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# *DICHOTHRIX GELATINOSA* SP. N.

## ITS STRUCTURE AND RESTING ORGANS

BY

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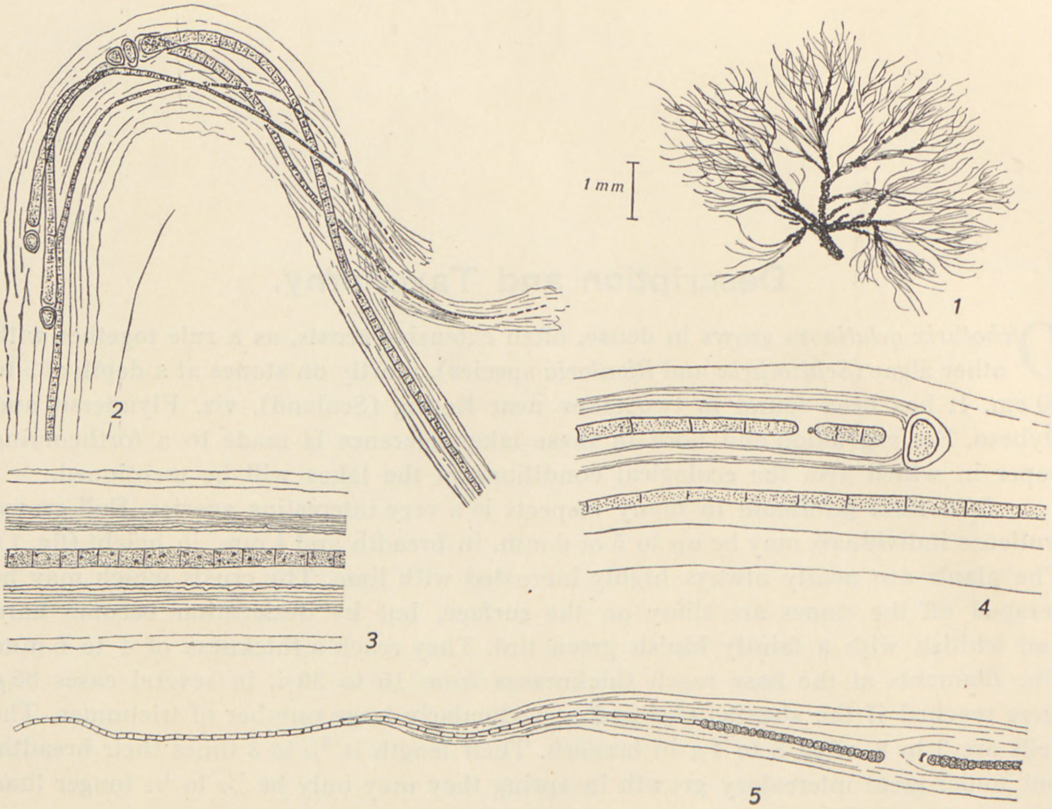
## Description and Taxonomy.

*Dichothrix gelatinosa* grows in dense, often extensive crusts, as a rule together with other algae (*Schizothrix* and *Rivularia* species), mostly on stones at a depth of 5 to 20 cm. It has been found in two lakes near Rørvig (Sealand), viz. Flyndersø and Dybesø. For vegetation and flora in these lakes reference is made to a forthcoming paper in which also the ecological conditions of the lakes will be mentioned.

*Dichothrix gelatinosa* in many respects is a very interesting species. Full-grown fruticose individuals may be up to 5 or 6 mm. in breadth and 4 mm. in height (fig. 1). The plants are nearly always highly incrusted with lime. The crusts which may be scraped off the stones are slimy on the surface, but by desiccation become hard and whitish with a faintly bluish green tint. They reach a thickness of 4 to 6 mm. The filaments at the base reach thicknesses from 15 to 30 $\mu$, in several cases 35 $\mu$ were reached if the sheaths enclosed a particularly large number of trichomes. The cells are 2 to 8, often 3 to 4 $\mu$ in breadth. Their length is $^3/_4$ to 3 times their breadth, but under great intercalary growth in spring they may only be $^1/_3$ to $^1/_2$ longer than they are broad. They may be both constricted and non-constricted at the transversal walls. The occurrence of constrictions practically always appears in particularly broad and short cells, as also in hormogones and hormocysts (hormospores), and in spring in the germinating hormocysts. The heterocysts are 7 to 10 $\mu$ in breadth and 6 to 8 $\mu$ in length, mostly nearly hemispherical. Intercalary heterocysts, however, as a rule are rounded-off cubic. The heterocysts in old sections of filaments are placed in rows of 3 to 6 or more together. Between the old heterocysts and the usual bluish green trichome cells there are often one or a few more or less extended cells with yellowish contents. Resting-spores are found. These have double walls and are 14 to 24 $\mu$ in length and 5 to 8 (rarely 10) $\mu$ in breadth. Also hormocysts occur. These consist of 2 to 10, rarely more cells, which are particularly broad (8 to 9 $\mu$ and 5 to 7 $\mu$ in length). The sheaths as a rule are very thick. The interior parts are colourless above, but downwards generally of a brown colour. Very often the hormocysts are enclosed by particularly transformed sheaths with annular inspissations. Annulation may also occur in filaments without a resting function, but here it is rare and never very pronounced. The sheaths are markedly laminous and are splitting upwards, while widening like a funnel. This is not, however, a rule, as they may taper in connexion with the often long distal hairs. Hormogones are frequently formed in the same way as in other *Rivulariaceae*.




Figs. 1—5. 1: Habitus of fruticose individual detached from the crust. The dark portions are highly incrusted with lime. — 2: Upper part of filament showing ramification. A vigorous lateral branch continues in the direction of the principal branch. — 3: Piece of filament showing interior brown and exterior gelatinous sheath. — 4: Area from lower part of filament with long basal cells. — 5: Tip of filament ending in hair. Formation of hormogone. — All figures rendering material from the autumn. Fig. 1 × 7.5, figs. 2 and 5 × 400, and figs. 3—4 × 780.

## Diagnosis.

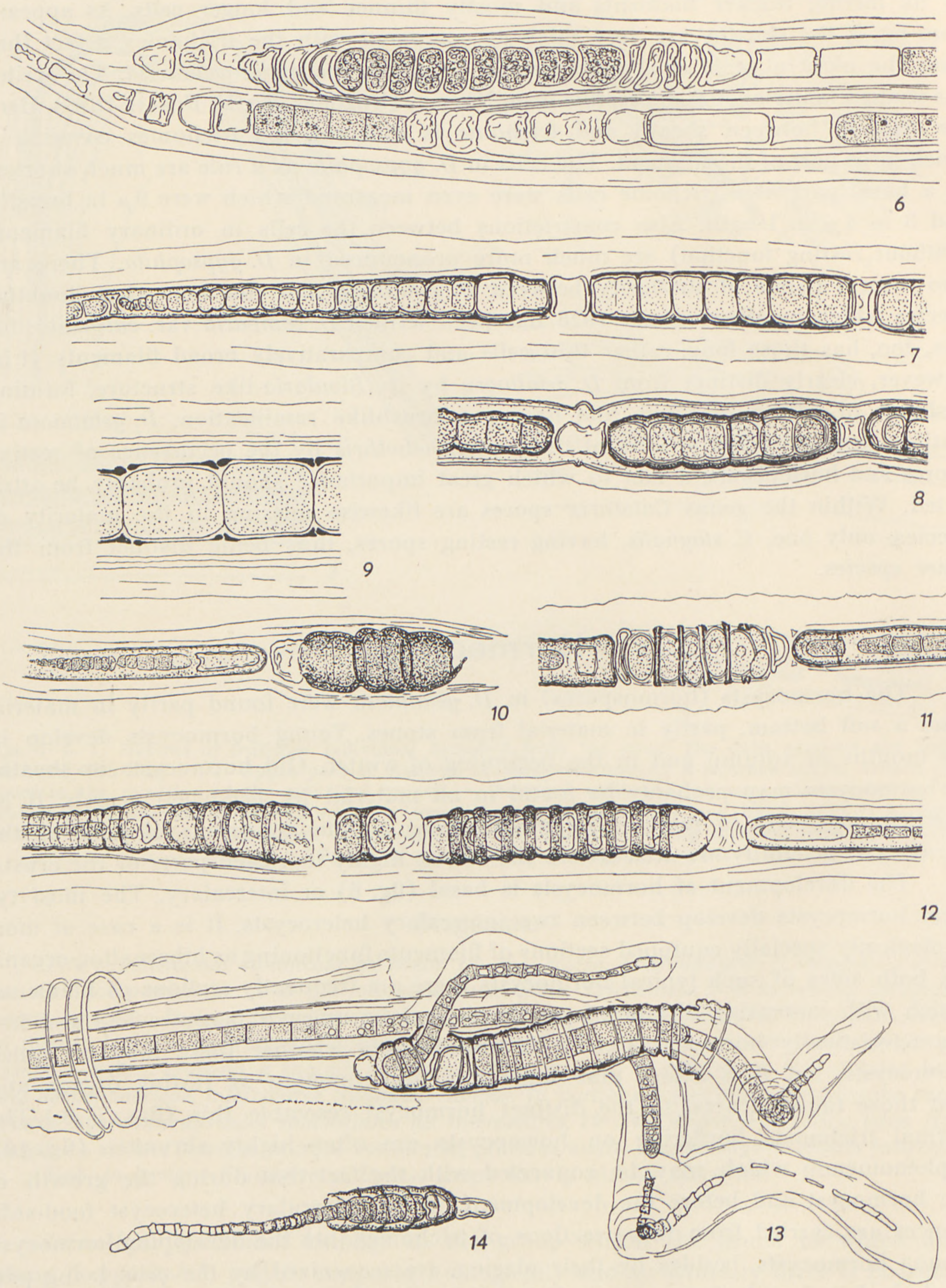
*Fila subdichotome ramificata ad basim 15—35 μ crassa. Trichomata 2—7, saepissime 3—4 μ lata. Cellulae plerumque $^3/_4$—3—plo longiores quam latae. Trichomata 2—6 in vagina communi, crassa, lamellosa inclusa. Lamellae interiores solidae, in partibus inferioribus aureo-fuscescentes vel fuscae; lamellae exteriores gelatinosae vel mucosae. Fila universa crustam constituentia extensam, 4—6 mm. crassam, a summo mucosam, deorsum calce ± induratam. Heterocystae basilares hemisphaericae, intercalares rotundato-cubicae, in partibus inferioribus veteribusque saepe seriatae. Sporae perdurantes subbasilares vel intercalares, 14—24 μ longae, 5—8 (10) μ latae. Hormocystae e cellulis brevibus latisque constitutae, pluribus anulis cinctae vaginis crassis instructae*
*Hab. in lacubus Flyndersø et Dybesø prope Rørvig insulae Selandiae.*

*Dichothrix gelatinosa* belongs to the group within the genus to which also belong *D. gypsophila* and *D. compacta*. Ecologically and structurally there is no small similarity to *D. gypsophila*. *D. gelatinosa* is, however, clearly distinct from the latter by its having thicker filaments and mostly thinner and longer cells. As appears from fig. 3, the sheaths are very thick as compared with the trichomes, a fact that gives the plant a peculiar appearance and suggested the name *gelatinosa*. The writer has studied material of *D. gypsophila* from Norway (Bugönes, leg. M. Foslie 1889). Here the relation between sheath and trichome is different, the trichomes covering a much large part of the filament. The cells in *D. gypsophila* as a rule are much shorter. In a basal part of a trichome cells were even measured which were 9 $\mu$ in breadth and 3 to 4 $\mu$ in length. Also constrictions between the cells in ordinary filaments (without resting function) are much more pronounced in *D. gypsophila*. There are also several points of similarity between *D. gelatinosa* and the somewhat doubtful species which by Woronichin 1923 has been termed *D. compacta* var. *calcarata*; for this, too, has those long, rather thin cells and comparatively broad filaments. It is, however, clearly distinct from *D. gelatinosa* by its *Rivularia*-like structure, forming cushions of only 3 to 4 mm. and having a brushlike ramification. *D. gelatinosa* is distinct from all other species of the genus *Dichothrix* by the occurrence of resting spores and hormocysts, a fact to which great importance should probably be attributed. Within the genus *Calothrix* spores are likewise missing in the majority of species, only one, *C. stagnalis*, having resting spores, thus being distinct from the other species.

## Hormocysts.

The hormocysts (hormospores) in *D. gelatinosa* were found partly in material from a soft bottom, partly in material from stones. Young hormocysts develop in the months of autumn and in the beginning of winter. Old hormocysts or sheaths of hormocysts may probably be found in all material by persistent search. It is, however, necessary to remove the incrustations of lime by means of acid, as the hormocysts generally are attached to the lower, highly incrusted parts of the crusts.

The development of hormocysts is basal (fig. 6) or intercalary. The most typical hormocysts develop between two intercalary heterocysts. It is a case of morphologically specially equipped sections of filaments functioning as hibernation organs. On both sides of such typical hormocysts there are frequently sections of filaments, which with increasing distance from the typical hormocysts becomes less and less characteristically hormocyst-like. It is questionable whether these may be called hormocysts, but it is a fact that sometimes they function as resting organs and that those found nearest to the distinct hormocyst resemble this (figs. 7—8.) The normal trichomes bordering on hormocysts are often highly shrunken (fig. 10), a phenomenon which may be connected with the fact that during the growth of the hormocyst and before the development of the intercalary heterocyst food-substances are carried from these sections of trichomes into the developing hormocyst. Young hormocysts, besides by their placing, are recognized by the cells being particularly broad and short, and constricted at the transversal walls, and the walls




Figs. 6—14. (Explanation see p. 7).

being fairly thick. Add to this the special conditions of the sheath to be mentioned below. There is a great difference in the thicknesses of original and newly-formed walls. In fig. 8 four thick walls and two quite thin ones are seen, developed after intercalary divisions in the hormocyst. The formation of new cells which may take place during the development of the hormocyst in some cases causes the hormocyst to apply pressure to the heterocysts placed on both sides of it (fig. 6). Hence, these heterocysts become flattened or cup-shaped. During the growth of cells food reserve is accumulated. The cells may contain one or many fairly large granules. These show negative reaction to iodine and faint reaction to Millon's reagent, and they also react to thymol and sulphuric acid. Hence the granules perhaps have a content of gluco-proteids.

The formation of sheaths round the hormocysts is very peculiar. In a few cases the sheath is clear and transparent (fig. 6), but as a rule it develops a brown colour and is provided with annular inspissations opposite to the transversal walls of the cells. The formation of the rings is seen in figs. 7—9, particularly fig. 9, which shows an optical longitudinal section through the axis of the filament in great magnification. In these cases the inspissation can practically be seen only in profile of both sides of the filament, while in the middle, where the light passes through it transversely, it may at most be barely visible as a slightly darker, brownish transversal zone. As appears from fig. 9, the annulations from the beginning are always double, both adjacent cells taking part in their formation. Sometimes the double annulation may also be seen at later stages (figs. 11 and 13). In fig. 8 the trichomes are surrounded by two thick brownish sheaths. Of these the interior one is darkest and is developing annulations outside two of the thickest cell-walls.

Similar annulations have been described in detail in *Hydrocoleus lyngbyaceus* (Schönleber 1937), but not in connexion with resting organs here. The annulations especially develop in old sections of filaments and always with a certain regular reciprocal distance suggesting that they have developed off the transversal walls in a filament which has later elongated by intercalary cell-divisions. The annulations probably develop off the original, oldest transversal walls. This explanation, which was offered by Schönleber, is strongly supported by conditions in *Dichothrix gelatinosa*. In *Hydrocoleus* the annulations apparently are single, as often in *Dichothrix gelatinosa* (see, e. g. fig. 12). For that matter there is a great difference in the annulation taking place in the two species; in *Hydrocoleus* it only occurs in the gela-

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Figs. 6—14. Hormocysts. 6: Basal section of filament with four trichomes, two of them partly covering the others. In one a hormocyst is developing. — 7: Young hormocyst and hormocyst-like piece of trichome. Incipient annulation. — 8: Young hormocyst surrounded by two sets of brownish sheaths, the interior one of which is developing rings. — 9: Optical longitudinal section showing incipient annulaiton. — 10: Old case of hormocyst. — 11: Hormocyst with double rings and interjacent transparent parts of sheaths. — 12: Row of three hormocysts, one of which (extreme left) has germinated or died away so that only the case is seen. — 13: Germinating hormocysts. Annulation in the gelatinous sheath on the extreme left. — 14: Germinating detached hormocyst. — Figs. 6—12 render material from the autumn, 13—14 material from April. All figures except no. 9 × 780. Fig. 9 × 1330.

tinous sheath, and in *Dichothrix* it is closely connected with the innermost, harder portions of the sheaths. In the latter we may, however, as appears from fig. 13 left, find rings of apparently the same type as those found in *Hydrocoleus*, even though they do not seem to constrict the sheath. I have observed such clear transparent rings in very few cases. The only plant actually resembling *Dichothrix gelatinosa* as regards annulation of the sheath, is the South African *Calothrix gelatinosa* F. E. Fritsch.

Thus there is much to indicate that the sheaths in the *Cyanophyceae* are secreted through the cell-walls. If so, it is peculiar that the thickest portions (the rings) are found just outside the transversal walls, from which we cannot imagine the occurrence of a secretion. However, it proves that the rings from the start are double, with a distinct partition between the portions of the rings outside the transversal wall. Hence we are led to the view that the protoplasm at the transversal walls secretes more sheath substance than the rest of it. In this connexion fig. 11 is of particular interest; for in this case only the rings were brown, while the broad interjacent sections of the sheaths were clear. So there may also be chemical differences between the rings and the rest of the sheath. The rings in *Dichothrix gelatinosa* may no doubt be apprehended as a further development of the incurvations in the layers of the sheaths seen outside the original transversal walls in *Petalonema alatum* (see fig. 18 in Geitler 1936). Their signification in connexion with the hormocysts is quite uncertain. Probably they offer a further support against pressure from the outside to the interior layers of sheath, which are strong beforehand. By freezing over the lakes a rather high pressure may no doubt be brought to bear on the sheaths against which the incrustations of chalk cannot alone protect the living cells.

In material collected in the beginning of April numerous stages of germination were observed. No very early stages, however, were found; in most cases the hormocyst had already formed a long filament tapering into a hair (fig. 14). In the specimen rendered in fig. 13 the basal, originally two- or three-celled, hormocyst has dissolved the sheath-wall and formed a filament at the outermost end of which a vacuolization is taking place. It is being transformed into a hair. The other hormocysts, on the other hand, has germinated in continuation of the old filament. This is probably one of the not quite typical hormocysts, only the cells nearest to the base having been fitted as resting organs. It probably corresponds to the part of the filament in fig. 7 situated farthest to the left and in which the cells become smaller the farther they are from the genuine hormocyst. It seems that the distal part of filaments in fig. 13 is being thrown off. This, as regards the hormocyst-like part means the part situated outside the inspissated sheath. In some cases it seems that the trichomes of the hormocysts may behave as hormogones and leave the cases of the sheaths. At any rate the specimen rendered in fig. 12, which originates from the autumn, shows an empty hormocyst and two hormocysts with trichomes in them. One of these is two-celled, the other multi-celled. In the latter the boundary of the cell does not fit exactly with the very distinct rings. Probably this is a very old hormocyst which has not germinated and where, therefore, a shrinking of the trichome has taken place inside the sheath. The plant shown in fig. 10 also originates from the autumn and also shows an empty hormocyst-case. In fig. 11, likewise material from the autumn, the ring found farthest to the right is visible on one side only, the one facing downwards. On the side facing upwards a small extra cell of a width like the ordinary trichome cells was found. This perhaps indicates a germination which has stopped.

The existence of hormocysts is mentioned for few *Scytonemaceae* and *Stigonemaceae* (Geitler 1936, p. 69). These differ from the hormocysts in *Dichothrix* by being without rings and, as in *Westiella lanosa*, by being without heterocysts. Also the hormocysts described from *Tolypothrix distorta* by Cholnoky (1935) differ, the heterocysts in this species perishing during the formation of hormocysts. This, for that matter, is introduced by a dying of cells in front of and behind the hormocyst. Even though very similar phenomena may no doubt be found in the *Dichothrix* species (fig. 6), there are often cases here where no cells seem to be dying or only to be particularly starved in the nearest surroundings of the hormocysts. In *Dichothrix* only such hormocysts as develop as small lateral shoots near the distal end of a vigorous filament (figs. 13—14) now and then are detached so that they may contribute to the dispersal of the species. In other species with hormocysts it frequently occurs that these are detached.

## The Spores.

Spores are found in material from all seasons, Still, young spores are seen only in material from late summer and from the autumn. In spring, after the germination of the spores has taken place spores may still be observed in old sections of filaments. These, however, as a rule are without any contents (fig. 15) and must be interpreted as ungerminated spores.

The resting spores may be basal or intercalary. As seen in fig. 22, there may be three spores in one trichome. The occurrence of intercalary resting spores is very remarkable, as all other resting spores in the *Rivulariaceae* are placed basally, at the heterocyst. Basal resting spores in *Dichothrix* are always adjacent to heterocysts. Ordinary trichome cells adjacent to resting spores are often remarkably shrunken or no more visible at all, because they have almost completely degenerated. In such cases only a plasmatic cord is seen in the middle of the filament, which near the spores is extended infundibularly. It seems to be fairly obvious that the growth of the spore takes place at the expense of these cells.

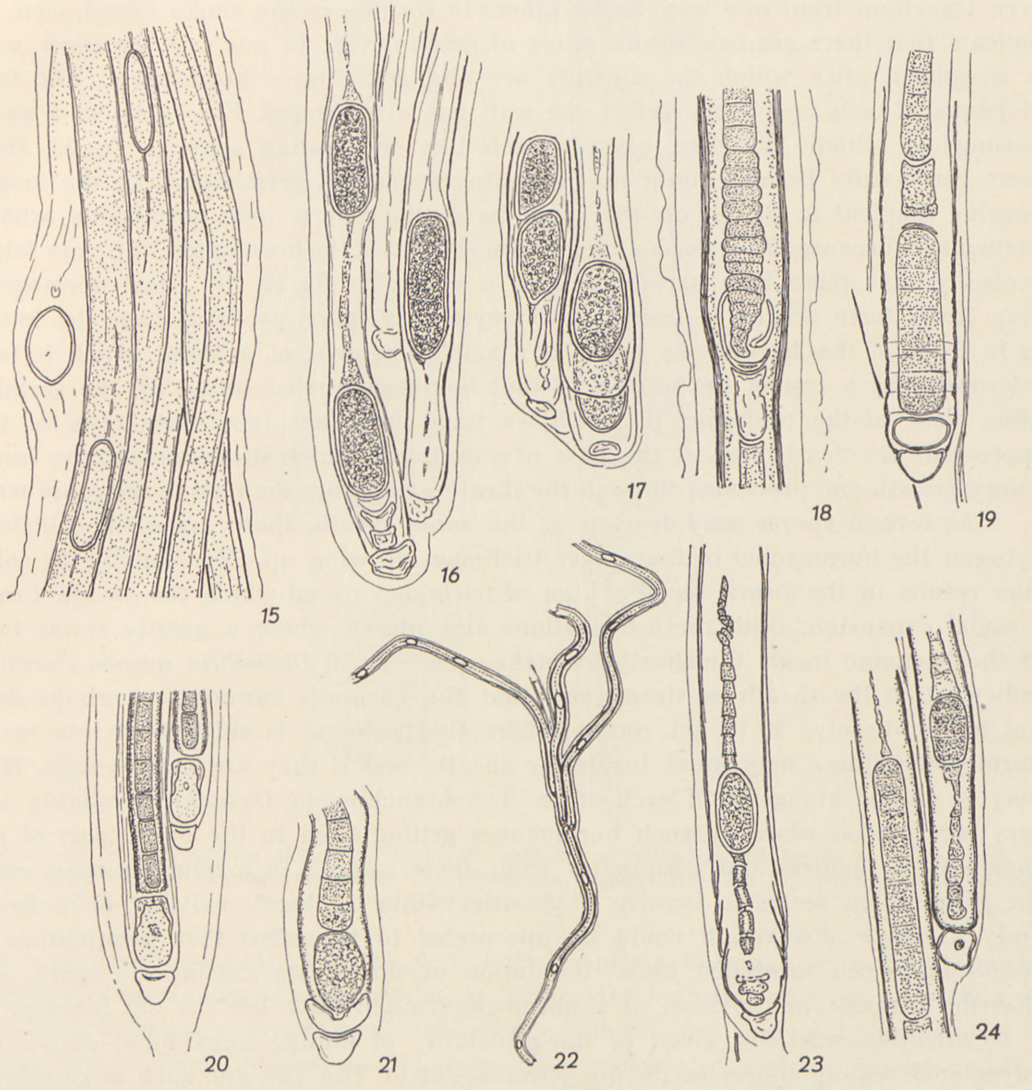
The wall of the spore consists of two layer, which, however, cannot always be distinguished. By addition of chloro-iodide of zinc besides the walls of the heterocysts, also the substance of the sheaths and the outermost layer of the spore wall are stained, a fact which greatly supports the view advanced by Fritsch (1904) that this layer has arisen by transformation of the innermost part of the sheath.

The cells which seem inclined to develop into resting spores are yellowish. But not all yellow cells become resting spores. They may either degenerate or be transformed into heterocysts. The large cell in fig. 21 has a fairly thin cell wall and yellow contents. It will probably develop into a spore; but it seems that, if anything, the corresponding large cells in fig. 20 are rather developing into heterocysts. Each of these has a peculiar cell wall, half of which is greatly inspissated and lamellose, and has a central pore. Perhaps these are cells which originally wanted to develop into spores, but later for some reason developed into heterocysts. The rather long yellowish cells are found at the base of many filaments (fig. 6), often probably neither developing into resting spores nor into heterocysts, but degenerating and at last, when they have died, they may collapse completely or partly, or be pressed together or aside by the living trichomes. Basally in the filaments there may be long rows of such long degenerated or dead cells. Closest to the green cells are the yellow ones, farthest from these there are colourless cells.

The fully developed resting spores are faintly yellowish and like the hormocysts full of food reserve. The cells of the hormocysts, however, are green and differ from the spores by having a clearly seen chromatine apparatus (fig. 27), which in the spore seems to be changed into substances which are little stainable. Once a spore was found in a trichome and a hormocyst in the adjacent trichome in the same filament; as a rule the two organs do not occur in the same filament and often it is possible in one sample af algae to find a majority of hormocysts, in another a majority of spores.

The spores mostly are situated in the lower parts of the filaments, where the sheaths are brownish, thick, and strong. The lower parts of the crusts of *Dichothrix* frequently chiefly consist of brownish sheaths, the cells of which are dead. In these there may by numerous walls of dead resting spores which have not germinated (fig. 15). In the specimen depicted there is one spore in the exterior gelatinous part of the sheath. It originates from a trichome long completely dead, of which only a single rest of a cell may be observed. The other spores are found within strong brown sheaths, and here several rests of cells may be seen near the spores. By observing the rests of the trichomes it was possible to make out that those with brown sheaths (five in all) had developed as lateral shoots from the colourless trichome now completely degenerated. In some cases an incipient annulation could be observed in the sheaths round the spores.

In material from the month of April it was tried to find stages of germination. Unfortunately the time was not suitable as it proved that the majority of the resting spores had germinated. In fact it was very difficult to estimate the large number of germinating trichomes correctly. Some of them, however, had evidently developed from hormocysts, others from spores, while the origin of the great majority was more doubtful. Fritsch (1904) in *Anabaena* has shown that the germination of the spores can take place in two ways. The contents are either "protruded from the ruptured spore-membrane by the formation of mucilage from the

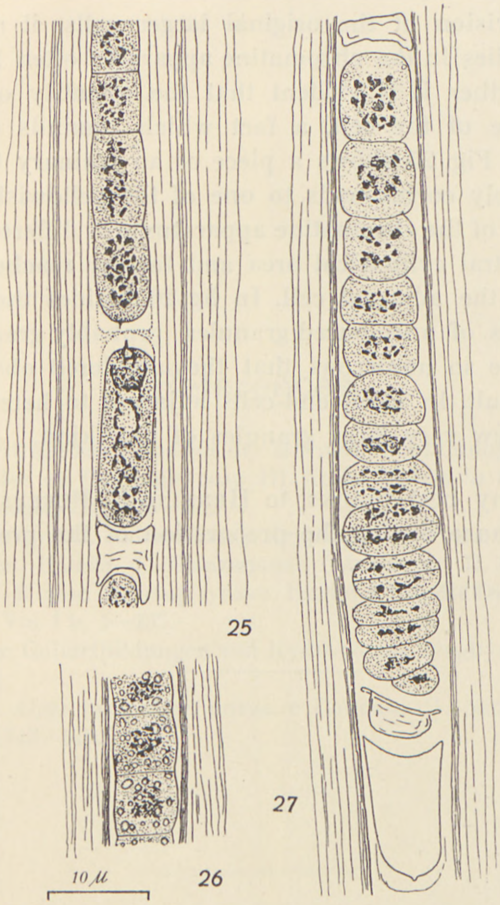



Figs. 15—24. Spores and germination of spores. 15: Basal section of filament with empty sheaths and spore-walls of ungerminated spores. — 16: Basal piece of filament with three spores full of food reserve. — 17: The same with four spores. — 18: Germinated spore, the spore-wall burst. Strong intercalary growth, probably as an introduction to formation of hormogones. — 19: Basal vigorous piece of trichome, probably originating from a spore. — 20: Basal piece of filament with cells in a transitional stage between heterocysts and spores. — 21: Young spore. — 22: Piece of filament with 11 spores, of which at any rate the lower ones are dead and empty. — 23: Intercalary spore surrounded by degenerated cells. — 24: Short vigorous trichome probably developed by germination of an intercalary spore. Degenerating cells are seen on both sides of it. — Figs. 15—17, 20—23 render material from the autumn, the others material from April. — All figures except fig. 22 × 780. Fig. 22 × 45.

protoplast; or the spore-membrane itself becomes mucilaginous, whilst the contents retain their original position in the thread." Fritsch also states that there is an even transition from one way to the other. In *Dichothrix* the stages found seem to indicate that there are two similar ways of germination. In one case the spore wall is dissolved, after which the contents are changed into a hormogone. The first divisions of cells may occur before the wall has disappeared. Fig. 24 shows a short hormogone, which has been placed much like the resting spore in fig. 23. Here there can hardly be any doubt that a resting spore has germinated. In the neighbouring filament in fig. 24, on the other hand, there is a long hormogone, where, particularly because of the degenerated plasm in front of the hormogone, it is only fairly probably that there is a germination of a spore. In fig. 19 the basal hormogone may either have originated from a hormocyst or a spore, probably from the latter, as in front of the hormogone there is a small fragment of a shell, which is best interpreted as a rest of the spore-wall that has been carried along by the germinating piece of the trichome. In fig. 18 we have the other type: here rests of the spore-wall are clearly seen at the base of a trichome which shows an intense intercalary growth and protrudes through the sheath away from the rests of the spore-wall.

As several spores may develop in the same sheath, there are often collisions between the hormogones or the longer trichomes growing up. Occasionally the collision results in the formation of a knot of trichomes round which the sheath forms a nodal expansion. Both these formations and places where a greatly spiral turn of the trichome inside the sheath has taken place as in *Dichothrix spiralis* Fritsch, indicate that the sheath is strong and that the trichome cannot, or perhaps does not try to dissolve it to get room. Where the trichome is subdivided into many hormogones, these may move inside the sheath, and if they are thin enough, they may be shifted alongside of each other. The branching in *Dichothrix* probably not very rarely takes place by such hormogones getting fixed to the upper part of the sheath of a filament and germinates from there, ending in a hair. In most cases the germination seems to involve a growth within a sheath existing beforehand. Only a single observation could be interpreted to the effect that germination of spores had been succeeded by a dissolution of the sheath outside the spore, and that the contents in the form of a hormogone might have left the old filament.

Attention was also given to the possibility of finding transitional stages between such resting spores as do not germinate at all (fig. 15), and such as germinate into a vigorous trichome. Even though such stages could not be demonstrated with certainty, they are very probable. Many old basal sheaths in some sections may be empty, while in other places they contain some faintly observable hormogone-like bits of trichomes. These may have arisen by the germination of some spore, but they are of no material importance as the assimilation in the basal portion behind the brown sheaths and the crusts of lime presumably are too small. Perhaps the completely missing germination of some spores is also conditioned by the failing light. Besides, it should be emphasized that hormogones or the more or less isolated




Figs. 25—27. The chromatin apparatus. In fig. 25 probably a yellowish cell beside the heterocyst. — In fig. 26 granules of cyanophycin round the chromatin apparatus. — Fig. 27: Young hormocyst. All the figures × 1330.

pieces of trichomes in the sheaths do not always originate from spores. They should probably often simply be apprehended as further developments of pieces of trichomes that have hibernated in sections of filaments without any special equipment as a resting organ. Thus hormocysts and spores only become of decisive importance for the continued existence of the species in particularly unfavourable winters.

## The Chromatine Apparatus.

As mentioned above the hormocysts contain a chromatine apparatus corresponding to that of the ordinary trichome cells. Fig. 27 shows its appearance after fixation with Nawashin's fluid and staining with Heidenhain's haematoxylin (differentiation with picric acid). In the cells which in the young hormocyst (fig. 27) have developed by division of the original larger cells, it may be seen how the individual coloured bodies in the chromatine apparatus often have a partner in the corresponding cell. Further it is evident that the quantity of stainable substance decreases with the size of the cell, a fact which reminds of the nucleo-plasmic ratio in nuclear plants. Fig. 25 shows a piece of an ordinary trichome and a rather long cell which probably corresponds to one of the yellowish cells mentioned. In this cell the appearance of the chromatine apparatus was different as it chiefly seemed developed round a central cylindrical area and further reached as far as the plasmatic connexion with the adjacent cell. In fig. 26 besides the chromatine granules there are large numbers of uncoloured granules, probably granules of cyanophycin. In some cells they were so numerous that they rendered observation of the chromatine apparatus difficult. In basal end-cells adjacent to heterocysts we may often observe one or some few very large granules of that kind.

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