

Observations on the Cytology of the Chroococcaceae.

BY

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With Plates XXXIII and XXXIV.

I. INTRODUCTION.

THE controversial question of the structure of the cell in the Myxophyceae (or Cyanophyceae) has formed the subject of many papers. None of these, excepting Chodat's ('96) on *Chroococcus turgidus*, has been restricted to unicellular forms, a few have mentioned them incidentally along with filamentous forms, while the remainder neglect them entirely. Yet it is in the study of the unicellular species, as being the most primitive, that one might hope to find the key to the confusion which prevails at the present time on this question.

A detailed investigation of *Chroococcus macrococcus*, taken up partly in order to determine the systematic position of this Alga by finding out whether it possessed a fully organized nucleus or not, yielded such unexpected results with regard to the structure of the cell that it was thought advisable to examine other species of *Chroococcus*. Later, the investigation was extended to other members of the Chroococcaceae. Other species examined in addition to *Chroococcus macrococcus* were—

Chroococcus turgidus
„ *limneticus*
„ *minor*
„ *schizodermaticus*
Gloeocapsa sp.
Aphanothece prasina
Merismopedia elegans
„ *glaucia*
Gomphosphaeria lacustris
Coelosphaerium Kützingerianum
Dactylococcopsis sp.

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II. RECENT LITERATURE.

The numerous papers which have appeared dealing with the cytology of the Cyanophyceae have been reviewed by so many authors, that it seems unnecessary to give an account of any but the more recent.

The papers of Kohl ('03), Phillips ('04), Olive ('05), and Guilliermond ('05), all contain excellent reviews. A criticism of these four papers forms the subject of a special paper by Zacharias ('07).

Interest chiefly centres round the question of the presence or absence of a nucleus, and its behaviour during division if present. Kohl, Olive, and Phillips agree on the presence of a nucleus which divides mitotically; though they differ on the question of the arrangement of the chromatin and on the details of division. It seems probable that both Olive and Phillips have mistaken central granules for chromatin.

The rod-like chromosomes of Kohl can hardly be explained as central granules, though stained preparations, in which under a $\frac{1}{12}$ " objective only central granules can be seen, do sometimes under a $\frac{1}{8}$ " objective show structures simulating karyokinetic figures with rod-like chromosomes; with the greater magnification the rod-like bodies are resolved into granules. Zacharias, who through the kindness of Dr. Kohl has been permitted to examine some of his preparations, believes that they are projections, &c., from the central body.

Gardner ('06), who has investigated *Oscillatoria limosa* and *O. splendida*, two species upon which Kohl worked, states with regard to mitosis in these: 'The process in the nuclei of these two plants is purely and simply amitotic.' He also makes the following statement in the course of a comparative review of the papers by Kohl, Olive, and Phillips: 'After very careful and prolonged investigation I am unable to subscribe to any of the conclusions just quoted concerning the mitosis of the nucleus in any of the Cyanophyceae upon which they have worked.'

Kohl believes that there are numerous small granular chromatophores, while Olive and Phillips agree that there is a single large chromatophore. All find granules of two kinds—central granules and cyanophycin granules.

Fischer ('05), on the other hand, denies the existence of a nucleus, though he admits the presence of a single chromatophore. He maintains that the chromatin-like, pseudo-mitotic masses in the centre of the cell are due to the presence of 'anabaenin', which is found sometimes in chromosome-like masses and sometimes in granules (central granules of other authors). The anabaenin is a transformation product of glycogen, and there is a steady stream of glycogen from the chromatophore to the central body. He believes that this laying down of anabaenin in the centre of the cell is a means of removing the excess of carbohydrate material formed by the chromatophore. He states that anabaenin is a carbohydrate, thus

differing from the majority of authors, who believe that the central granules, with which he identifies anabaenin, are of a proteid nature. He has observed cyanophycin granules in the central body as well as in the chromatophore.

Gardner ('06) finds that there is a small refractive structure in the centre of the cell with a definite outline for each species, and regards this as a true nucleus. It does not, however, divide mitotically. He describes nuclei of three types. 'The diffuse type is characterized by having the chromatin distributed throughout the nucleus in the form of thin, plate-like or small angular pieces, or more or less branched and knotty thread-like masses, according to the species and to the shape and size of the cell in which it is located; or even a combination of these may be found in the same species. In whatever shape the chromatin is found, the essential characteristic is its quite equal distribution throughout the nucleus. . . . Within this type may be found quite a continuous series, showing how a very primitive nucleus becomes modified by gradual steps in the direction of the nuclear structure found in higher plants. . . . The differentiation seems to be proceeding towards the spireme formation found in the dividing of the nucleus of the higher plants.'

The second type he finds only in the genus *Dermocarpa*. 'The type is characterized by having the chromatin united into a very definite fine network, on which, particularly at the junction of the threads, are small granules or knots, presumably of chromatin, since they stain like the remainder of the thread. This whole network occupies a very large part of the cell, leaving only a very narrow zone outside, next to the cell-wall.

'Only a slight modification of the first type is necessary to produce the third or primitive mitosis type, which has been found in a single species only (*Synechocystis*). In this type the chromatin unites into a single contorted thread, quite irregular and indefinite in shape. This thread breaks into a definite number of pieces preparatory to cell division.

'In all the species studied, with the possible exception of *Synechocystis*, the nucleus divides amitotically.'

He describes granules of two kinds, which for convenience he terms α and β granules. These presumably correspond to central granules and cyanophycin granules respectively. There is no definitely organized chromatophore.

It will be seen that Gardner strikes out quite a new line with regard to the form and arrangement of the chromatin. At the same time, judging from his figures, the amount of chromatin present in proportion to the size of the cell is very great, and it is difficult to believe that so much chromatin is really present in the cells of such a primitive group as the Cyanophyceae.

Guilliermond ('06) states that the central body consists of a hyaloplasm with a deeply staining reticulum. The arrangement of this reticulum

would seem to correspond to that of the chromatin in the more advanced of Gardner's diffuse type; but Guilliermond describes this reticulum as consisting of an achromatic ground substance containing granules of chromatin. He thinks that it ought to be considered as a true chromatic network, and compares it with the 'appareil chromidial' described by zoologists in certain *Protozoa*. Division is amitotic. There is no spireme nor chromosomes, neither do the chromatin granules divide. The only suggestion of mitosis is that in some more highly developed species the threads of the network tend to become drawn out in parallel lines, though the anastomoses do not disappear and the reticulum is not broken.

The cortical layer does not constitute a true chromatophore, but contains the pigment in a state of solution. In the central body he observes (a) 'corpuscules métachromatiques' (Kohl's central granules); (b) 'corps nucléoliformes' of A. Meyer; and in the cortical cytoplasm cyanophycin granules.

Swellengrebel ('10), who has investigated only one species, *Calothrix fusca*, describes a central body consisting of an alveolar achromatic ground substance in which are embedded granules and filaments of chromatin. There is no very marked distinction between the groundwork of the central body and the surrounding cytoplasm, and sometimes the chromatin is diffused throughout the whole cell, so that the distinction between central body and cytoplasm seems to be lost. Division is amitotic.

Brown ('11) in a species of *Lyngbya* finds a nucleus which, in the stages between division, resembles the resting nucleus of higher plants, except for the absence of a limiting membrane. It contains a mesh of fine fibres, along which small granules are scattered; the fibres are embedded in a clear substance resembling nuclear sap.

Dobell ('12) in a paper on Spirochaetes briefly mentions three species of Blue-green Algae, and states that there is a definite central body in these species.

It will be seen from the foregoing account that, with the exception of Fischer, all investigators since 1902 conclude that there is a definite nucleus. Since, however, none of them agree on the details of its structure even when examining the same species, the value of these conclusions seems somewhat questionable. The evidence in support of the theory of a nucleus which divides mitotically is certainly inconclusive. With regard to the structure of a nucleus which divides amitotically there seems to be more agreement. Judging from figures, the achromatic ground substance of Guilliermond and the chromatin of Gardner occupy similar positions in the cell. Both stain more deeply than the surrounding cytoplasm, yet Guilliermond believes the substance to be achromatic, while Gardner thinks it is chromatin. As stated before, it is difficult to believe that there is as much chromatin present as Gardner describes.

The results described above are based almost entirely on work done on filamentous forms. On comparing these with my own results, it appears that the structure of the cell as described by Guilliermond most nearly approaches the condition in the Chroococcaceae.

III. TECHNIQUE.

The method of fixation employed naturally depended on the material to be examined. Both wet and dry methods were used.

In the case of gelatinous forms like *Aphanothece* and *Gloeocapsa* wet smears were made on the slide and fixed and stained without drying. If fixed in bulk, the material was afterwards embedded and cut with a microtome, as the fixing reagents caused coagulation of the gelatinous material, making it difficult to spread out on the slide.

Forms like *Chroococcus* were usually fixed by allowing a drop of the material to almost dry on the slide, and then fixing and staining in the ordinary way. These preparations were usually fixed in absolute alcohol. There was very rarely evidence of shrinkage or of plasmolysis in specimens fixed in this way, except in the earlier preparations of *Chr. macrococcus*, probably because the films were never allowed to become absolutely dry, and also possibly because the tough envelope afforded some measure of protection to the cell. Wet methods were always used as a control. *Chroococcus turgidus* and *Chr. macrococcus* were found in such enormous quantities among the flocculent material from certain sphagnum bogs that it was possible to fix in bulk, and also to embed and cut microtome sections of the material.

The fixing reagents used were absolute alcohol, Flemming's weak chrom-osmium-acetic, alcoholic-picric, alcoholic sublimate, and $2\frac{1}{2}$ per cent. formalin.

The thick outer covering of the cell proved a very great obstacle to satisfactory staining. Many stains, e.g. safranin and gentian violet, stained the membrane intensely, preventing examination of the cell contents, while others only penetrated with difficulty.

The stains that gave the best results were Loeffler's methylene blue, Delafield's haematoxylin, and iodine-green-fuchsin as used by Kohl ('03). This combination stain was very difficult to manipulate, as the exact proportion of the two solutions required seemed to vary with the condition of the material and the species under investigation. Repeated trials had to be made in each case, but when the right proportions were found the results were excellent. It was never found to be profitable to take back through the stains, if overstained with one or the other reagent. With Loeffler's methylene blue and Delafield's haematoxylin, preparations were overstained and the excess stain washed out.

Brilliant blue and Bismarck brown were used for the identification of cyanophycin granules and central granules respectively. Unfortunately, brilliant blue stains everything in the cell, and it was found best not to overstain to bring out the cyanophycin granules.

IV. THE PROTOPLAST OF *CHROOCOCCUS TURGIDUS* (Kütz.), Näg.

Although *Chroococcus macrococcus* is perhaps the most interesting member of the Chroococcaceae, it cannot be considered as typical of the group. Also it is impossible to discuss its systematic position without comparing its cytology with that of other members, so that it will be described separately at the end of this paper.

Chroococcus turgidus was examined in great detail, and as the structure of the protoplast in this plant appears to agree almost entirely with that of the remaining species except *Merismopedia elegans*, I propose to first describe fully *Chroococcus turgidus*, and then to deal with any facts of interest in connexion with other species.

Among the literature on the Cyanophyceae there only appear to be three papers mentioning *Chroococcus turgidus* as one of the species examined.

Palla ('93) makes special mention of it only with regard to the cyanophycin granules, the presence of which he was unable to demonstrate.

Nadson ('95) worked at several unicellular species, among which was *Chr. turgidus*. His conclusions were briefly as follows: The protoplast shows an alveolar structure in the sense of Bütschli, and the outer portion functions both as cytoplasm and chromatophore. The pigmentless inner portion is only distinguishable from the outer portion by the fact that it contains a more strongly staining substance, and that in this region the so-called chromatin granules (metachromatin granules) are exclusively or especially concentrated. There are three kind of granules in the cell—chromatin granules (metachromatin granules), reserve granules, and plasmatic microsomes. The latter are only found in *Merismopedia* and *Aphanocapsa*. They are small granules of plasmatic substance occurring at the junctions of the meshes of the alveolar protoplasm. The chromatin granules are especially, but not exclusively, concentrated in the central body, and are variously distributed in different species. The reserve granules are situated in the peripheral portion of the protoplasm. Division is usually direct.

Lastly, Chodat ('96) has a special paper on *Chroococcus turgidus*. He fails to find a definite nucleus. The central portion is quite often coloured in addition to the peripheral portion, and there can be no reason therefore to distinguish a special chromatophore. Mucilages, soluble amides, and cyanophycin can appear in the protoplast. The distribution of these substances varies exceedingly according to physical conditions, but they may

accumulate in the central region as a network, and confer on it the peculiarities which give it an appearance analogous to a nucleus.

The present investigation extended over a period of three years, and comprised material collected from three different sources at different periods of the year. The variation in the distribution and staining capacity of the granules in the different collections, due probably to physical conditions, as suggested by Chodat, was well shown.

As a result of these investigations, it has been found impossible to divide the work into descriptions of 'cytoplasm', 'nucleus', and 'granules', in the usual way. The best plan seems to be to state first the actual results obtained by staining reactions, and to discuss these results afterwards.

In the living cell one can only see that the protoplast is densely packed with rounded granules of a fairly uniform size. Some of these seem to be more refractive than others. Satisfactory results could not be obtained with living staining methods, on account of the tough thick membrane. In only one or two cases did there seem to be any indication of deeper staining in the central region.

In considering the results obtained from fixed material, observations which were made early in the work, before the structure of the protoplast was fully understood, will be mentioned briefly, since they appear to coincide with results mentioned by other authors. But it will be shown later that these results, in my own case at any rate, were only due to imperfect observations.

Staining with brilliant blue brought out deeply staining granules in the peripheral region of the cell, and with Bismarck brown granules in the central region. The number of granules reacting with Bismarck brown varied considerably. They were not confined entirely to the central region, but were chiefly concentrated there. These granules apparently correspond to the central granules of Kohl ('03), metachromatin granules of Guilliermond ('05), and volutin of Wager and Peniston ('10). These will be called metachromatin granules in the remainder of the paper. The granules reacting with brilliant blue correspond to the cyanophycin granules of Kohl and others.

Preparations stained with Delafield's haematoxylin showed the metachromatin granules stained a dark red colour, sometimes appearing almost black. In most cases these granules were almost uniform in size, but some material showed, in addition to these metachromatin granules, larger irregular granules which were apparently hollow. In the peripheral region could be seen granules very faintly stained of the same colour as the rest of the groundwork.

In addition to these granules, in several dividing cells, V-shaped threads, which might be compared to the spindle threads of a mitotic figure, were seen joining a few of the metachromatin granules.

Similar results were given with Loeffler's methylene blue. Granules in the central region stained dark blue, and in some cases there were apparently large irregular granules with a reddish tinge. The larger the granules, the redder they appear. Delicate threads could be seen here and there joining the granules. The groundwork and granules in the peripheral region were stained a very greenish blue.

With the iodine-green-fuchsin combination the cells appeared to stain uniformly red, with reddish granules distributed throughout the cell. These were not quite so conspicuous in the central region, but could be seen in sections. In certain parts of the cell, however, not isolated threads, but a definite reticulum of fine threads could be seen joining the granules, giving the appearance of a nuclear network. Finally, it was seen that the reticulum extended throughout the whole cell (Pl. XXXIII, Fig. 6). This necessitated the revision of the whole work and preparation of new slides. The following conclusions were only arrived at as a result of careful examination of hundreds of specimens.

The protoplast undoubtedly consists of a ground substance traversed by a very regular reticulum of delicate threads. This reticulum extends throughout the whole cell right up to the cell-wall (Fig. 6). At the junctions of the meshes of the reticulum, granules occur of fairly uniform size. These appear to correspond to Nadson's 'plasmatic microsomes', although he only described them for *Merismopedia* and *Aphanocapsa*. They are a constant feature of every species examined by the author, and the name 'plasmatic microsomes' will be used for the sake of convenience in the remainder of the paper in describing these granular thickenings of the reticulum.

The reticulum, with its granular thickenings at the nodal points, is seen most clearly in slides stained with iodine-green-fuchsin. With this stain, the ground substance, reticulum, and plasmatic microsomes stain red. In the central region, however, the microsomes are not so clearly marked, sometimes appearing to take on a bluish tinge. In good preparations the reticulum can be seen with other stains, but more often portions of it only can be seen in different parts of the cell. This reticulum has a very small mesh and can only be seen with a high magnification (Fig. 2).

The granules in the central region, which stained deeply with Delafield's haematoxylin, were found to be situated at the junctions of the meshes, the fact that the reticulum was imperfectly observed giving rise to the idea that these granules are sometimes joined by V-shaped threads. It is interesting to note in this connexion that Gardner ('06), in speaking of the α (central) granules, says: 'There often appears to be a connexion between two granules, but I presume that this is simply the deeply stained protoplasm, the colour of which is not washed out.' Since they occupy the same position, i. e. at the junctions of the network, the metachromatin granules must be identical with the plasmatic microsomes in the centre of the cell, or, in other words,

the plasmatic microsomes in the central region have the characteristic reactions of metachromatin. They stain deeply with haematoxylin, Loeffler's methylene blue, &c., and take on a reddish blue tint with iodine-green-fuchsin.

The latter stain does not appear to differentiate very clearly the various granules in the cell. Even the large metachromatin granules can often only be recognized because they are refractive.

Kohl ('03) states that the metachromatin granules are not stained with this combination, and that chromatin should stain green blue to blue violet. I find that metachromatin is often uncoloured, but at times gives the colour reactions which Kohl ascribes to chromatin. In Fig. 6 the dark granules stained a distinct green blue, yet they correspond in size and number to the metachromatin granules in other cells of this collection. Many of them contained very little metachromatin. At the same time the author does not deny the possibility of these granules being true chromatin, especially considering the position of the chromatin in those forms which have a distinct central body.

The large irregular, apparently hollow, metachromatin granules were found to be simply large accumulations of metachromatin, or, perhaps more correctly, clusters of metachromatin granules filling up a whole mesh of the network (Fig. 2). The granules are very refractive and thus give a dark edge on focusing, causing the appearance of a darkly stained rim (Fig. 2). Possibly these correspond to the hollow granules of Kohl and others.

It is possible that these clusters of granules, taken in conjunction with the imperfectly observed network, may have given rise to the statement of Phillips ('04) that 'The chromatin is aggregated in hollow vesicles in the resting cell. These vesicles give out chromatin to the net spireme very much like the nucleolus of the higher plants, and they may represent it.' He also describes on division a network of threads connecting chromatin granules. It will be shown that the metachromatin does actually diffuse into the ground substance.

It has been stated before that the number of metachromatin granules in the central region varies with the condition of the material. When the number is large, the groundwork seems to stain equally in all parts, but if the number of deeply staining granules is comparatively few, the ground substance differs in parts in its capacity for staining. This is especially well shown in slides stained with methylene blue. Certain parts of the ground substance stain more deeply than the rest, but they do not stain nearly as deeply as the metachromatin granules. Plasmatic microsomes, faintly stained like the remainder of the ground substance, can be seen in the central region.

These deeply staining areas are generally circular or elliptical in shape, and sometimes appear to be surrounded by a colourless rim. There may be

either numerous small areas (Fig. 4), a few larger areas, or in some cases the whole central region seems to stain.

The very regular shape of these areas seems to suggest that they are of the nature of vacuoles. If so, they are certainly not due to degeneration, for they are almost always found in dividing cells. Since the cells in which they occur always contain few metachromatin granules, while the majority of the cells in the collection are rich in metachromatin, it is probable that they are due to diffusion of the metachromatin when accumulation of reserve has reached its limit.

With iodine-green-fuchsin these areas remain colourless or slightly blue, which is another argument in favour of diffusion of metachromatin (Fig. 5).

The cyanophycin granules were also found to be situated at the nodal points of the reticulum in the peripheral region of the cell. They are not situated in the absolute periphery of the cell. There is always a region immediately within the cell-wall in which the majority of the plasmatic microsomes stain only with cytoplasmic stain (Fig. 3).

Division is brought about by the constriction of the cell into two approximately equal halves, caused by the ingrowth of the cell-wall. There is perhaps a tendency for the reticulum in the central region to be drawn out into more or less parallel lines, though the cross-connexions are not lost (Fig. 7). Again, the threads of the reticulum appear to stain more deeply in some cases in the central region, but this may only be accidental.

V. OTHER SPECIES OF THE CHROOCOCCACEAE.

All the remaining species examined are characterized by a reticulum with plasmatic microsomes. The size of the mesh of the reticulum is approximately the same in the different species, so that in the smaller ones there are necessarily fewer granules (Figs. 12, 13, and 14). The other species of *Chroococcus* offer no features of particular interest.

The species of *Gomphosphaeria*, *Coelosphaerium*, and *Dactylococcopsis* examined were too small for a clear interpretation of results, but do not appear to differ from the *Chroococcus* type.

Aphanothece prasina is interesting because the envelope in this species is easily penetrated by stains, and so intravital methods of staining were possible. The action of methylene blue on the living cell is as follows: the metachromatin granules in the central region take up the stain almost immediately, and in a short time have stained so intensely that the central region is marked off as a dark mass; one or two granules in the periphery also stain. In the peripheral region of the cell the reticulum can clearly be seen stained a little darker than the ground substance, and also the unstained cyanophycin granules (Fig. 14).

Gloeocapsa sp. is worthy of special mention, because Olive ('04) has definitely described mitosis in *Gloeocapsa*. He says: 'The segmented spireme in *Gloeocapsa* appears to consist of a simple more or less spiral thread, having about eight 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'; and again, '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 threads divide lengthwise, beginning at the two ends and splitting thence progressively to the middle of the thread.'

Now in *Gloeocapsa* sp. the reticulum connecting the plasmatic microsomes in the centre of the cell appears to stain more deeply than the remainder of the reticulum (Fig. 13). This certainly simulates a segmented spireme stage, and is of quite frequent occurrence in some preparations. It probably accounts for Olive's 'mitosis'. It may be a stage in the specialization of the central portion of the network, but is possibly an artifact due to slight shrinkage of the central region.

The genus *Merismopedia* differs rather strikingly from the species described up to the present, since one species, at any rate, has an 'incipient nucleus'¹ in the dividing stages. Two species only were available—*M. elegans* and *M. glauca*. The former occurred very sparingly in some plankton material from Sutton Park, which also contained *Chr. limneticus* in fairly large quantities. *M. elegans* was unfortunately overlooked when the slides were prepared, and this species was in consequence overstained in most preparations. One slide, however, stained with Delafield's haematoxylin showed a fairly large colony with cells in all stages of division. Some of these are shown in Figs. 8 and 9. The stain had been completely washed out, with the exception of a deeply staining portion which is evidently the 'nucleus'. The position of this in the dividing cells is shown in Fig. 8. The cells also contained a few colourless refractive granules, which were probably metachromatin granules.

The 'nucleus', before division takes place, is situated in the centre of the cell. Constriction of the cell takes place in two planes simultaneously, but the 'nucleus' appears to constrict, if not to actually divide, before it is reached by the constriction of the cell-wall (Fig. 9, *b*).

The structure of the 'nucleus' is somewhat similar to that of *Chr. macrococcus*, which will be described later. It is a restriction of deeply staining plasmatic microsomes to a small definite area in the network. There is some indication that this substance is distributed along the network

¹ The term 'incipient nucleus' has been suggested by Professor G. S. West, in his forthcoming publication in the Cambridge University Press, 1914, to describe the nuclear structure found in the Myxophyceae, and the word 'nucleus' will be used in this sense in the remainder of the paper. The reasons for using the term are given in detail by Professor West, and it is therefore unnecessary to discuss them here.

after division, and collects again in the central region when the next division is about to occur.

In this way, diminution in size by repeated division is prevented, and the central position of the 'nucleus' accounted for, since it is obvious that the 'nucleus' as a whole cannot travel to the centre. This suggestion needs to be more definitely established, but is supported by the following facts: In a group of four cells which were separating after division and increasing in size, the 'nucleus' was not so deeply stained, while the network was fairly distinct, especially round the region of the 'nucleus' (Fig. 9, *a*), but in cells showing a deeply stained 'nucleus' the network and plasmatic microsomes could hardly be distinguished (Fig. 9, *b*). Again, in one cell the network and microsomes were quite distinct, but no 'nucleus' could be distinguished (Fig. 9, *c*).

Merismopedia glauca, on the other hand, appears to have no definite nuclear body. This species occurred in abundance in some collections from North Wales, and though most of the colonies showed cells in a state of active division, no trace of a nuclear restriction of the network could be seen.

The cells only appear to divide in one plane at a time by a gradual constriction of the cell-wall, as in *Chroococcus turgidus* (Fig. 10). Most of the cells contained a few very large metachromatin granules, which almost filled the cell and at first gave rise to the idea that the cells were reproducing by gonidia.

No indication of a single chromatophore or of numerous small chromatophores was seen in any of the types examined.

VI. *CHROOCOCCUS MACROCOCCUS*, Rabenhorst.

Chroococcus macrococcus is a member of the Chroococcaceae of frequent occurrence in sphagnum bogs. The cells are solitary or associated in groups of two or four. They are spherical or somewhat elliptical in shape, and have a thick lamellose sheath. The diameter of the cells, including the sheath, varied from 30μ to 64μ , and of the protoplast from 24μ to 30μ in the specimens examined, but they were mainly from cultures and rather less than the usual size. The colour of the protoplast is not constant, but varies from a golden brown to a dark brown. At times an orange tinge is given to the cells by the presence of several blood-red pigment spots.

(a) *Cell-wall.*

The cell-wall is lamellose and often very thick, the outer layers frequently splitting away very irregularly (Pl. XXXIV, Figs. 20 and 21). It appears to consist of alternate layers of two substances.

In cuprammonia it swells rapidly but is not dissolved in thirty minutes, while in concentrated sulphuric acid only the darker layers remain. The

whole wall is soluble in caustic potash solution. On testing with picrosulphuric acid the thin darker layers take on a yellow tinge, showing the presence of chitin. No colour reactions for cellulose could be obtained.

Generalizations as to the nature of the cell-wall in the Cyanophyceae seem to have been derived from studies of filamentous form only. Hegler ('01) states that the sheath is chitinous, the gelatinous coat pectose in nature. Kohl ('03) observes that in the Cyanophyceae the membrane and sheath consist largely of chitin with some cellulose, the gelatinous sheath of pectose. The amount of cellulose present is often not sufficient to give the usual colour reactions. Speaking of *Tolypothrix*, he says, p. 92, '... in der Scheide das Chitin, in der Membran der Cellulose prävaliert'. Massart ('02) says the walls are not of cellulose, and a gelatinous sheath is often present. These observations cannot include forms like *Chroococcus macrococcus*, in which the envelope consists of alternating layers of pectose and chitin.

The irregular splitting away of the outer coats is probably due to the fact that the chitinous layers are incapable of rapid expansion, while the pectose layers swell with ease. The ecdysis of the older layers is always more noticeable in cells which are actively dividing after a period of rest.

Culture experiments were made to ascertain the effect of various media on the development of the cell-wall. It was found that solid media produced a one-sided development of the cell-wall. Cultures on damp earth showed, in three weeks, a considerable increase in size of the cell-wall on the side nearest the earth (Fig. 22). This was shown also in agar cultures, but not to the same extent, development being much slower.

The most striking results were obtained by removing material which had been for eighteen months on an agar culture to a 2 per cent. KNO_3 solution. The cell-wall elongated rapidly on one side, and, as the cells divided, corrugated stalks were formed (Fig. 23).

(b) *Protoplast.*

In the living condition the protoplast is too densely coloured to show much structure. Often it is not evenly coloured in all parts, and so an appearance simulating a star-shaped chromatophore arises. Cells which are golden brown in colour are frequently crowded with large refractive globules. These appear to be yellow, but whether they contain pigment or not has not been determined. They are not soluble in alcohol and do not blacken with osmic acid. One or more blood-red pigment spots can also be seen.

The pigment is not easily dissolved out of the cells, so all the earlier preparations were cleared in a 2 per cent. solution of KOH before staining. There was too much shrinkage in these preparations for detailed investigation, but they were useful in showing that a portion of the protoplast is definitely marked off from the remainder by its staining properties, having

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an affinity for chromatin stains. These two parts of the protoplast will be distinguished as 'nucleus' and cytoplasm.

Sections cut from carefully prepared material showed that the 'nucleus' is reticulate in character. With Delafield's haematoxylin the whole 'nucleus' appears to stain deeply, but at the nodal points of the reticulum granules are present which stain more intensely than the body of the 'nucleus' (Figs. 16 and 17). With iodine-green-fuchsin the body of the 'nucleus' does not appear to stain at all, or else takes on a slight bluish tinge, but the granules stain an intense blue and so the 'nucleus' is sharply defined. The reticulum stains red, as does the cytoplasm.

In cutting the material, sections thinner than 10μ were found to be unsatisfactory. Unfortunately, this means that the section does not necessarily pass through the 'nucleus', and, in fact, only two sections through the 'nucleus' were found. These are shown in Figs. 16 and 17, and prove beyond doubt that the portion of the 'nucleus' which reacts with chromatin stains is confined to the surface. Traces of a network could be seen inside the 'nucleus', but this may have been the inside view of the surface reticulum.

The author is inclined to think that the interior of the 'nucleus' consists of a sap vacuole, but a thin slice through the 'nucleus' which would show this clearly has not been obtained. This suggestion is supported by the fact that the 'nucleus' frequently appears flattened and in a state of collapse.

At first sight the cytoplasm appears to be coarsely reticulate, except in the neighbourhood of the 'nucleus', but closer investigation shows that a fine reticulum is present which passes insensibly into the reticulum of the nucleus. This certainly corresponds to the reticulum present throughout the ground substance of the other members of the Chroococcaceae, but its definite character is disappearing.

The coarsely reticulate appearance is due to the presence of cell-sap vacuoles, which break up the ground substance. The amount of vacuolization varies, being much more marked in the older cells; the region immediately surrounding the 'nucleus' is the last to be broken up. The effect of this vacuolization is to destroy the regularity of the finer reticulum, which becomes very much distorted, and in parts can hardly be distinguished (Fig. 15). The thickenings at the nodal points, which are characteristic of the network in other types, have almost disappeared, but traces of them still remain, especially near the 'nucleus' (Fig. 15). Metachromatin granules are scattered through the cytoplasm.

In *Chroococcus macrococcus* there is evidently a distinct morphological differentiation in the protoplast. The fine reticulum, characteristic of the whole protoplast in simpler types, has been restricted (in its definite character) to one portion of the cell, and associated with it is a very definite

accumulation of chromatin at the nodal points. In the remainder of the protoplast this fine reticulum is disappearing, and a coarser one, characteristic of higher plants, is arising.

This distinction appears to me to be sufficient to warrant the separation of the protoplast into 'cytoplasm' and 'nucleus'. Division, although it takes place by constriction of the cell, is initiated by the 'nucleus'. This divides and separates, and then the constriction in the cell-wall appears (Fig. 19). So that there is a physiological differentiation as well as a morphological one.

The 'nucleus' is certainly primitive. It divides by constriction, and there is no evidence whatever of mitosis (Fig. 18). It seems to permanently remain in a condition resembling the resting stage of the nucleus in higher types.

Repeated division of the cell seems to diminish the comparative size of the 'nucleus', for in the older cells the 'nucleus' is always small. Possibly reproduction takes place in some other way, as it seems essential that rejuvenescence of the 'nucleus' must take place sooner or later. No trace of fragmentation of the nucleus, as described by Gardner ('06) in *Dermocarpa*, has been seen in *Chroococcus macrococcus*, although in examining living material I have occasionally observed groups of eight daughter-cells free within the old cell-wall.

(c) *Systematic Position.*

Though the protoplast of the Alga described above is more highly developed than that of other Chroococcaceae, the connexion between them is clearly shown, and there is no reason therefore to separate it from the group on this account. It is undoubtedly a member of the Cyanophyceae, and agrees in all essentials with *Chroococcus macrococcus*, Rabenh.

Hassall (45) describes an Alga under the name of *Haematococcus insignis*, which he suggests should be placed in a separate genus, '*Urococcus*', characterized by transversely corrugated prolongations. The cells are described as being spherical and blood-red, with numerous vesicles and ringed prolongations. It is not unlikely that this Alga is identical with *Chroococcus macrococcus*. It has been shown that the *Chr. macrococcus* is capable of developing a ringed prolongation on being removed from a solid medium to a liquid one. The two algae are identical in size, but though *Chr. macrococcus* often contains several large blood-red pigment spots, it has never been described as completely blood-red in colour. This may possibly result from a prolonged resting condition.

Kützing ('49) refers to *Haematococcus insignis* as *Urococcus insignis*, and states that the stalk is short or absent, and the 'nucleus' blood-red. This is, of course, still more in agreement with *Chr. macrococcus*.

It is also possible that *Chr. macrococcus* is identical with a *Chrootheca* described by Hansgirg ('84). Hansgirg's description of *Chrootheca Richteria-num* is in exact agreement with the characters of *Chr. macrococcus*, except as regards colour. He finds in the cell an orange-coloured, *rarely blue-green* 'chromatophore' with ray-like processes spreading into the periphery of the cytoplasm. The apparent star-shaped orange 'chromatophore' can often be seen in *Chr. macrococcus*, but it has never been described as blue-green. He finds in the older cells of *Chrootheca* a one-sided development of the cell-wall on damp earth, but not in the younger cells, nor in the water forms. As the colour of *Chr. macrococcus* is very variable, it seems not unlikely that the two species are the same.

VII. SUMMARY.

It is not surprising that in many of the Chroococcaceae there should be no nucleus, not even a primitive one. In the course of evolution there must have been a stage in which there was no nucleus, or in which the cell was all nucleus, depending upon the point of view, and we should expect to find this condition in some of the more primitive unicells. The Cyanophyceae are undoubtedly a very primitive and ancient group, and it is not unlikely that some of the early stages in the evolution of the nucleus may be shown in these plants. The type represented by *Chroococcus turgidus* may be a very early stage, in which the difference in function between the inner and outer region of the cell has not yet produced any morphological differentiation.

There is in this species a protoplast consisting of a matrix or ground substance traversed by a reticulum of delicate threads, and at the junctions of these threads small granular thickenings occur. There is no difference in the size of the mesh of the reticulum in any part of the cell. Thus there is no differentiation in form. But there is a difference in composition. In the portion immediately beneath the cell-wall the plasmatic microsomes do not stain deeply with any stains except cytoplasmic ones, and seem to be of the same nature as the reticulum. Further inwards, the plasmatic microsomes appear to contain accumulations of material, and stain deeply with brilliant blue, and in the central region the microsomes contain accumulations of metachromatin, which, although it is not true chromatin, is undoubtedly allied to it. The close connexion between the metachromatin granules and chromatin has probably been the cause of much confusion in the earlier papers on the Cyanophyceae. They react with most chromatin stains, though not all,¹ and Macallum ('99) has shown that they contain

¹ Iodine-green-fuchsin, which invariably stains the nuclei intensely blue and the cytoplasm red in other forms which may be found on the same slide, such as Desmids and Protozoa, never seems to give more than a purplish tint to the central granules.

masked iron and organic phosphorus, and are therefore nuclein compounds, but states that they are not true chromatin.

Fischer denies the nuclein nature of these granules, and says they are simply accumulations of a carbohydrate reserve to which he gives the name 'anabaenin'. It is quite possible that in some forms, and at certain times in other species, the granules in the central region are entirely carbohydrate in composition. The plasmatic microsomes served probably first of all as centres for the accumulation of the excess of food-material elaborated by the pigment in the peripheral region. This would naturally be in the first place of a carbohydrate nature. But as evolution proceeded we might expect that the central region would become a centre for further metabolic activity, and so gradually nuclein compounds would be found as reserve, and finally true chromatin would be evolved. It would be somewhere about this stage that such a form as *Chroococcus turgidus* arose. A few of the plasmatic microsomes in the central region may consist of true chromatin, but this has not been definitely proved.

The difference in staining capacity shown by various parts of the ground substance at certain times has still to be considered. These deeply staining areas correspond in arrangement sometimes to the chromatin shown by Gardner ('06) in his Figs. 4, 5, 9, but the arrangement is not constant; it varies from numerous small areas to one or two large ones, as though the smaller areas gradually merged into each other. They do not stain nearly as intensely as chromatin should, and they are not a constant feature, so that there is no reason to regard them as nuclear structures.

It would appear that when the accumulation of reserve material reaches its maximum the central granules begin to diffuse into the ground substance. A similar diffusion of the central granules into the ground substance has been mentioned by Gardner,¹ and diffusion of both volutin and chromatin into the cytoplasm has been described by Wager and Peniston² in the Yeast-plant.

The majority of the cells showing this diffusion are in a state of active division, and so the dissolved material would gradually be utilized in growth. Then, possibly, a resting period occurs in which nuclein material is again stored up in the central granules. When this accumulation reaches its maximum, active division again sets in, and so eventually the formation

¹ These granules (a granules) disappear before the spore reaches maturity. 'They do not disappear at once, but become gradually smaller and finally disappear entirely.' The a granules probably give up their material either to form chromatin or to form the β granules, and the former is more likely, since they are so closely united to the chromatin.

² 'It is possible that in the yeast-cell there is a constant interchange of chromatin between nucleus and cytoplasm.' Speaking of volutin granules, he states: 'there can be no doubt that they become dissolved in the cytoplasm, which becomes intensely stained with methylene blue just at this stage.'

of chromatin becomes connected with the part division and is confined, in highly developed cells, to that part of the protoplast which is connected with division, i. e. the nucleus.

If this suggestion as to the lines on which nuclear structures have evolved be true, then a stage should exist in which part of the reticulum is definitely marked off and true chromatin should be present, also this portion of the reticulum should have a definite relation to division of the cell.

This stage is actually found in *Chroococcus macrococcus* (Fig. 15). In this species a small portion of the reticulum always differs very markedly from the rest of the protoplast. The plasmatic microsomes contain true chromatin, and this portion of the reticulum has the power of dividing before the cell-wall constricts.

The peripheral portion has also begun to change in structure. The fine reticulum can still be seen in places, especially in the neighbourhood of the nucleus, but the plasmatic microsomes have almost disappeared, and the reticulum is very much broken up and distorted by cell-sap vacuoles.

The nucleus in *Chr. macrococcus* seems to remain permanently in a condition which resembles the resting nucleus of higher plants, except that a nucleolus and nuclear membrane are not present.

The exceedingly small and delicate reticulum found in these unicellular forms has not been described for filamentous species. Probably it does not occur, though this needs verification.

Vacuolization may have entirely crushed out this fine reticulum in the cytoplasm, giving rise to a coarser one, and at the same time the reticulum of the nucleus may have become irregular and thickened, giving rise to the achromatic reticulum of Guilliermond ('05) with chromatin granules embedded in it, or to something approaching the highest modification of Gardner's diffuse type of nucleus, in which 'differentiation seems to be proceeding towards the spireme formation found in the dividing nucleus of the higher plants'. It is not unlikely that the 'spireme' condition of the nucleus is a permanent one in some low types. This is, however, merely a suggestion, and the filamentous forms have yet to be investigated from this point of view.

VIII. CONCLUSIONS.

1. In the Chroococcaceae a highly specialized nucleus of the type found in higher plants does not occur.
2. There is a gradual transition in the structure of the cell from an almost undifferentiated condition in the lower types to a somewhat specialized one, of which *Chroococcus macrococcus* represents the highest type examined, and *Merismopedia elegans* an intermediate stage.
3. The protoplast consists of a ground substance traversed by a reticulum of delicate threads, with thickenings at the nodal points. These are

'plasmatic microsomes' and serve as centres for accumulation of reserve materials elaborated by the pigmented parts of the protoplast. The nature of the accumulation varies in the different regions of the cell.

4. In the majority of species examined by the author there is no definite demarcation of central and peripheral regions, but roughly speaking the microsomes in the central region accumulate metachromatin and correspond to the 'Centralkörner' of Kohl, and in the peripheral region accumulate cyanophycin and correspond to cyanophycin granules.

5. *Chroococcus turgidus* may be taken as an example of this type; though it is possible that it may represent a slightly higher one, since a few of the plasmatic microsomes in the central region occasionally give a true chromatin reaction. There is also in this species a region, just within the cell-wall, in which the plasmatic microsomes are undifferentiated, reacting only with cytoplasmic stains.

6. In *Chroococcus turgidus* the number of metachromatin granules varies greatly in different specimens. If the accumulation of metachromatin is excessive, it appears to diffuse into the ground substance, and a period of active division sets in. At this time the majority of the microsomes in the central region react only with cytoplasmic stains, but one or two very large metachromatin granules can usually be seen.

7. Division takes place in this type by the constriction of the cell into two approximately equal halves, caused by the ingrowing cell-wall. Occasionally the reticulum in the central region seems to stain a little more intensely in dividing cells, and there is a tendency for the threads to become drawn out in parallel lines, though the cross-connexions are not lost.

8. Metachromatin may represent a stage in the formation of chromatin.

9. In *Gloeocapsa* sp., many of the cells show a deeper staining of the network in the central region simulating the 'spireme' stage described by Olive ('05). This may be a more advanced stage in specialization of the central region, but it is probably an artifact.

10. In *Merismopedia elegans*, which represents a higher type, there is a definite 'central body' or 'nucleus' at the time of division. This is not of the same type as the nucleus of higher plants, but is simply an accumulation of chromatin, or some substance allied to it, at the nodal points of a small definite area in the centre of the cell. There is some evidence to show that this 'nucleus' gradually distributes itself along the reticulum after division, to appear again in the centre of the cell prior to the next division. Division of the 'nucleus' takes place before it is reached by the ingrowing cell-wall.

11. *Chroococcus macrococcus* represents the highest type found. Here there is a definite 'nucleus' and cytoplasm. Only the peripheral portion of the 'nucleus' stains deeply with chromatin stains, and contains a fine reticulum with chromatin at the nodal points. The interior of the 'nucleus' is

probably a sap vacuole. The cytoplasm is simply the ground substance of the former types broken up by cell-sap vacuoles, which give it a coarsely reticulate appearance. The fine reticulum is present but is very much distorted, except in the neighbourhood of the 'nucleus'. The plasmatic microsomes are very small and indistinct.

12. It is suggested that evolution of nucleus and cytoplasm has taken place along the following lines: The excess of food-material elaborated by the pigment was first stored in the plasmatic microsomes as a carbohydrate—cyanophycin. As more and more material was elaborated, the reserve in the central region became more complex, and the proteid metachromatin granules were formed. In time, the accumulation of nucleo-protein became restricted to a very limited area in the cell, so as to ensure its equal distribution on division, and this restriction only occurred on division, as in *Merismopedia elegans*. In this way, part of the cell became physiologically and morphologically separated on account of its function in connexion with division. This area may be called the 'nucleus'. At a later stage the 'nucleus' became stable and was always present, as in *Chroococcus macrococcus*. The ground substance also altered in character, forming a definite cytoplasm as described above.

In conclusion, I wish to express my thanks to Prof. G. S. West for suggesting the investigation, for continual help and encouragement in the work, and for providing the greater part of the material examined.

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EXPLANATION OF PLATES XXXIII AND XXXIV.

Illustrating Miss Acton's paper on *Chroococcaceae*.

All figures $\times 2,500$ approximately, unless otherwise stated.

PLATE XXXIII.

Fig. 1. *Chroococcus turgidus*: A, actual appearance of cell; B, semi-diagrammatic optical section showing protoplasmic reticulum. *p.m.* = plasmatic microsomes, *m.* = metachromatic granules. Stained with Loeffler's methylene blue after fixing with absolute alcohol.

Fig. 2. Portion of reticulum, showing accumulations of metachromatin at nodal points. Stained with Delafield's haematoxylin after fixing with Flemming's solution.

Fig. 3. *Chroococcus* [? *Chr. minutus* (Kütz.), Näg.] stained with brilliant blue after fixing with absolute alcohol. *c*, cyanophycin granules.

Fig. 4. *Chroococcus turgidus*. Cells which contain few metachromatin granules, showing differential staining in the ground substance; A, actual appearance; B, semi-diagrammatic optical section. Stained with Loeffler's methylene blue after fixing with absolute alcohol.

Fig. 5. As Fig. 4. Stained with iodine-green-fuchsin after fixing with absolute alcohol. The unstained portions of the ground substance correspond to the deeply stained portions in Fig. 4.

Fig. 6. Section showing that reticulum extends throughout the cell. The darker granules are metachromatic granules, stained with iodine-green-fuchsin after fixing with formalin.

Fig. 7. Cell showing slight elongation of network in central region which sometimes occurs in dividing cells. Stained with iodine-green-fuchsin after fixing with absolute alcohol.

Fig. 8. *Merismopedia elegans*, $\times 1,500$; a colony showing the position of the 'nucleus' in dividing cells; *n* = nucleus. Stained with Delafield's haematoxylin after formalin.

Fig. 9. A, Cells which are enlarging and separating after division; the 'nucleus' is beginning to disappear. B, Cells about to divide; in one cell the 'nucleus' has actually divided, though the cell is only just beginning to constrict. The reticulum is very indistinct and plasmatic microsomes are small. C, Cell in which no 'nucleus' could be seen. The network and plasmatic microsomes are very distinct.

Fig. 10. *Merismopedia glauca*, $\times 1,500$; dividing cells—no 'nucleus' can be distinguished.

Fig. 11. Dividing cells, showing large metachromatin granules and distinct network.

Fig. 12. *Gomphosphaeria lacustris*, stained with iodine-green-fuchsin after formalin.

Fig. 13. *Glotocapsa* sp., stained with iodine-green-fuchsin after Flemming's solution. The apparent thickening of the network in the central region is probably only an artifact due to slight contraction.

Fig. 14. *Aphanolthece prasina*: A, stained living with methylene blue, showing metachromatin granules and network in central region, cyanophycin granules in peripheral region; B and C, stained with iodine-green-fuchsin after fixing with chrom-acetic.

PLATE XXXIV.

Fig. 15. $\times 2,500$. *Chroococcus macrococcus*. Section through protoplast, not cutting the nucleus. *v*, vacuole; *m*, metachromatin granules; *n*, 'nucleus'. Stained iodine-green-fuchsin after fixation in weak chrom-acetic.

Figs. 16 and 17. $\times 2,500$. Sections through 'nuclei' stained with Delafield's haematoxylin. The interior of the nucleus is seen to be unstained and free from granules.

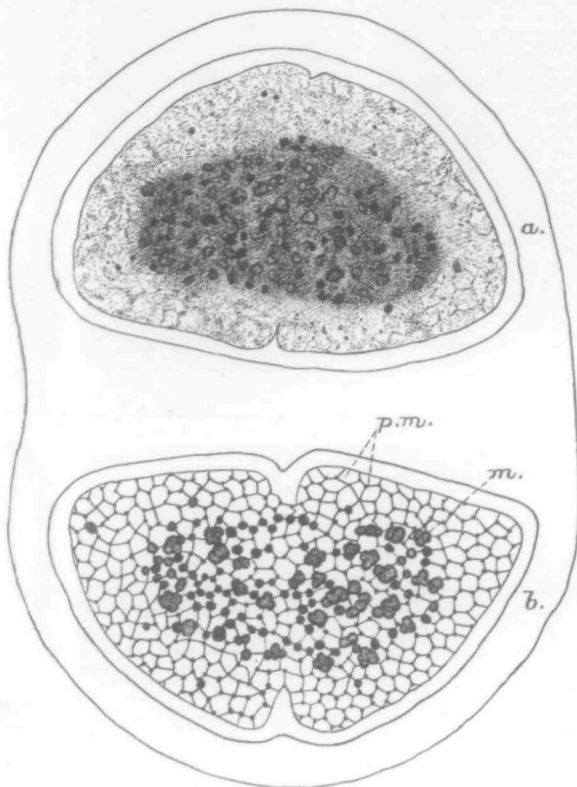
Fig. 18. $\times 2,500$. Nucleus stained with Delafield's haematoxylin, showing division by constriction. The nuclear reticulum is still quite distinct.

Fig. 19. $\times 1,450$. Cells showing nuclei dividing previous to constriction of cell-wall. Stained with Delafield's haematoxylin after absolute alcohol.

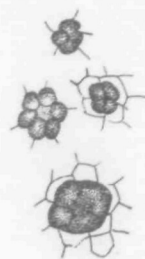
Figs. 20 and 21. $\times 540$. Illustrating the splitting away of the outer envelope of the cell in the normal condition.

Fig. 22. $\times 540$. From a three-weeks-old culture on damp earth. The cell-wall shows a one-sided development.

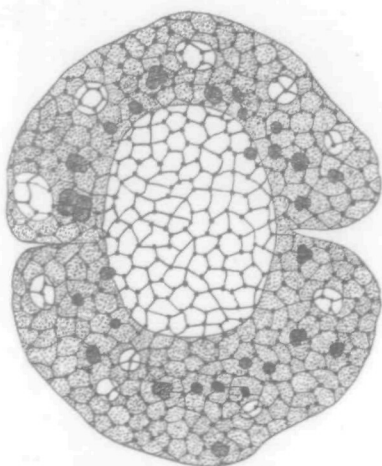
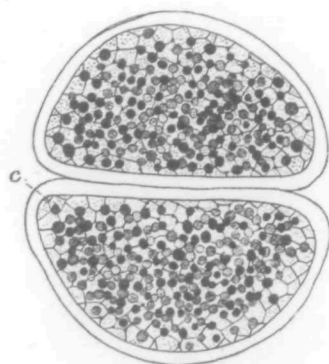
Fig. 23. $\times 540$. From an eighteen-months-old culture on agar after three weeks in a $\frac{1}{2}$ per cent. KNO_3 solution. A distinctly corrugated stalk is formed.



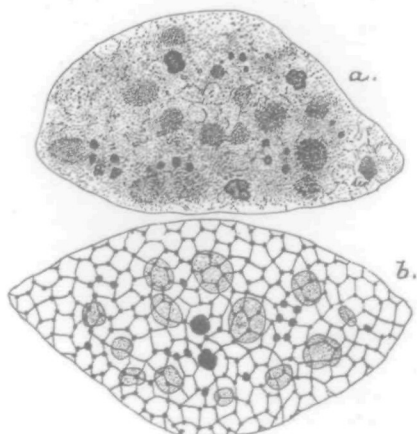
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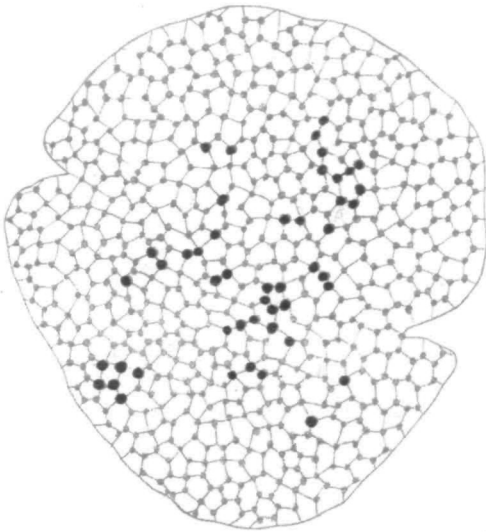
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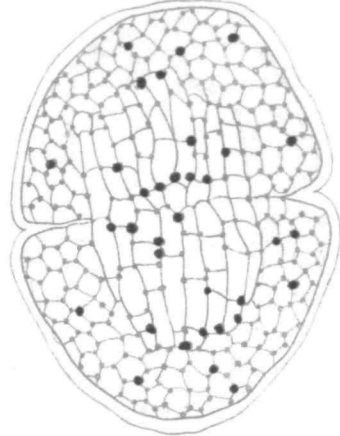
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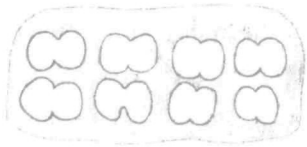
CYTOLOGY OF THE CHROOCOCCACEÆ.



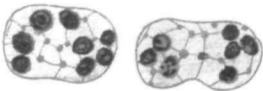
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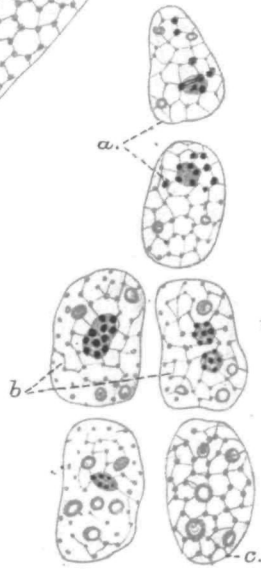
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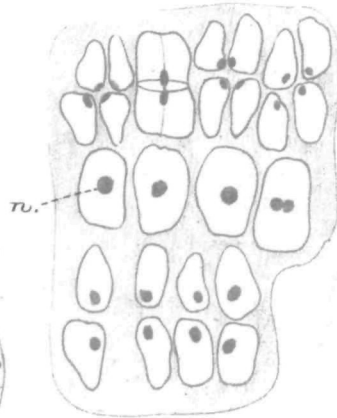
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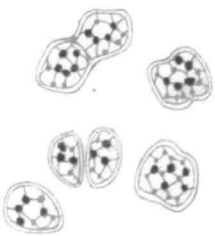
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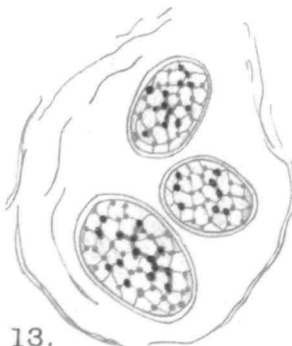
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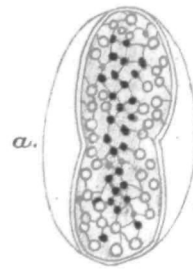
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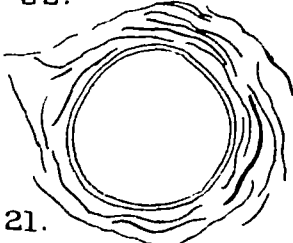
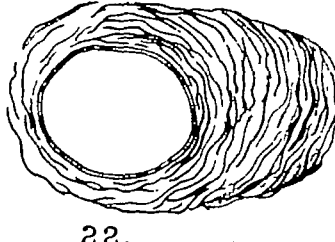
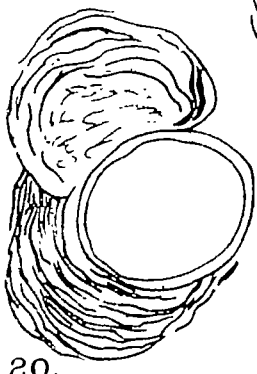
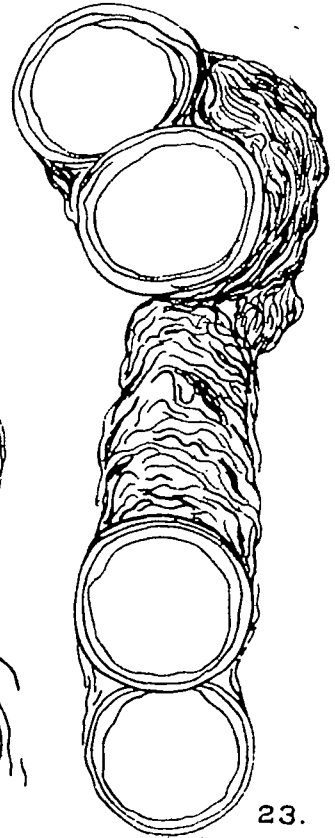
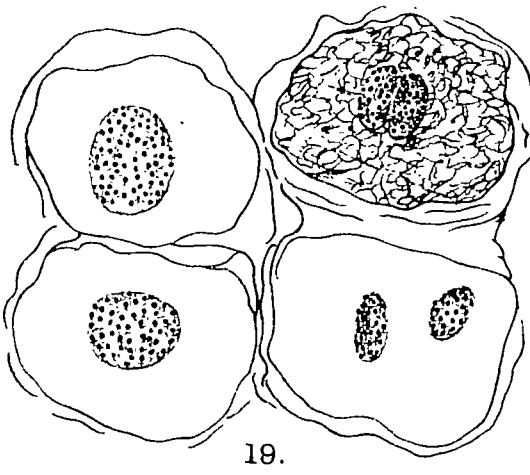
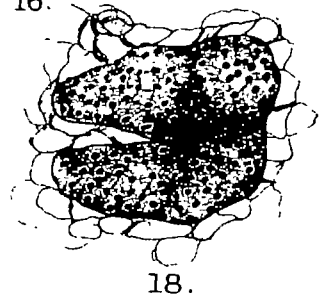
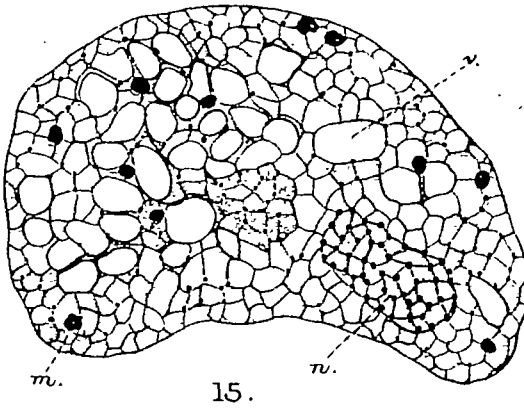


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CYTOLOGY OF THE CHROOCOCCACEÆ.