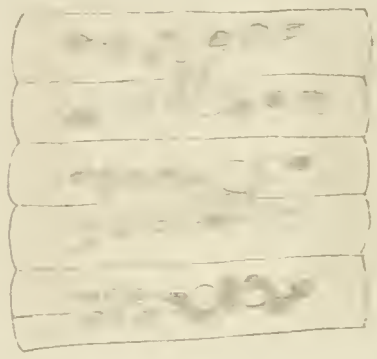

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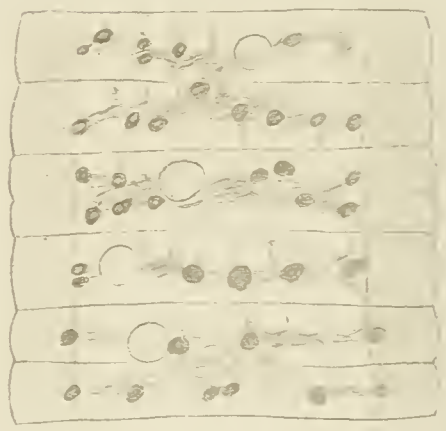

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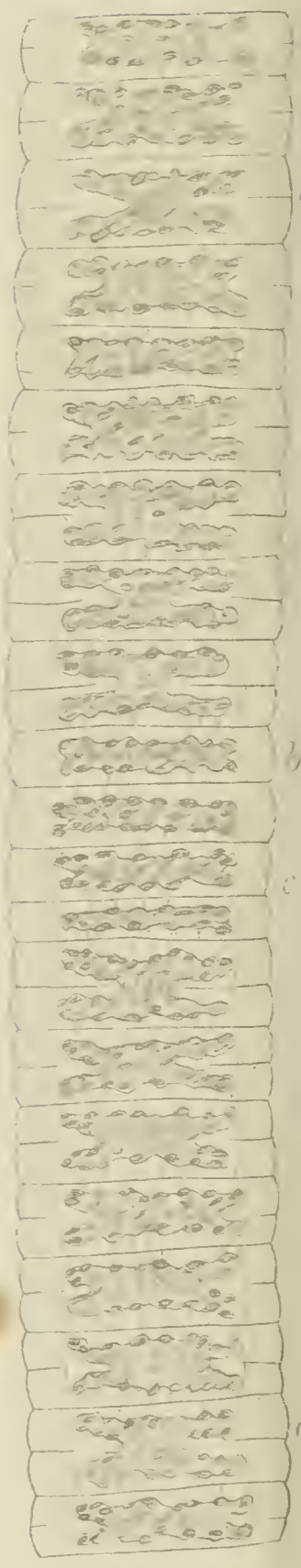

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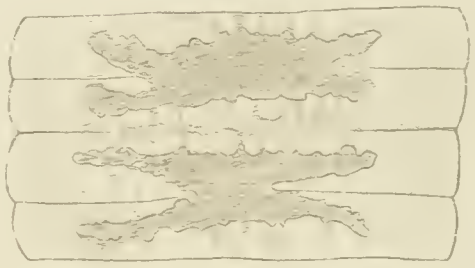

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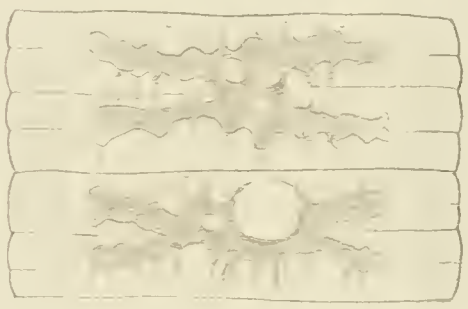

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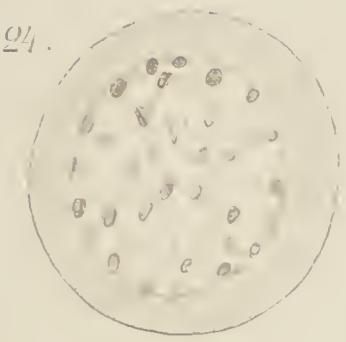

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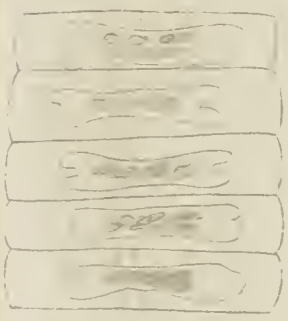

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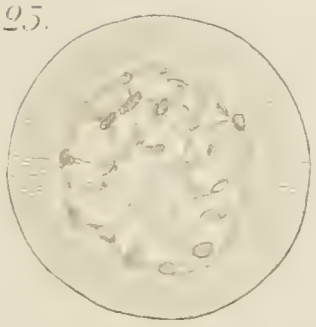

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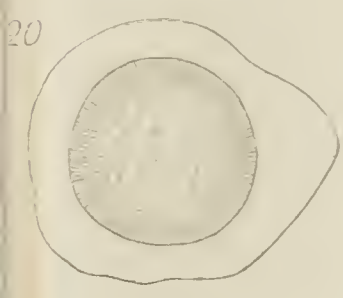

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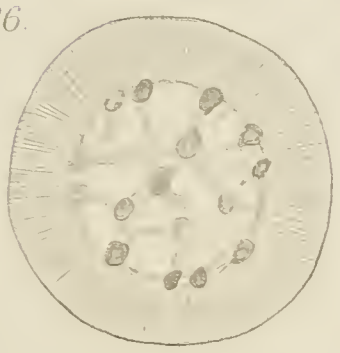

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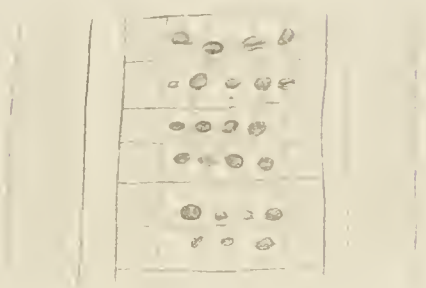

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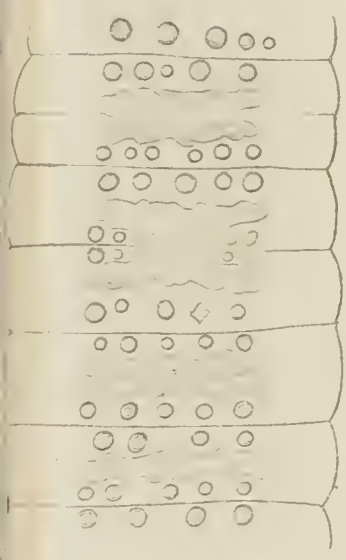

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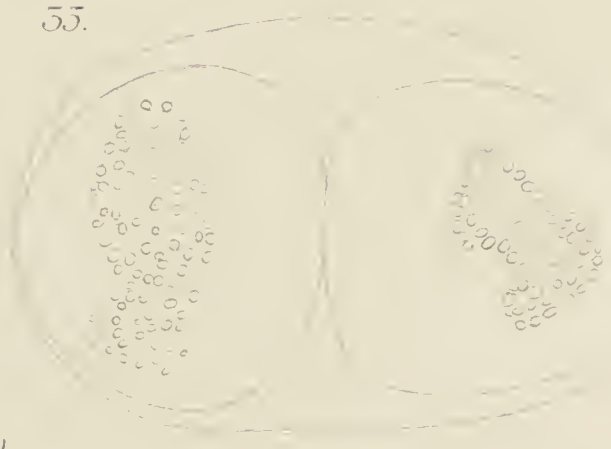

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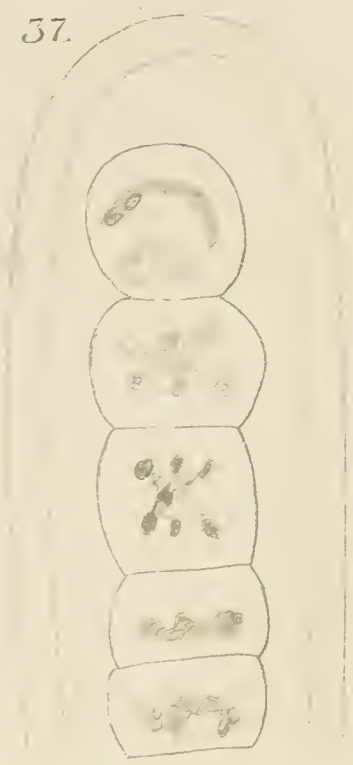
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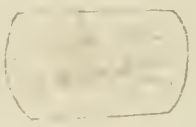
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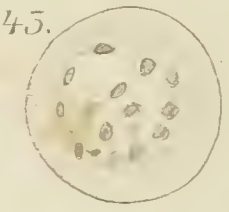
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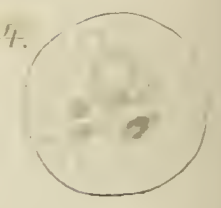
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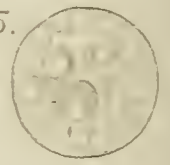
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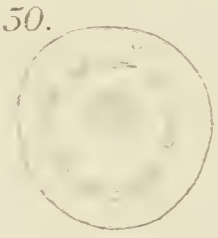
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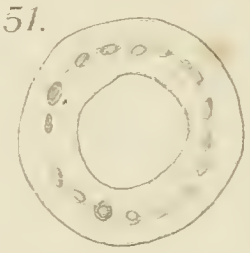
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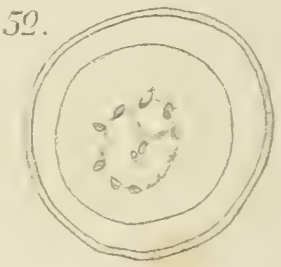
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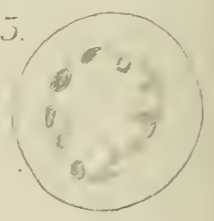
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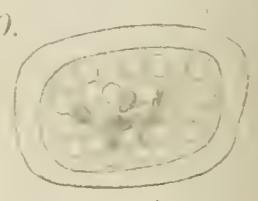
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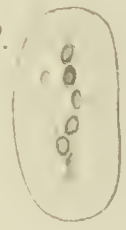
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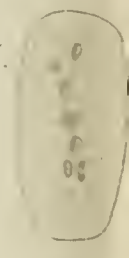
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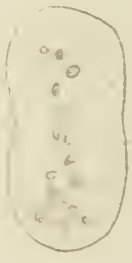
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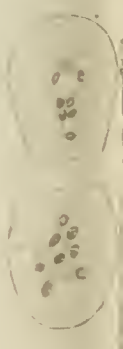
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