Nutritive Changes in Spirogyra.

G. Mann.

1/3/89.

Nutritive Changes in Spirogyra-species as representatives of Fresh-water Algae.

The reasons I chose Spirogyra were these.

1. It is the commonest fresh water alga about Edinb?, and a fresh supply could easily be procured when required.

2. Its comparatively simple structure made it specially suitable for microscopical and microchemical investigations.

Spirogyra belongs to the Order Conjugatae and forms one of the subdivisions of the Zygnemaceae.

Spirogyra consists of long unbranched filaments forming large floating patches, specially in ponds and slow waters. Each filament consists of a series of cells, cylindrical and with the proportion of the thickness of the cell to its length greatly varying. Taking any large celled species, e.g. Spirogyra crassa, Sp. nitida, Sp. jugalis etc we notice a peripheral layer of protoplasm embedded in which are one or more spirally arranged chlorophyll bands; in the centre of the cell we find a nucleus suspended in the cellsap by threads passing from it towards the chlorophyll bands.

In my paper I shall go minutely into the different constituents of the cell and try and point out what function each plays in the nutrition.

To save myself the trouble of being constantly on the hunt for fresh material I endeavoured to grow Spirogyra material in the room in which I was working. The material I got from Luddington lock, which abounded specially in Spirogyra nitida and jugalis, and in September there were, specially on the south side of the lock, large banks of Sp. nitida in splendid condition. I may as well state here shortly the condition of this alga during the present mild winter; up to the end of November both Spirogyra nitida and jugalis were healthy but about middle of December Sp. jugalis began to decay, and about end of December 90 % of the threads I gathered were dead or in a decaying condition. Spirogyra nitida had kept remarkably well, only a few threads, about 10 % showed any sign of disease; - The large masses of dead Spirogyra material which were lying on the bottom of the lock showed a pinkish brown colour.

due to pigmentary deposit.
ation, which was situated at the periphery of the cell and which seemingly had been derived from the chloroplasts. I have never seen this colour in any of the material that decayed while I was cultivating it.

To preserve spirogyra in cultivation I found Strasburger's method answer very well: The material is placed in shallow vessels with opaque walls to prevent unilateral light acting on the plant, but at the same time the vessel is placed in a light place protected from the direct action of the sun, & a room towards the north is to be preferred. From time to time pieces of turf, soaked in following nutrient fluid, are placed in the vessels containing either river or spring water.

water	100 ccm.
potassium nitrate	1 gram.
sodium chloride	½ gr.
calcium sulphate	½ gr
magnesium sulphate	½ gr.
calcium phosphate	½ gr.

I also tried the formula given by Detmer

1 grm Ca_2NO_3
0,25 g KCl
0,25 g $MgSO_4$
0,25 g KH_2PO_4
} in one liter of distilled water.

but found this solution to be too strong for Spirogyra, if I took 1/2 liter instead of one I got a solution in which Spirogyra grew vigorously. To either solution I added a few drops of ferric chloride.

In all the cultivation methods I found it to be of the highest importance to give the plants daily a good supply of carbon dioxide, which I prepared in the usual way with nitric acid and pieces of marble, and which I purified by caustic potash. The way I proceeded was this: from the bottle with the KOH-lie an indiarubber tube passed to the vessel in which the Spirogyra grew, and into its end was fixed a glass tube which was drawn

out into a fine point, to allow the gas to escape in a stream of minute bubbles, when the glass tube was introduced into the respective vessel with the Spirogyra material.

This process of providing CO_2 lasted for 10 minutes in a vessel containing 5 liters of water.

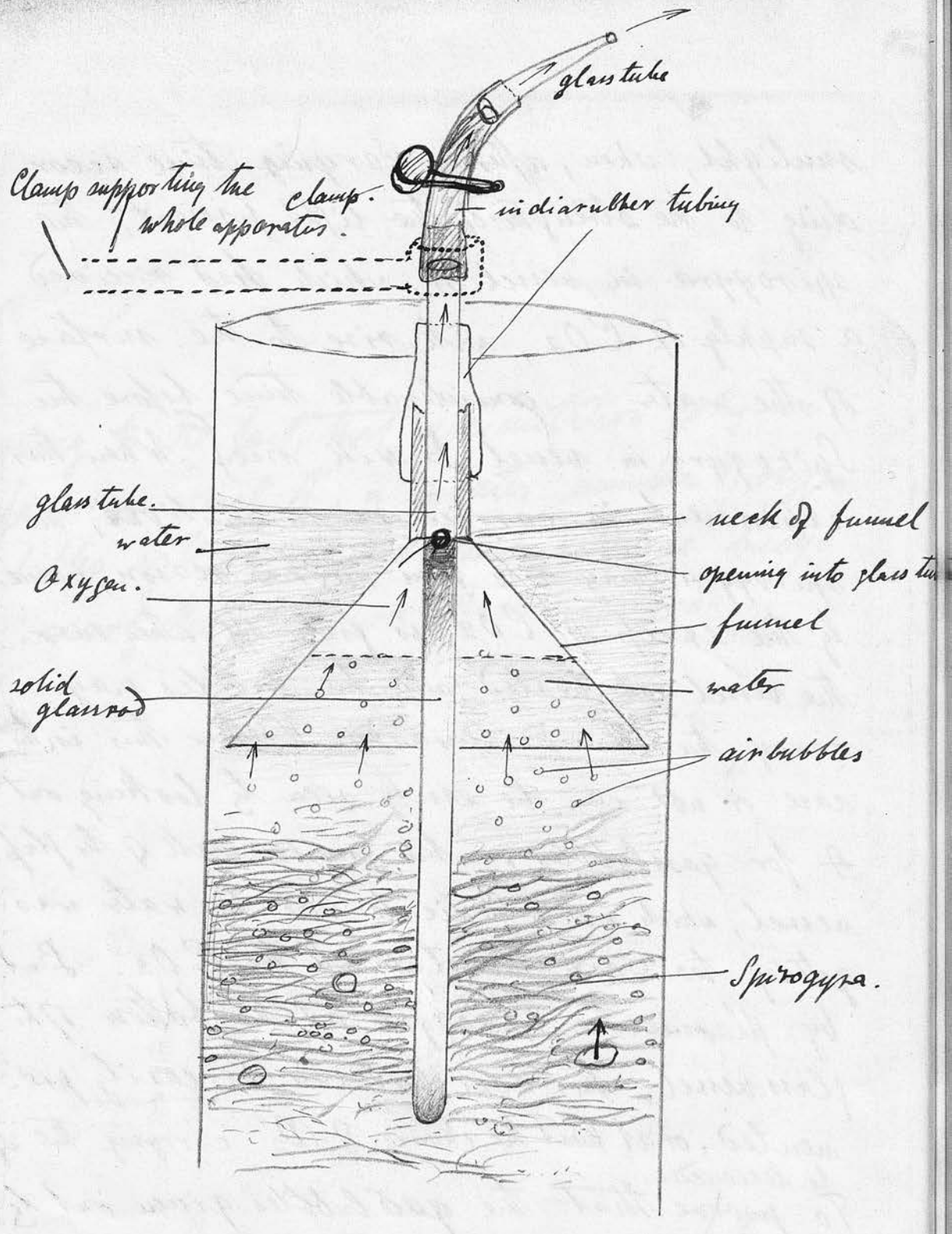
The effect produced by the CO_2 was very well seen in following simple experiment.

Take two glass jars 4 inches broad by two feet high, fill them with ordinary water up to 4 inches from the top, pass through vessel A, a stream of Carbon Dioxide for 10 minutes, while vessel B does not receive any CO_2, besides what is present in the water. Next take an equal quantity of Spirogyra and with a glass rod which is flattened at the base push the Spirogyra to the bottom of vessels A & B; Then expose both vessels to bright daylight or to direct

Owing to the fact, that, assimilation going on faster in vessel A, a greater number of oxygen bubbles will be set free which acting as buoys carry the spirogyra threads upwards.

sunlight, when, after a varying time according to the strength of the light present, the spirogyra in vessel A, which had received a supply of CO_2, will rise to the surface of the water a considerable time before the Spirogyra in vessel B will rise. When this experiment is carried on in sunlight, the spirogyra may rise from another reason, namely the excess of CO_2 is given off when ever the vessel gets heated and the bubbles may carry the threads upwards. Whether this is the case or not can be easily seen by looking out for gas bubbles on the inner wall of the glass vessel, which would indicate that the water was getting too warm to retain all the CO_2. But by placing the Spirogyra at the bottom of the glass vessel, this accident can be easily prevented, or at least the chance of O_2 carrying the Spirogyr. upwards be lessened.

To prove that the gas bubbles given out by Spirogyra material are Oxygen, I found following way the best, since the bubbles are en-


glass tube
Clamp supporting the whole apparatus.
clamp.
indiarubber tubing
glass tube.
water
Oxygen.
solid glass rod
neck of funnel
opening into glass tu
funnel
water
air bubbles
Spirogyra.

tangled amongst the threads of the ascending mass and are not easily separated.

A funnel, one inch less in diameter than the vessel in which Spirogyra is assimilating, has a glassrod placed in it, which is solid up to the neck of the funnel, and thence hollow and communicating with the interior of the funnel by a small hole. Funnel and glass tube are connected by a piece of indiarubber. The upper part of the glass tube is connected by a short piece of indiarubber tubing to a glass piece, 1 1/2" long and drawn out into a fine point. The indiarubber tubing connecting the 2 glass tubes is provided with a clamp.

The apparatus is placed on the surface of the water immediately after the Spirogyra has been placed at the bottom of the jar, next the clamp is opened when the air out of the funnel escapes through the glass tubes. As soon as the water has risen to the level of the hole in

the glass tube, the clamp is shut, and the apparatus which was floating at first is now immersed up to the neck of the funnel. To prevent it sinking down it may be kept in this position by a wooden clamp as used for holding test tubes, or 3 pieces of wire may be twisted round the indiarubber holding the glass-tube and the funnel together, and the ends of the wire placed on the glass-jar. When the Spirogyra has risen high enough to be reached by the glass rod, it is gently moved to disengage the bubbles, most of which will be caught by the funnel. After a considerable portion of gas has been collected in this way, it may be drawn off by opening the clamp, when the weight of the apparatus by sinking into the water will force the gas out. While the gas is escaping, a match, put to the end piece, by burning brighter will show that the gas is Oxygen.

To proove that light is an essential factor in assimilation, a third jar was prepared as just described and after Spirogyra-material had been placed at the bottom of the jar, it was placed in the dark room, and to make sure that even not the slightest trace of light was present the jar was covered with a box. If now after the Spirogyra in vessel A, which had received an excess of CO_2 had risen to the surface, and also the meterial in glass B after a longer period had reached the top, the Spirogyra in vessel C which had been placed in the dark was still remaining at the bottom and even after 24 hours showed no sign of rising. If we now judge by the amount of Oxygen given off, the amount of assimilation going on in the plant, we may from above experiment see that an alga with a larger supply of CO_2 will assimilate more rapidly than one which has only a little CO_2; and that to start assimilation and to keep it going light is necessary.

Besides judging by the amount of gas given off we may come to the same end by watching the most visible product of assimilation, namely starch, being formed in the Starch centres.
Strong species of Spirogyra, e.g. Sp. nitida were placed for 3 days in the dark, to allow all the Starch stored up in the pyrenoïd to be consumed; to ascertain whether this was the case or not, some of the material which had been kept in the dark was tested in following way: It was placed (partly I) in a solution of 5 parts of Chloral hydrate to two parts of water, to which a little iodine in iodide of Potash was added. The starchgranules round the pyrenoïd if present swell up and showed a blue colour, — another part was treated with a solution of one part of Iodine, 3 parts of KI, and 60 parts of water, when the starchgranules turned a bluish black.
If both these treatments gave negative results, 3 vessels, A, B, C, were filled with either springwater or with nutrient solution, and CO_2 gas passed through A & C, while in B only the gas which

was normally in the vats, was present.
The material freed from starch was placed quickly in vessels A, B, C, and the thread separated as much as possible, so that each thread held a considerable portion of water to itself. Next the vessels A & B were exposed to diffuse light, while vessel C was placed again in the dark.
In my experiments after 2 hours distinct starch-granules were to be seen in vessel A, and after 3 hours in vessel B, while the material in vessel C did not form any starch.
I have never been able to see any traces of Starch before 1 hour and 30 minutes, the reason being that in winter the activity of the cells seems to be decreased, partly due to the low temperature, and partly to the sun being less active. Detmer in his Practicum states that already after 30 minutes large quantities of Starch can be seen.

To study the relation of the starch granules to the protein crystal in the hypertrophied tissues the best ways are these:

1/ Detmers method :

Spirogyra is placed in strong warm Alcohol which in about 10 minutes will have extracted the whole of the Chlorophyll. The bleached threads are laid either for a short time in hot or for 20 hours in cold into much concentrated caustic potash (a 5 % solution I find best), next the threads are carefully washed out with dis. filled water, then treated with dilute acetic acid to neutralize the potash completely, again washed in water and placed in a solution of

0,05 Grm Iodine
0,2 Grm Potassium iodide
15 Grm. water.

—

2./

Treat the threads for about 2 minutes with liquor chlori when the starch granules will become very evident.

Some observations made while cultivating Spirogyra.

When a mass of Spirogyra material was placed in a vessel it always sank at first to the bottom, then the more vigorous threads began to grow upwards, i.e. towards the surface of the water, & very often the threads formed a ribbonshaped mass or masses, and thus it was possible to distinguish a foot, a shaft, and an apex in each thread, I make this division for following reason. In no book have I seen a drawing of an apical cell of a fully formed Spirogyra thread. Pringsheim in his study of the germination of the zygote gives drawings of the apical cell in a young Spirogyra thread. The apical cells as my cultivated specimens showed, it is a typical one, it is slightly bulged out on the free end, and the chlorophyll band often also broaden out somewhat, and it is the most vigorous in growth, as I conclude from the fact that I have twice got division occur.

in it, while in one case only the 2nd cell and in the other case no other cell of the Spirogyra thread was dividing.

The cells of the shaft are typical ones as I shall describe fully afterwards.

The cells as we approach the lower end of the thread are always the first to show signs of disease, and by their decay we have soon a layer of débris lying at the bottom of the vessel in which Spir. grows.

The cells of the shaft in a normal condition undergo regular division, while the cells as we approach the lower end become less and less active.

Have we got a similar condition when Spir. is growing under its normal conditions?

My experience in gathering Spirogyra in Denbighton lock points to the same fact, namely at first I only used to look out for masses of Spirogyra floating on the water and to col-

lect these masses, but one day when trailing for pike I felt that I had caught some weed, and expected anything but Spirogyra, but got the most beautiful specimen of Sp. nitida. The water at this place was 4 1/2 feet deep, and taking a long oar and searching for Spir. I found it to grow in large banks, the threads not at all entangled as in the specimens I got on the surface of the water. Therefore this seems to be the normal condition under which Spir. grows, and the masses floating on the water have become detached from the bottom by the large quantity of bubbles, given out in assimilation, acting as a buoy.

The apices of the threads have further a metaline power as will become evident from Fig. 4, page. 4. which represents a mass of threads which has formed a band, growing upwards towards the surface of the water, but not in a straight line, for at a/ and b/ the threads have grown towards the source of light, or rather after growth

i.e. cell division had taken place during the night, the ~~cells~~ thread exhibited during day a heliotropic tendency, but as night came on again, and the cells could grow no longer in this direction, we had nutation occurring and the thread began to grow towards the opposite side and upwards, till they were pulled again towards the side from which the light came, etc.

[The diagram further illustrates that a stronger species, i.e. in this case a species with darker chlorophyll band will grow under somewhat unfavourable condition more vigorously than a weaker species, for the lower part of this band consisted of Spirogyra nitida and Sp. jugalis in the proportion of about 40 :: 60, but at the upper part hardly any threads of jugalis were to be found.]

In treating threads of fresh Spirogyra with absolute alcohol, the thread supporting the nucleus ruptured and in many cases I was

1.

struck with the different positions the nuclei assumed in a row of cells; they tended either to go to opposite sides in neighbouring cells, (v. Figs, 2 & 3, page 3 & 4) or seemed even to make a spiral if a line was drawn through their centres. This phenomenon was too constant to be regarded simply as an accident. I dont venture to say whether these different tensions between the nuclei and the peripherally placed spiral bands have any thing to do with nutatory movement or the facilitation of upward growth, or not.

Causes tending to produce individual growth of cells rather than their division.

The four main factors in this respect are

1/ Cold
2/ Want of light during day.
3/ Want of Oxygen.
4/ Excess of Carbon Dioxide.

How does cold act as promotor of individual cell growth and retarder of cell division?

Cold acts probably mainly by restricting the power of respiration in a plant, as following experiment would tend to show.

Two vessels A & B are filled with ordinary spring water, and the water in both vessels is oxygenated for the same length of time with pure Oxygen gas; vessel A is placed in a cold room, F while vessel B is placed / 42° Fh.
in an evaporating chamber in which a Bunsen is burning to warm the air, [the temperature I used was 70° Fah.,] next Spirogyra material rich in Starch is placed in vessels A and B. After 30 hours no trace of starch can be found in the vessel B which was placed in the evaporating chamber.

The individual cells are shorter than the normal ones, and not very well formed, i.e. the cells in the same thread have not a uniform appearance. In some cells the chlorophyll band show signs of degeneration, which I shall describe more fully afterwards. The threads give us the impression that the act of cell division had taken place suddenly, and that the chlorophyll band and the cell walls could not have kept step with the division.

Vessel A which had been placed in the cold room, when examined showed the threads to have partly undergone no division when the individual cells were larger than the normal ones, and partly threads, which seemingly had undergone division, for they contained normal cells; in no thread was the starch

entirely consumed, and the Chlorophyll band showed no sign of degeneration.

Thus all factors being, as far as I could judge, the same except that one factor "cold", I believe this to be a retarder of combustion in the cell and also of the transformation of Starch into material, which could be directly used in the formation of new cell walls, but only a retarder provided the cold is not too great to prevent any metabolism occurring.

Want of light during day I would consider the second factor, for, in a shaded place or in a darkened room, very little reserve material can be formed compared to what is formed in diffuse light, although a good deal more is formed than I expected at first. This small amount of nourishment in the form of starch deposited during the day is not sufficient to start cell-

division.

Want of Oxygen affects the metabolism of starch into ~~available~~ directly assimilable substances in a marked degree.

Ordinary water is boiled to expel all gas and while still hot it is filled into a vessel the mouth of which is tightly covered with a sheet of indiarubber, when ever the water has cooled, Spirogyra rich in Starch is placed into it, and the vessel put in a dark place, after 48 hours the cells are in an unhealthy condition, the chlorophyll bands tend to run together in pairs, and the Starch granules have not decreased in size.

In normal water the change after 48 hours is very marked, the starch granules being much less in size.

While, as already stated, excess of Oxygen will

hurry the disappearance of starch provided the temperature is a suitable one for proper growth of the plant.

Excess of Carbon Dioxide seems to prevent the starch being made use of for after 16 days in complete darkness. I found starch in cells of Spirogyra placed in water through which I had passed CO_2 for 1/2 an hour. Most of the threads were dead, and the protoplasm of each cell had formed a globular mass in the centre of the cell, with the chlorophyll bands still plainly visible, little decomposed and with starch granules, for which I tested as above stated. The formation of the balls of protoplasm was very peculiar and reminded me of the Zygote formed after conjugation; this formation of balls I did not see in the material I had treated with excess of Oxygen, for in them there seemed to be

general decomposition, and no drawing together of the protoplasm round the nucleus. This may be due to the thread having decayed in the Oxygen material while in the CO_2 - material the thread contracted round the nucleus, but I could not satisfy myself as to this.

At present I have simply mentioned the facts and shall refer to them again where I speak of cell Division.

4.

To estimate the amount of water, combustible and non combustible solids I proceeded thus.

Newly gathered material consisting of about equal quantities of Spirogyra nitida and jugalis was carefully and repeatedly washed to remove all traces of animal life, then the material was washed in distilled water to remove the salts which are in ordinary water; next the mass of thread was placed on muslin in a large filter and allowed to drain till it assumed a light green colour, showing that the water between the thread had drained off, then one kilogram was weighed off and placed in a large shallow porcelain dish, whose weight had previously been ascertained. This dish was exposed on a sandbath at a temperature of 100° Fah, and after 48 hours the mass had evaporated and formed a hard crust in the dish, then exposed to 120° for 3 hours

As soon as the dish had cooled it was weighed with the dried Spirogyra. and the result marked down. If instead of weighing at once we wait for some hours and the atmosphere is damp, the dried material increases considerably in weight, the moisture from the air being sucked up as in a sponge.

The result of the weighing showed that of the 1000 gr. we started with 968 gr. had been evaporated, giving us a percentage of 96.8 of water, and a residue of 32 gr. of solids.

This residue was placed in a platinum vessel and burnt to calculate the amount of pure ash, and of the 32 gr of air dry material the amount of ash equalled 4.8 gr, or with other words 15 %.

By substracting the pure ash from the air dry material we get the amount of volatile products.

Therefore in 1000 parts of Spirogyra we get.

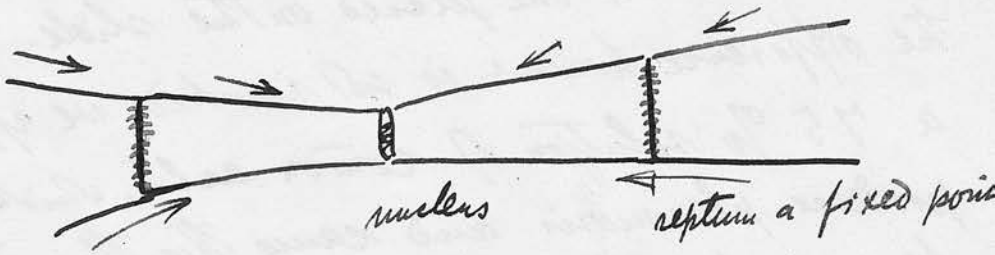
968 parts of water
27.2 volatile substances.
4.8. non volatile " "

1000. =.

This large amount of water led me to following experiment, showing to what degree turgor can stretch the elastic cells:

One thread of Spirogyra is placed on a slide by immersing the slide in water, and fixing one end of the thread with the index finger and gently with drawing the slide, when the thread will lie in a perfectly straight line. The slide is placed under a low magnifying power, a power just sufficient to allow the cells being counted easily, and the ordinary eyepiece is replaced by one with a micrometer, and the number of cells lying betw.

between the 2 ends of the micrometer are counted, and the number marked down, then the slide with the thread is moved along and another set of cells are counted and their number marked down, and we find that in normal cells the difference between the two numbers will only be a very slight one, up to ½ the length of the normal cell, should there be a difference of one or 1½ cell the thread should be rejected and a new one placed on the slide. If the difference however is not marked, we apply a 7.5 % solution of common salt, which will produce plasmolysis and cause the thread to become shorter, and the average of shortening in my experiments was about 10 %, say e.g. if we have 20 cells corresponding to the length of the micrometer before plasmolysis and 22 cells afterwards, we may roughly calculate the shortening to be equal to 10 %.


nucleus
septum a fixed poin

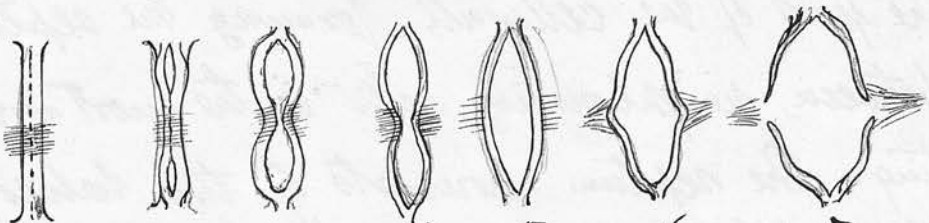
When making these experiments with Sp. × amongst others, the peculiar change in the appearance of the cells as represented in Fig. 1, page. 6. interested me very much, but a satisfactory explanation I am not able to give; it may be that the sudden withdrawel of water from one cell by causing its collapse, most marked round the nucleus, sets up a tension running along the elastic cell wall, which finds a in the septum, which resists the plasmafis, a fixed point and thus pulls out the neighbouring cell. on the side next to it, while the other half of the cell which is undergoing widening will be pulled out by the other contracting cell on the opposite side.

The Cell-wall.

The structure of the cell-wall seems to differ considerably in different species of Spirogyra. In Spir. nitida if seen on surface view it exhibits a tuberculär appearance, while if seen in section it is possible to distinguish two distinct layers, an outer one showing a fine striation at right angles to the periphery of the cell, and an inner layer with lamellae parallel to the periphery of the cell. If vegetating cells are treated with a 10 % saltsolution and so plasmosis is caused, it is often possible to see very shallow depressions in the cellwall just opposite the spirally arranged chlorophyll band, and the chlorophyll band seemingly has a distinct influence on the cellwall, in what respect I shall endeavour to explain when I speak about the relation of nucleus to cellwall.

The part of the cellwall forming the septum between neighbouring cells is the most interesting. The septum consists of two halves, each half belonging to cell on its own side. It is possible to proove this by bringing fresh Spirogyra into a large test tube with absolute alcohol and then by shaking it, when the thread will break up into short pieces, and this is brought about by clearage occurring in the centre of the septum, at a place corresponding to the interval between the two rows of granules deposited by the action of the nuclear thread on the cytoplasm.

This clearage may now also occur in normal Spirogyra and then causes the replicate condition, as figured on pages 6, Fig. 3, and page 7, Fig. 1-5. but since these figures are not arranged in the order in which the clearage appears to me to occur, I have represented the successive stages diagramatically on the following page.



This view is wrong, vide figure below

Fig. 1. represents a cell plate in a very young condition, the dotted line indicating the place where cleavage will occur. The transverse lines show the attachment of the protoplasm to the centre and there is good ground to believe that we have here to do with continuity of protoplasm between neighbouring cells, brought about by the thread of the nuclear barrel passing to the position of the dotted line and there joining; the figures 1, 2, 3, 4 page 7, tend to confirm this point.

If we assume the protoplasm of each cell to be pulling on the septum, we might account for its cleavage, and if there was greater strain in the middle it is quite likely that we might get a series of changes as represented on the opposite page.

Crystals.

When Dr. Macfarlane was working on Cell-division he noticed already crystals to occur in Spirogyra. but he did not publish the fact and since the crystals are of a very peculiar shape I thought it worth while to look into the matter. First as to their composition; they seem to be oxalate of calcium crystals since they are insoluble in acetic acid, and since in nitric acid they dissolve without the evolution of gas. They are of very common occurrence in Spirog. nitida & jugalis, in the normal cell about 2 or 3 in number, but they may increase in number up to 17 in slowly growing vegetating cells. I never saw them in material which was undergoing cell division, and what becomes of them I have not been able to make out; — the commonest form is that of a cross with slender pointed arms as represented on page 5, Fig 1, b.1. By a fusion of a number of

these crosses figure d & f are brought about.

The figures "g" and "h" I saw only once in a thread, which I spoiled in the attempt of mounting. How they could assume this shape I cant explain, and I think I was quite sure they had that knobbed appearance.

5

Chlorophyll.

Since G. Kraus' time most elaborate investigations into the nature of Chlorophyll, – "the green colouring matter of plants" – have been carried out by a great number of observers, & specially by Sachse, Hansen Tschirch, Schunck, who worked at the chemistry of Chlorophyll, and Pringsheim who investigated its physiological action in the plant. I shall refer to their papers when I compare the results I got with their results.

My investigations into the Absorption-Spectra of Chlorophyll were entirely carried on in Prof. Rutherford Labarotory under the kind assistance of Dr Hayecraft, who made me familiar with the working of the "B"rowning Spectroscope and specially with the calculation of wavelengths in a very convenient form; The method used to determine

the wave-lengths I have explained on the back of the charts showing the curve to calculate wavelengths in millionths.

In all the absorption-spectra which I drew I endeavoured to be as accurate as possible in giving the different absorption bands their true extent and the respective degree of intensity of absorption.

The first spectrum to define was the spectrum of the chlorophyll in the living condition, and I proceeded thus.

Spirogyra was the species specially used, for its fine threads allow a more uniform light to come thro' and not so much white light as is the case in the species with thicker thread.

A thin layer of Spirogyra was spread out on a thick coverglass, 2 × 1½ inch, and covered by a similar glass, allowing no air-bubbles to be entangled amongst the thread, then the two glasses were held by a clamp in such a way that the light entering the

spectroscope had to pass through the green layer of thread. Following bands were seen. (vide № 1 of my diagrams).

1. In the middle between the lines B & C appeared a darkish band with wave-lengths: λ-678 – 662.
2. To the right of C, beginning at C a second very faint band appeared, its wave lengths are λ654 – 638.
3. General absorption towards the blue end of the spectrum, commencing at λ531, and very marked at λ508.

This result so quite different from the absorption spectrum usually given, in as much as band 2, has to my knowledge never been observed before, made me at first dubious about the material I was working with, therefore I got some fresh material from the pond in the Botanical Gds and repeated the process, but got the same result. This band became

even more evident, when I modified my proceedings by gently shaking up some Spirogyra material in a ~~test~~ tube and arranging the test tube in such a way, that the light passing through it, threw a green light upon the slit in the Spectroscope. Dr. Haycraft saw this band also quite distinctly, and so all doubt was removed, that, what Kraus has described as the first band of the Chlorophyll spec. trum really consists of two bands.

This second band can only be seen if the layer of green material is very thin through which the light comes, for a thicker layer makes band, "I," become more evident, it broadens out towards the right, and the second band itself becomes dark, so that these two together form one band.
The second band is most evident if instead of sunlight, a gaslight is used e.g. in the form of an Argand burner. I may mention in this place, that, although all authorities who have

been working at Chlorophyll, state that the bands in the blue end of the Spectrum can only be seen in sunlight, ~~their~~ it is not only possible to see them in artificial light ~~light~~, but that to me they are as evident as in sunlight, if not more so. —

Schunck in his paper: Chemistry of Chlorophyll in the Annals of Botany Vol. III, No. IX, shows in fig. 2, representing a very dilute alcoholic extract, a band corresponding very much to my band "I." If a thick layer of fresh material is taken, only one band appears in the red, and this band differs from the red band of a moderately strong alcoholic solution in being gradually shaded off towards the right and left, and does not show the sharp margins of the band to be described presently. .

Fig. 2. shows a freshly prepared alcoholic solution of medium strength with following bands:

1. Band I of Kraus, wavelengths 678 – 628
2. — II — , centre = 604.
3. — III — centre = 575.
4. General absorption commencing at 518, dark at 504.

This solution was made from Spirogyra material which had been carefully washed in distilled water to remove all traces of impurities, which might be in the common water, and which then had been placed on filter paper in a funnel, for 5 minutes, to allow most of the water to run off, without causing at the same time any changes in the thread themselves. Ten minutes were sufficient to extract all the chlorophyll, the alcoholic solution or water extract was filtered and examined at once.
The solution was to the naked eye dark green, by transmitted light, and showed the well known fluorescence of a colour like fresh blood clot.
It will be seen that in this figure the fourth band of Krauss has been omitted, and a solution which will show the 3 first bands as plainly as shown in Fig. 2 will not show the slightest trace of a band in the green.

Fig. 3.

Extract with benzol, freshly prepared, too dark to show the two bands in the red distinctly, but shows the 2 bands in the blue end, the left half of each band shows the intensity of absorption, the right half is drawn darker to bring out the centre.

Band 1. wavelengths 674 – 632

— 2. centre of band = 486

— 3. — - — = 453.

The extract was made by ~~triturating~~ purified Spirogyra material with benzol, after 10 minutes hard work a yellow extract with a slightly greenish tint was got, showing very slight fluorescence. – From the figure it will be seen that band one, although not so well marked as in Fig. 2 is present in the benzolic extract, and that this band shows a distinctly darker left part, and a lighter ~~left~~ right part. The two bands in the blue end differ

considerably from the 2 bands of Kraus; both bands being shifted in my extract to the left. The centres of my two bands are about 52 & 71 respectively if we divide the spectrum into 100 equal parts, while in Kraus' bands the centres are about 60 & 85. My bands seem to correspond rather with Schmucks: Chrysophyll in Ether.

It is remarkable, that by triturating benzol should take up the yellow colour principally, while if chlorophyll has been extracted by alcohol and the alcoholic solution be shaken with benzol, latter takes up the blue green colour, and leaves the yellow to the alcohol. An explanation I shall venture in treating the chlorophyle from its physiological aspect.

Fig. 4. of the Absorption Spectra.

Very concentrated extract of Chlorophyll 48 hours old; the layer is so thick that the ex. ~~tract~~ on transmitted light appeared dark red to the naked eye.

Band 1. λ. 690 – 555
—— 2. λ. 543 [centre = 535] to 527.
—— 3 begins at λ. 515.

Fig. 5. Solution of Nr. 4 evaporated on a water bath, the black green residue dissolved in as much benzol as to have the same bulk as used in Fig. 4.

Band 1. λ. 690 – 550
—— 2. λ. 544 [centre = 535] to 525
—— 3. commences at λ. 515.

Fig. 6. An alcoholic extract after boiling the material for 10 minutes.

Band 1. λ. 690 [centre = 643] – 635.

— 2. λ. 618 [centre = 607] – 596.

? — 3. λ. 570

— 4. λ. 542 [centre = 538] – 530.

— 5. commences at. 517.

Fig. 7. Same as in Fig. 6 after treatment with Liquor ammonii.

Band 1. centre at λ. 706

— 2. λ. 690 – 644

— 3. ——————— λ. 618 – 596.

— 4. λ. 542 – 530

— 5. λ. commences λ. 514

Fig. 8. Same as in Fig. 6 after treatment with nitric acid.

Band 1 λ. 682 – 635

— 2 λ. 618 — 596.

— 3 λ. centre at 570

— 4. λ. centre at 538

— 5. commences at 517.

Compare Fig. 2, 4, 6, 8 for the band in the green.

Schunck notices in his paper that the band in the green according to some authors is very faint, while according to others it is considerably darker than the band in the yellow, and Schunck is of the opinion that this band in perfectly pure chlorophyll would be absent. In figures 4, 5, 6, the band in the green will be noticed, but while in number 4 the band has only become evident, when so thick a layer was used as to cause fusion of the three first bands of Kraus into one absorption, in Fig. 6, we have the band perfectly evident, without however any fusion of the first mentioned bands, the reason being that Fig. 4. represents the spectrum of a very thick layer of alcoholic extract from material which had been washed in distilled water to remove all impurities, and which had been treated at once with absolute al-

cohol, while Fig. 6. represents a thin layer of an extract made from material, which had been treated just as that of Fig. 4 but which in addition had been boiled for 10 minutes, washed in distilled water and then extracted with absolute alcohol.

The probable explanation accounting for the difference between the two extracts I consider to be the following: In fresh material the green colouring matter has a protoplasmic, hence an alcaline basis, and is thus protected against the action of the acid cellsap. Alcohol now extracts the colouring matter so rapidly as to restrict the action of the cellsap almost entirely, therefore only the slight absorption in the green part of the Spectrum al, though so thick layers of extract are used as to appear dark red to the eye by transmitted light. – In material on the other hand which has been boiled, the cells have been killed, the acid cellsap has had time

to permeate the alkaline protoplasm and to act on the chlorophyll, therefore when an alcoholic extract is made of material thus treated, the absorption band in the green part of the spectrum becomes very evident.

Now one might ask the question did not the boiling "per se" affect the chlorophyll, cause its decomposition and the appearance of the green band? This is not the case since if we boil an alcoholic extract made from fresh material, no change occurs in the band in the green, therefore the change must occur during the act of boiling.

By comparing Fig. 6 & 7 it will be seen that this band is dark in Fig 6, while it is much fainter in Fig. 7, latter representing an alcohol extract made from boiled material, after adding a few drops of liquor ammonii to half a test tube full of extract and heating for one minute up to the boiling point. It is

therefore evident that a strong Alcali as caustic potash or Ammonia will decrease the amount of absorption in this special band, while an acid, e.g. nitric acid on the other hand will increase the intensity of absorption as shown in Fig. 8, but care must be taken not to add to much of the acid or the whole of the Spectrum will be changed, this special band will become lighter again.

This band in the green part of the Spectrum, better known as the fourth band of Kraus does not belong to the spectrum of chlorophyll but is a decomposition product, caused probably by the acid cell sap acting on the alcaline chloro-phyll.

I may state here the different action of an alcali & an acid on an alcoholic extract as shown in Fig. 6. with regard to the band in the red.

By referring to the diagr. Fig. 7 it will be seen that if an alcali be added to an alcoholic

extract a new band is formed to the left of band I, the centre having a wavelength of λ. 706. This peculiar action of an alcali was first described by J. Chautard (Comptes Rendus, Tom L XXVI, 570.) and we may consider this band the analogue to Krauss' band IV, the one being due to an overdose of alcali, the other due to an acid.

But while an alcali has the effect of producing a new band on the left, an acid has the reverse action, it makes the spectrum to the left of B brighter and shifts the position of the left margin of the band in the red towards the right side, as will be seen by comparing the Fig. 6 & 8, the difference in wavelengths being λ. 690 for Fig. 6, and λ. 682 for Fig. 8.

While the II band of Krauss does not seem affected by either alcali or acid, the III^{d} band is affected it becomes more evident if the extract be treated with an acid. This band is just as band IV very faint in a freshly prepared extract but it can be seen in an extract which to the eye appears dark green or brown.

Band I : wavelengths from 488 – 469
the centre = 479

Band II : wavelengths from 456 – 431 ←
the centre = 441

The least absorption between the two bands in the blue = 461.

mitted light.

Fig. 9. represents an extract of fresh material made with Xylol. At first the light was allowed to shine through a thin layer to be able to define the two bands in the blue, and then through a thick layer to bring out the bands in the red and orange.

Band one : wavelengths from 676 – 656
the centre = 666.

— two : wavelengths = 656 – 643

— three : centre = 613.

— four. : centre = 492

— five : — = 458.

Fig. 10.

Represents an extract with Petroleum-ether with the peculiarity that it does not dissolve out any material which produces a dark band in the ~~green~~ red. The extract was quite free from

any greenish tinge, as for example all the ex. tracts with Benzol and Xylol had;.
Whether this extract is identical with Schunks Cryosophyll I am not as yet prepared to say, but I must repeat that in very thick layers not the slightest absorption occurred in the red.
When in the College of Physicians Labarotory I got some Petroleum ether through Instrument maker Baird which gave me an extract very like that of Benzol with absorption in the red. The petrole ether, I used of late, I bought at Duncan & Flockart, Chemists, who were not prepared to state definitely who the manufacturer of it was, but that the two Petrole Ethers were not the same I knew whenever I opened the 2nd bottle for the smell was different.

To extract a pure yellow colouring matter

which will give no absorption band even if concentrated it is best to triturate till the petrol-ether has taken on a faint yellowish tinge, which is easily ascertained by pouring off some of the petroletther into a test tube and by holding it up to daylight, for which purpose the dark room has to be left of course for a second.

[If material which had been triturated to day is allowed to stand for 24 hours in contact with Petroletter, the latter takes up substances which will give absorption in the red with a comparatively dilute extract.]

I just mention this fact put in brackets to warn anybody who is getting tired of triturating to trust to the petroleter extracting pure yellow colour if it is left in contact with the triturated material.

After we have got 4 to 6 ounces of the pale yellow extract we allow it to evaporate spontaneously in an evaporating chamber,

taking great care that no light is near the evaporating petroleether for latter is very ~~apt to~~ to become ignited by the vapours passing towards a light of any description,

To recapitulate shortly I consider the correct spectrum of chlorophyll for Spirogyra to be this:

1/ a band with wavelengths R. 678–662 } } I.
2/ - —— - —— 654–638 } } II.
3/ - —— - —— centre = 604 } = } III.
4/ - —— - —— —— = 575 } } IV.
5/ - —— - —— —— = 486 } } V.
6/ - —— - —— —— = 453 } } VI.

= Sorby's bands.

8.

The Chlorophyll bands.

The Chlorophyll bands are spirally arranged in the peripheral protoplasm. In different species their number varies, but even in the same species variations occur, as I have found 3 and 4 bands in Sp. nitida, 2 and 3 bands in Sp. . . . . .

They further differ with regard to the amount of green colouring matter stored up in them, so Sp. nitida has distinctly greener threads than Spir. jugalis, and this fact may have something to do with the greater resisting power of Spirog. nitida, compared with Spir. jugalis, to conditions unfavourable to their healthy growth; I have found at least that in culture Spir. jugalis will show signs of disease before Sp. nitida will do so, although the individual threads of Sp. jugalis are thicker than those of Sp. nitida.

With high powers of the microscope we see the bands to have a wavy outline with a clear protoplasmic

bounding layer but with no distinct membrane. Specimens treated in various ways, stained and unstained, never revealed a membrane similar to that round the nucleus; the ground substance seemed to me to consist of a granular substance, the granules at some parts being arranged so as to form a dense network, while at other parts distinct vacuoles appeared surrounded by these granules. The ~~thread~~ band round the pyrenoid showed a denser construction than at the intervals between the pyrenoid.

When watched in the living condition, I was able, with a 1/12 millimeter Prazmowski immersion lense and a № 12 apochromatic eyepiece of Zeiss, to see distinctly the granules in the ground substance to move as in Brownian movement; this movement was best seen near the abovementioned vacuoles which in the living condition contain an oil which is red green as the substance surrounding the vacuoles but is either colourless or has a yellowish tinge and only twice was I able to make out a greenish tinge, and perhaps

this latter condition was brought about by reflected light from the green pigment substance.

To see these vacuoles to the greatest advantage, it is best to place a bundle of thread on a slide and to cut the thread up into fine pieces by rocking a razor to and fro, (and if this is done with a sharp razor, the weight of the razor is sufficient to cut the thread, and the razor itself wont suffer much,) then to cover the minced mass and to observe at once.

Very careful observations have led me to believe that the granules which can be seen between the chlorophyllbands and which have no distinct motion are directly given off by the bands in question, for by looking along the clear protoplasmic envelope of the bands it is possible to see it form bulgings and after a time the bulgings will disappear, the actual cutting off of these bulgings I have not been able to make out, but I made out that these bulgings occur frequently close to the pyrenoid centres. These observations during the living condition of the cell, seem to be verified by

by the use of various staining reagents, the three I found of most value with regard to the Chlorophyllband were 1/ osmic acid = 1%.

2/ quinolein, 6 drops of a 36% solution in absolute alcohol. to a liter of water

3/ Tincture of alkanna root, concentrated till a precipitate was beginning to form.

Quinolein has the remarkable property of having great affinity for all fatty bodies, hence the above mentioned vacuoles with the oily contents will be especially stained, but the whole of the band undergoes a distinct change in colour from the normal green to a bluish green or pure blue. This staining occurs during life of the plant and is therefore of most value to ascertain where exactly fatty material is stored up, while in using the tincture of Alkanna in many cases the fatty particles are dissolved in the alcohol and may be deposited in all possible places.

How great the affinity of Spirogyra for Quinolein

is, becomes apparent, if the water in which Spir. grows is stained blue by a few drops of the alcoholic solution of Cuinolein. In a few hours up to 24 hours not the slightest trace of colour will be noticed in the water, the whole of it having been taken up by the Spirogyra.

Not only the chlorophyll bands but also the granules at least many of them are stained blue also, so revealing their fatty nature.

If concentrated tincture of Alcanna is used the alcohol dissolves the fat out of the chlorophyll. bands and deposits it in large globules outside the thread, or in the protoplasm or just at the margin of the chlorophyll bands, if latter is the case, we get an appearance exactly similer to the hypochlorin reaction, namely bigger and smaller globules are deposited along the margin of the band, only they have a dark red almost black appearance, but show under the microscope the characteristic appearance of fat-globules.

The chlorophyll band itself is stained faintly pink.

I may note here that the chlorophyll band differs in its shape during day as compared to its condition during night.
During night it represents on cross section an oval shape, while during bright daylight it assumes the shape of a  . I am not sure as to the cause of this change, it may be that in strong light the band has the power of contracting, and so exposing less surface to the light, but I rather think it is due to the mechanical reason of the pyrenoid swelling as the starch is being deposited, and, as the increase occurs in a downward direction, so causing the lower aspect of the band to assume a ridge like elevation between the pyrenoid.

For the just stated fact it is best to observe material which has been for a night kept in

a room and not exposed to light till half an hour before the microscopical examination.

—

The ground substance consisting of very fine granules has the characteristic green colour, but as to the arrangements of the granules I cant speak as yet definitely, but what I would like to lay stress upon is this: I believe the green colouring matter not to be of an oily nature but just a green protoplasmic and hence alcaline substance, which would be affected at once if any acid could reach it, as I stated when speaking of the difference in the absorption spectrum between alcoholic extract from fresh and from boiled material. I further venture the suggestion that this green ground substance for its own nutrition elaborates the oily material which is gathered up in the vacuoles, and the excess of which is given off from the chlorophyll band in the shape of the granules moving about between the chlorophyll bands.

It is this oily material which I believe I get when I triturate fresh Spirogyra with petrolether, benzol, xylal and turpentine, and that these extractives in the order mentioned extract more or less purely the different stages in the transformation of the yellowish oil into the green protoplasmic matrix: the petrolether extracting a pure yellow oil, while the turpentine extracts the yellow oil as well as that which is on the point of undergoing the change into a green substance. What led me first to this view, even before I had made any spectroscopic examinations, was the remarkable fact, that an extract of Spir. made with benzol and with only a faint trace of green, after standing for some days in the dark, or after warming for 2 hours in a water bath at a temperature of 100° Fahr. assumed distinctly a greener appearance than it had at first. I thought at first that it might be an optical delusion, but two

test tubes, one with a freshly prepared extract, and one with an extract which had been warmed ~~to~~ handed to Dr. Woodhead, Dr Wood, etc and they were able to see the difference in colour as well as I did, and this settled my mind.

I believe the oil-vacuoles to correspond to the starch-centres, the one to be the storehouse for the Chlorophyll-bands, just as the other is the storehouse for the nucleus as I shall try and explain afterwards.

Degenerative Processes due to deficiency of light in the Chlorophyll bands.

The coils of the spirals tend to become confluent, forming pairs, and so we seem to have a double row of pyrenoids in a single band. Then we get vacuolation occurring in the bands close to the centres. At a later period the bands form irregular masses towards one side of the cell, and the green matrix between the pyrenoid is gradually absorbed, and by its absorption the starch-centres are brought into actual contact.

The formation of the masses of chlorophyll bands is due to the threads of the nucleus pulling on them after the nucleus has lost its central position, probably by some of the threads supporting it giving way on one side.

S after 16–20 hours.

Effect of Oxygen on the Chlorophyll.

If fresh material be placed in a bottle with a narrow neck and Oxygen gas passed through the water for 20 minutes, we find the threads to have lost almost entirely their green colour, and they have the same appearance as Pringsheim threads ~~described~~ exposed to the action of intense light, and we may therefore conclude that light causes oxidation to be carried on more vigorously in the Chlorophyll bands.

In Sp. . . . . a very peculiar arrangement of the chlorophyllbands round the nucleus exists. There are usually 3 bands in each cell and as these bands approach the nucleus. the coils become steeper and are applied close. ly to the surface of the nucleus, a very peculiar broadening of the bands occurring at the same time; thus a complete bag is formed for the nucleus and it is securely moored, the bag formed by the bands replacing the thread which supports the nucleus in Sp. fusalis for example. This bag surrounding the nucleus is densely packed with pyrenoid, so keeping a supply of starch in close proximity of the nucleus.

The Nucleus.

The nucleus in a typical cell has always a central position, and may be a spherical structure as in Sp. nitida or of spindle shape as in Sp. jugalis. It is moored to its position in either of two ways, 1/ by threads passing from it to the pyrenoid in the Chlorophyll band, or 2/ by the formation of a bag by fusion of the chlorophyll band round the nucleus as already described.

There seems to be a difference in the various species as to the extent to which the nucleus is attached, in Spir. jugalis for example I have been able to trace a thread from each pyrenoid centre to the nucleus, while in vigorously growing Sp. nitida the pyrenoid toward the end of the cylindrical cells may be without threads, but again in vegetating cells with slow growth I have been able to see connections between most if not all the pyrenoids with the nucleus.

Although at first sight the main function of the thread might appear to be the mooring of the nucleus, it is really of only secondary importance, as will be apparent when I speak about the division of the nucleus.

These threads, as already stated, run from the pyrenoid to the nucleus; at the pyrenoidal end they form deeper or shallower cups enclosing the starchcentre or protein crystal as Strasburger terms it, while at the nuclear end the threads fuse to form a bag round the nucleus.

Let me first describe the pyrenoidal end of the threads with the protein crystal.

A. Meyer in Bot. Zeitung. 1883. Nro 30 describes the protein crystals as bodies with angular outlines Strasburger describes them similarly in his Practical Botany. Berthold denies the angular outline Schmitz also states that the pyrenoid consists of a substance identical with the chromatin of the nucleus, a fact denied by Berthold, A. Meyer & Schimper Zacharias considers the nucleoli and pyrenoid

to be similar, since both consist of digestible albuminoid.

I myself hold the proteincrystals to be both globular and angular at various times of the day according to the amount of the starch present.

On page 8 of the Drawings I have represented in Fig. 4, from a – f the various appearances of the pyrenoid centres, and it will be seen that if we have to do with the pyrenoid in a starved condition, then the proteincrystal has a rounded appearance, surrounded by a thin envelope which corresponds to the margin of the caplike dilatation of the thread already mentioned. As the starch-granules are being deposited in the form of biconvex plates at various places round the pyrenoid centre they give an angular outline to the protein-body if it is viewed from above as best seen in Fig 4, e/, where the plates have increased to so much in size as to touch one another laterally. If assimilation has been going on very vigorously

the formation of starch may go on to such a degree, as to form a swollen ring like mass round the centre when the protein body will assume again a circular outline, as is best seen in Spirogyra nitida from which I took the sketch f in Fig. 4, while the sketches a – e were taken from Sp. jugalis, which has larger pyrenoid and less colouring matter in the bands, two factors which facilitate the study of the formation of Starch considerably. Sp. jugalis has also to be preferred to Sp. nitida if the transfer of nourishment from the starch centres to the nucleus has to be studied, but it is not to be recomended for nuclear study, as it has a spindle-shaped nucleus.

The starch granules are deposited inside the cuplike expansion close to the starch centre, but it is only possible to see the cuplike dilatation well in specimens with little starch.

In Fig. 4, d' / is a sketch of an isolated pyrenoid showing 2 starch granules depos-ited laterally at the starchcentre but I have

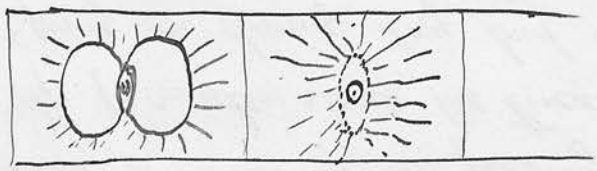
been able to make out 3 ~~rows~~ rows distinctly in Sp. fusalis by focussing downwards.

The shafts of the thread appear to me to be hollow structures for when the starch is being digested and is passing to the nucleus in the shape of minute granules, it is possible to trace these granules moving slowly along the thread and since on each side of the granule there is a very delicate line which seems to be continuous with the rest of the thread. I think we may consider the threads to be acting as feeding-tubes. The passage of granules along the thread seems also to be going on during day but the granules are so minute that even with high powers I was not able to make this out definitely.

As to the swellings occurring on the thread I am inclined to believe them to be extra store-houses for a material easier in reach of the nucleus, which has been formed out of the starch. I tested very carefully with the method already stated for Starch but could get no reaction, with

This ~~nourishing~~ material will ~~first of all~~ be consumed first by those elements
of the cell ~~which produce~~ are essential for the mainten
~~those the proper nourishing~~ which are essential for the
maintenance of the life of the cell as an individual,
any surplus of nourishing material ~~is~~ will be used for purposes of ~~multiplication~~
~~or fission~~ division or multiplication.

96



iodine the granules assumed a darkish brown colour.

Besides these granules passing along the interior of the thread, at the time of division we have the thread surrounded by a delicate layer of protoplasm derived from the peripheral layer and along this protoplasmic lining granules pass as well to the nucleus. These two different kind of granules seem to have different functions, the granules passing down the thread seem to be stored up inside the bag, while the granules passing down the protoplasmic lining are deposited outside the ~~the~~ bag formed by the portion of the thread. But what I would like to lay specially stress on, is the fact, that the material outside the bag is placed in the long axis of the cell, and, whenever it can be detected there, that the nucleolus seems to take advantage of the store-house and to take up as much material inside its nucleolar membrane as to cause these two balloonlike expansions. The nucleolus

being provided with more nourishment at the 2 sides corresponding to the long axis of the cell will react on the endonucleolus and when it has started division, its two halves retract into the nucleolar balloon like structures because there an excess of nourishment is stored up, and I dont think they are propelled by any magnetic or electrical force as Dr. Macfarlane is inclined to suppose. After the endonucleoli have receded to the two nucleolar storehouses, the nucleolus itself undergoes division, and it is pulled in the direction of most nourishment, i.e. it goes to the masses of protoplasm which were deprived outside the nuclear membrane, latter by this time having disappeared.

The 2 just mentioned protoplasmic masses at this time begin to be separated more and more, and as they are the storehouses the nucleoli follow them up and ultimately blend with them; as soon as the daughter nuclei

have been formed in this way, the nuclear barrel begins to broaden out more rapidly, the threads separating more and more till they have reached the original cell wall.

What causes the ring of granules to appear in the peripheral protoplasm of the cell I can't make out, it appears as if the granular protoplasm of the two ends of the cell aggregated mostly into the two masses round the nucleus, and that the line of granules seen in the middle of the cell was due to a remaining layer of protoplasm which being pulled on by both sides equally remained in the middle.

Anyhow it is to this row of granules the threads of the nuclear barrel stretch and it is here where the cellulose septum is formed by a direct action of the nucleus through the threads on the surrounding plasm, and I don't think that the threads have to be considered a mere scaffolding as Dr Macfarlane suggests in his paper. I tried to see whether any granules

passed from the daughter nuclei to the develo-
ping cell plate along the threads of the nuclear
barrel, but in vain, but still I hold the daugh-
ter nuclei to be the direct agents in the building
up of the new cell plate, for the formation of
the plate is most active as long as the nuclei
are close to the forming wall, and when the
& nuclei have done their duty they retract
to the centre of the 2 newly formed cells,
but now another and no less important duty
has to be performed, namely general nutri-
tion of the whole cell wall of the newly for-
med cell to allow it to grow vigorously and
evenly in all directions; after the cell has
reached its normal size the nucleus still
influences the cell, for after all the cell wall
represents the most important ~~function~~ part of the
cell, it has to prevent any injurious material
reaching the protoplasm, which by injuring the
protoplasm would cause the plant to be unable
to carry on its work.

An explanation "how" the nucleus influences the

growth of the cell wall we may find in the already stated fact that the cell wall shows along the lines of the spiral band is slowly growing thread a slight depression, and I believe the nucleus to act on the wall through the threads going to the pyrenoids.

Another fact noticed by Dr. Macfarlane long ago shows that the nucleus has a direct influence on the formation of new cellulose material, I refer to the facts worked out by Mr Ellis in his paper on cell growth (Bot. anical Society, Edinb'), where during conjugation the nucleus leaves its central position and approaches the cell wall on the side which forms the outgrowth.

Very interesting in this respect is also a paper by Haberland (Über die Beziehungen zwischen Function & Lage des Zellkernes in den Pflanzen. — Jena 1887.

a short sketch of his paper may be of value

at this place :

Haberland worked out, firstly, the relation of the nucleus in epidermal structures. He finds the nucleus always to be near the side which undergoes special thickening, so in Trades. cantia, Aloe & Agave where we have thickening of the wall on the outer side, the nucleus lies close to the outer side, while in Carex panicea, where thickening occurs on the inner aspect of epidermal cells we have the nucleus towards the inner aspect.

He also found, that as the ridge developed in stomatic cells for facilitating the closure of the stomatic openings that the nucleus was lying on the cell wall close to these ridges.

~~Mature~~ cells don't undergo any further changes the nucleus may take up any position.

In the roothairs of beans, peas etc the nucleus is not at the extreme end of the cell, but a little behind the apex where the main growth occurs.

In unicellular growing hairs of Geranium, etc we have the nucleus near the base, since here the intercalary growth is taking place, the growth towards the base being besides of longer duration than that towards the apex.

Haberland for the experimented with Vaucheria, which he cut into small pieces. Whenever the exuding protoplasmic masses come in contact with water, the chloroplasts retracted from the surface, while the nuclei remained near the periphery and formed a new cell wall.

The observer comes ultimately to the conclusion that the nucleus has more or less a direct influence on surface growth as well as growth in thickness of the cell wall.

In slowly growing threads of Spirogyra it sometimes happens that a nucleus undergoes division but whether it is want of nourishing material or simply mechanical causes we only get a very imperfect or no cell wall formed. That we

are dealing with 2 perfectly formed nuclei which are passed the stage for forming a cell wall is proved by the absence of a nuclear barrel. Such a cell with 2 nuclei I have figured on page 3. Fig. 1. and it can also be seen in the microscopic preparation.

Method for demonstrating the nuclei and thread.

The best method I know for demonstrating the endonucleolus is to treat fresh material with a solution made by mixing equal quantities of saturated alcoholic picric acid solution with saturated $HgCl_2$ solution also in absolute alcohol. Threads treated in this way show hardly any plasmosis, and the endonucleoli are sharply defined.

To stain the thread I found picronigrosine to answer best, it is prepared by dis.

solving in a saturated solution of picric acid as much nigrosine as it will take up. Place the material to be stained into this material for about 3 hours, then wash out the picric acid with distilled water, when thread and nucleus will be stained a bluish black, and the nucleolus almost black.

Another almost equally good method is this: Place Spirogyra for 24 hours in a one per cent gold chloride solution, and examine the material in this material, when the thread will be beautifully defined, no staining has occurred but the thread refract the light more and thus examination is made easier.

Saturated picric acid solution in water will show by itself the thread much clearer.

If Spirogyra material is placed in a 1:800 solution of gold chloride, the threads while still living have a peculiar action on the gold.

chloride; it is decomposed and a beautifully violet colour is given to the water on transmitted light, and in the cells we have the gold chloride deposited along the course of the thread, just as if some substance in the thread or the thread itself had caused this decomposition to occur just here.

G. Mann.

21 Charles Street.

An

account of various methods

used in the preparation of

so called pure Chlorophyll.

Methods employed by different Observers to Extract their Pure Chlorophyll.

I here add a list of the different methods employed for the extraction of what each writer considers pure chlorophyll. The methods mentioned are those of Gautier, Hansen, Hoppe-Seyler, A. Meyer, Pringsheim, Sachsse, Schunk, and Tschirch; along with Detmer's method of preparing a crude chlorophyll extract.

*Detmer's Method of preparing a Crude Chlorophyll Extract.**

Young wheat plants or *Elodea canadensis* are boiled for a quarter to half an hour in distilled water. The water is then poured off, and the plants are washed in water, and extracted with strong alcohol. To quicken the process, the alcohol may be warmed.

Gautier's Method.†

1. Green leaves are triturated with sodium carbonate and alcohol, and the green alcoholic product is treated with animal charcoal, which takes up the colouring-matter and leaves the impurities behind.

2. The charcoal is extracted with alcohol, which removes a yellow colouring-matter.

3. Next, the charcoal on treatment with common ether or petrol ether should give a green solution, which, on evaporation, allows the green colouring-matter to crystallise out. A. Hansen repeated the process, but was unable to get the same results, the charcoal not giving up the colouring-matter on treatment with ether or petroleum ether.

Dr Adolph Hansen's Method of preparing "Chlorophyll-green."‡

The material used is grass, because it contains no resins, tannin, turpenes, &c.

1. Boil a quantity of grass to get rid of soluble substances and yellow and brown colouring-matters, which on evaporation form a brown extract.

* *Das Pflanzen physiologische Practicum.*
† *Comptes Rendus*, lxxxix. 861.
‡ *Arbeiten d. Bot. Instituts in Würzburg*, iii. 123, 430.

The boiling, which must be gentle, lasts for half to three-quarters of an hour.

2. Wash till the water runs off quite colourless.

3. Dry the material at a low temperature as quickly as possible. When dry, the material may be used at once, or kept for future investigations in the dark.

4. Treat the dried material with absolute alcohol, and a dark green solution is got. After a time no more colouring-matter is taken up, and after the first quantity of alcohol is poured off, the material is extracted with a second, and it may be third quantity of alcohol, till most of the colouring-matter has been removed.

All these manipulations, as also the succeeding ones, have to be done in a darkened room, since the colouring-matter is very susceptible to light.

5. Evaporate the alcoholic extract to one-eighth of its volume.

6. Saponify by Kühne's method—

(*a.*) Make a solution of caustic soda by taking 1 part of NaOH, 5 parts of water.

(*b.*) Add of this solution 40 to 50 c.cm. to $2\frac{1}{2}$ litres of the concentrated alcoholic solution thus: While the alcoholic extract is boiling, add the caustic soda solution drop by drop, stirring all the time.

(*c.*) Drive off the alcohol and add water; evaporate the water to a small quantity. Then add alcohol for a second time, and the saponification is completed.

7. To get the soap in a solid form, evaporate the alcohol, add water and an excess of sodium chloride. Thus we get the soap separated in the form of black-green granules.

8. Add petrol-ether to the soap, and a dark yellow colour is extracted. [If I understand the author correctly, it is more advisable to treat the black-green granules (7) first with ether (9), and then with petrol ether (8), as less of the latter will thus be required for the removal of the yellow colouring matter.—G. M.]

9. Next, wash the soap with ether, which will remove foreign bodies, along with a small quantity of colouring-matter.

10. Treat the soap with ether, mixed with a few cubic centimetres of alcohol, and a clear green extract results.

Thus we get the yellow colour in petrol ether and the green colour in alcoholic ether. The yellow colour crystallises in dark yellow needles out of the petrol ether, or after this has been evaporated and the residue treated with absolute alcohol out of the latter.

The green colour crystallises in sphæro-crystals, and to get it pure we proceed thus :—

1. Filter the dark green ethereal solution.
2. After twenty-four hours repeat the process.
3. Remove all traces of yellow colour with petrol ether, after having evaporated the ethereal solution to dryness.
4. Redissolve and crystallise out of an alcoholic ethereal solution.

The sphæro-crystals the author calls "chlorophyll-green."

*Hoppe-Seyler's Method of preparing Chlorophyllan Crystals.**

1. Grass is washed three or four times with ether to remove the waxy covering of the leaves.

2. The leaves are then treated with boiling absolute alcohol, which extracts the green colour.

3. The green alcoholic extract is evaporated by heating it at a low temperature. The resulting residue is washed in cold water and redissolved in ether.

4. The ethereal solution is slowly evaporated in loosely-covered glass vessels, and thus are got :—

5. Granular crystals, brown with transmitted, dark green on reflected light. These crystals are purified by recrystallisation, and are called "Chlorophyllan" crystals.

A. Meyer's Method of preparing Chlorophyllan.†

Since chlorophyllan is readily soluble in hot glacial acetic acid, A. Meyer proceeds thus :—

Treat the leaves directly with hot glacial acetic acid, filter the extract, evaporate it, extract the residue with alcohol, and let the chlorophyllan crystallise out.

* *Zeitschrift f. Physiologische Chemie*, iii. 339, iv. 193, v. 75.

† Arthur Meyer says (*Das Cholorophyllkorn*, Leipzig, 1883) Pringsheim's hypochlorin is identical with Hoppe-Seyler's chlorophyllan.

*Pringsheim's Method of Preparing Hypochlorin.**

Pringsheim's method for the separation of the colouring-matter of chloroplasts from the ground-substance consists in warming green tissue for fifteen minutes to an hour in water heated up to 50°–80° C., or by exposing the tissue to steam of boiling water from fifteen minutes to several hours, when variously-coloured drops exude (the commonest colours are shades of green up to olive-green; rarer are blue-green, yellow, or reddish-brown). These are of an oily nature, and keep the colouring-matters in solution; they are soluble in alcohol and ether. When the exudation is completed the ground-substance of the chloroplasts shows up as a hollow sponge-like ball, and the oil is believed to have been contained in the meshes of this hollow ball.

Exposure to intense light has the same effect, for by bleaching the colouring-matter the ground-substance is revealed, and shows the hollow spongy nature of it.

The exuded drops the author calls "Hypochlorin."

Sachsse's Method of preparing Chlorophyll.†

Sixty kilogrammes of *Primula elatior* and the same quantity of *Allium ursinum* are boiled in water, well pressed to get rid of the water, extracted with 44 litres of boiling 90 per cent. alcohol to each 60 kilogrammes of raw material, then the material pressed again to get rid of the alcohol, and twice boiled in so-called "light" benzin (0·7 sp. gr.) After each boiling the material is pressed, and the benzin extracts, after filtering, are added to the alcoholic extract, when the benzin will take up most of the green colour, leaving the yellow colour to the alcohol.

After several days' standing the benzin solution is decanted, bottled, and treated with pieces of sodium.

After eight to fourteen days the benzin solution becomes cloudy, and ultimately a precipitate is thrown down. The process is completed when the benzin has assumed a pure golden-yellow colour, and when it does not show even in very thick layers the characteristic band of chlorophyll between the lines B and C.

* *Lichtwirkung Chlorophyllfunction in der Pflanze*, Leipzig, 1881.

† *Phytochemische Untersuchungen*, Dr Robert Sachsse (Leipzig, Verlag von Leopold Voss, 1880).

Should the precipitate formed be so fine as not to deposit readily, we may hasten the process by passing a strong current of carbon dioxide through the benzin.

The precipitate is separated from the benzin by filtration, is washed repeatedly with benzin, and evaporated on a water-bath. When dry the precipitate forms a nearly black pulverisable mass, readily soluble in water and alcohol, imparting to them a dark green colour. This precipitate is "the green colouring-matter."

*Schunck's Method of preparing two Colouring-Matters identical with Frémy's Phyllocyanin and Phylloxanthin.**

Fresh green leaves—preferably grass—are treated with strong boiling alcohol, and the dark-green extract having been poured off from the exhausted leaves, is allowed to stand for a day or two, when it deposits a quantity of wax, fatty matter, and other impurities, which had been extracted along with the colouring-matter. The deposit being filtered off, a current of hydrochloric acid gas is passed through the filtrate. This produces a dark green, nearly black, voluminous precipitate which increases in quantity on standing. The precipitate is separated by filtration, and washed with alcohol along with an excess of acid.

The precipitate contains, with impurities, chiefly of a fatty nature, two distinct colouring-matters, identical with the phyllocyanin and phylloxanthin of Frémy.

Tschirch's Method.†

To prepare the chlorophyllan of *Hoppe-Seyler*, which Tschirch believes to be identical with Pringsheim's hypochlorin and Gautier's crystallisable chlorophyll, he proceeds either according to Hoppe-Seyler's method, or according to A. Meyer, or ultimately according to his own method, namely, thus—

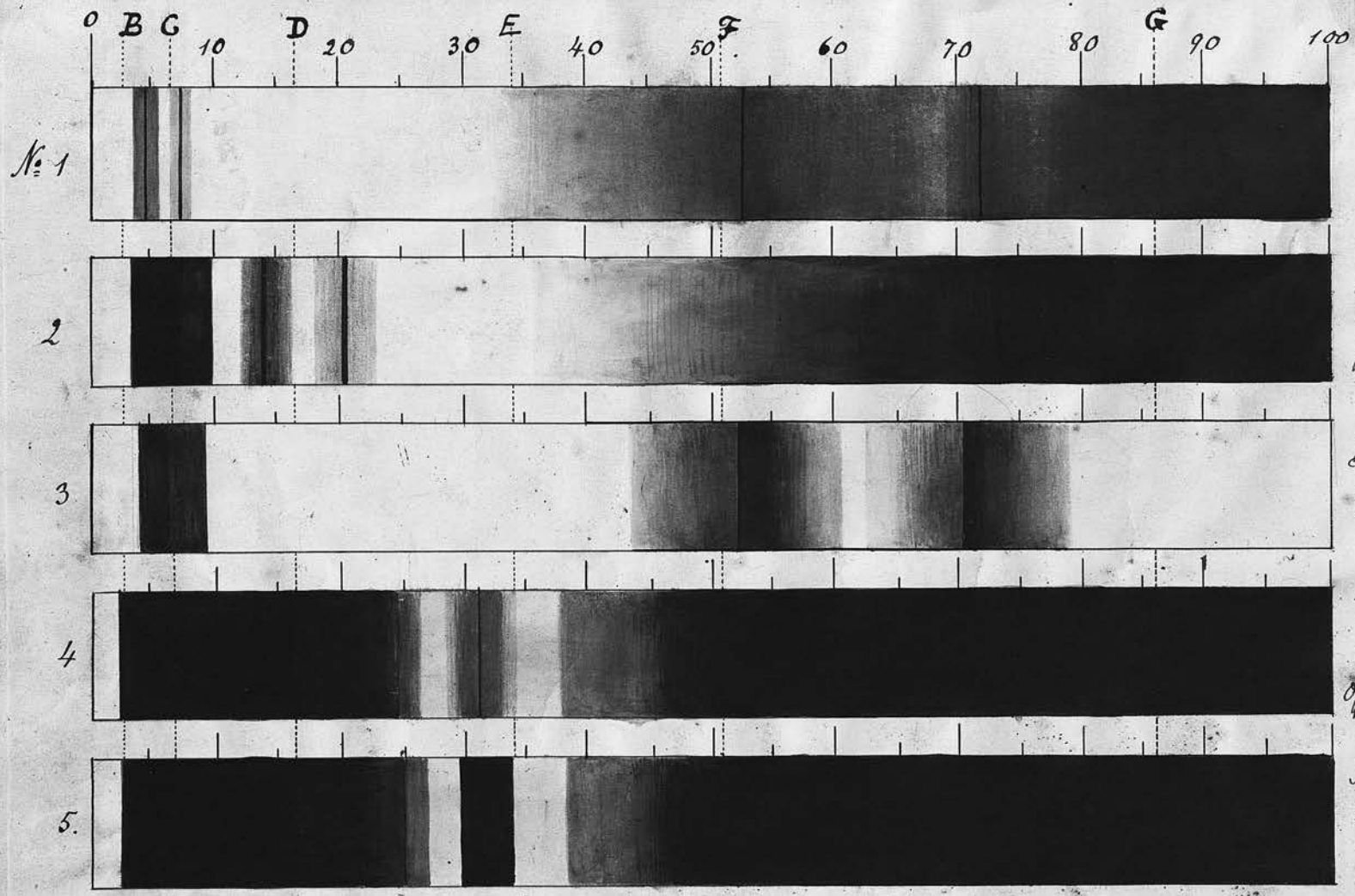
Wash the leaves (grass) with ether, treat them with diluted hydrochloric acid, wash, make an extract with boiling alcohol, filter it and evaporate down to half its original volume, and on cooling hypochlorin will be deposited in considerable quantities.

* *Annals of Botany*, vol. iii. No. 9. page 88.

† *Berichte der deutschen Botanischen Gesellschaft*, i.

Figures
illustrating paper on
Nutritive changes in
Spirogyra.

G. Mann



Normal

Dilute alcoholic extract from fresh material

Benzol + fresh material.

Concentrated alcoholic solution of fresh material.

Same as Fig. 4, but evaporated and redissolved in Benzol

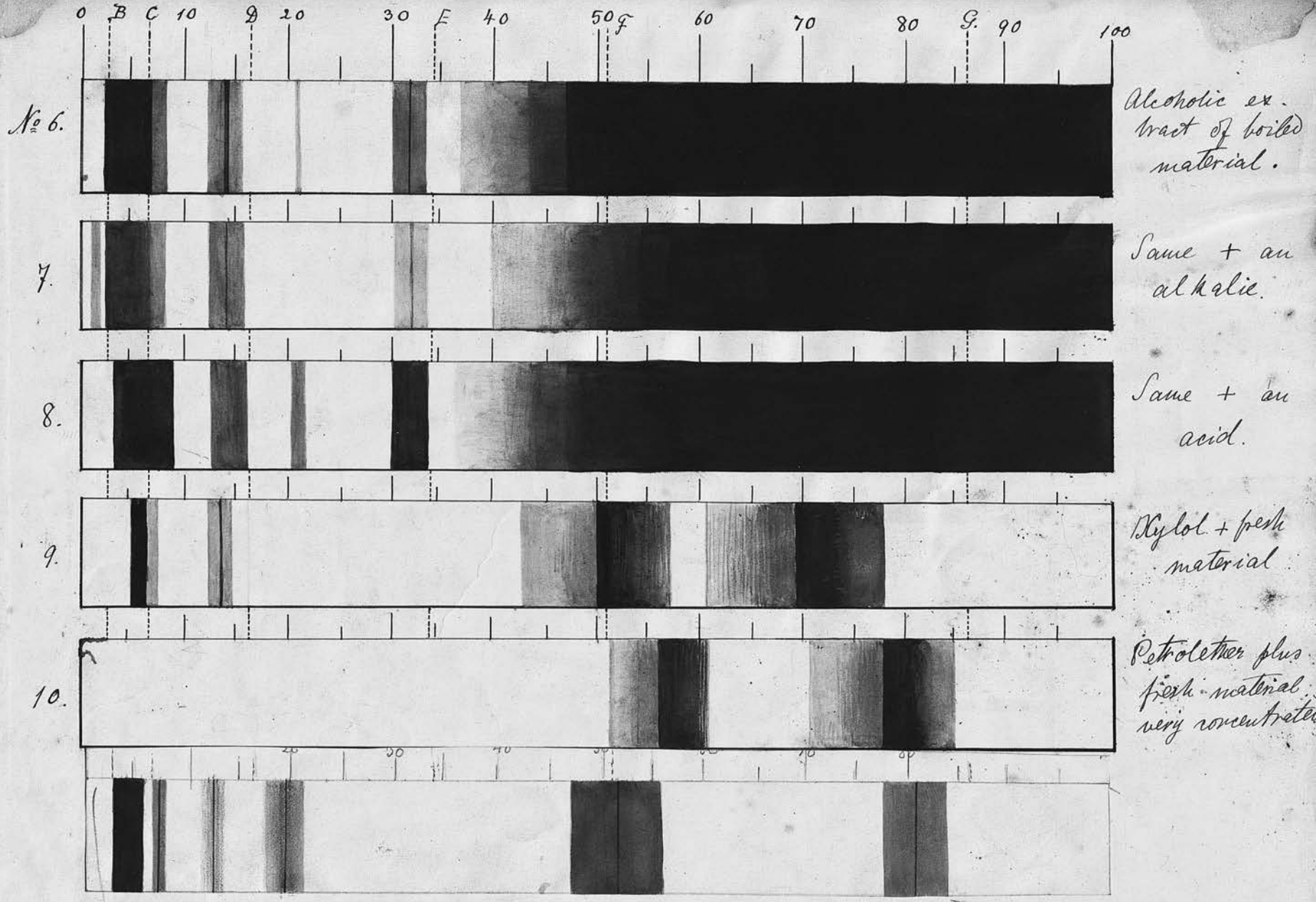

0
B
C
10
D
20
30
E
40
50
F
60
70
80
G
90
100
№ 6.
Alcoholic extract of boiled material.
7.
Same + an alkalie.
8.
Same + an acid.
9.
Xylol + fresh material
10.
Petroletter plus fresh material very concentrated

Fig. 3

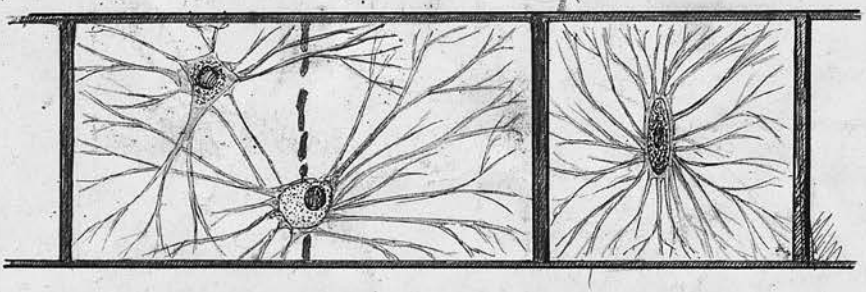


Fig. 1.

Abnormal cell of Spirogyra jugalis with imperfect formation of cell wall probably due to the bad position of the two newly formed nuclei. The 2 nuclei are lying in different planes, and the new cell wall is represented by a much interrupted ridge on the inner surface of the old cell. To the right is a normal cell. The threads running from the pyrenoids to the nucleus are deeply stained by picro-nigrosine. — Viele slide № 1.

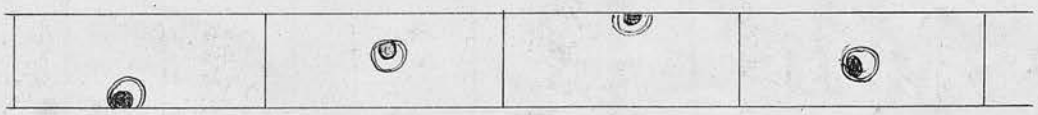


Fig. 2.

Diagram showing the various tensions in a filament, the nuclei are represented in their respective positions, after the threads running from the pyrenoids to the nucleus have been made to rupture on the weakest side and to contract on the opposite side by an alcoholic solution of Eosin

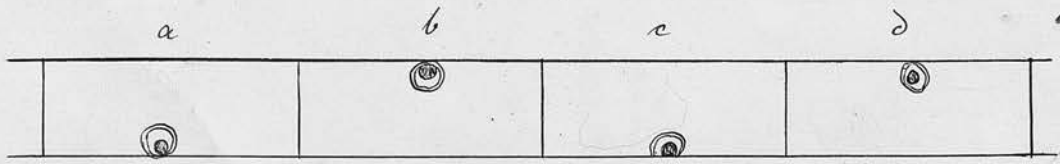


Fig. 3.
Reduced 1/3

The nuclei have gone to opposite sides in adjacent cells, the filament is viewed from the side, if viewed from above and nucleus in (a) is focussed the nucleus in c will be seen with the same focus, but not the nuclei "b & d" since these will be at a higher or a lower level, but both on the same level.

Fig. 4. Fig. 2

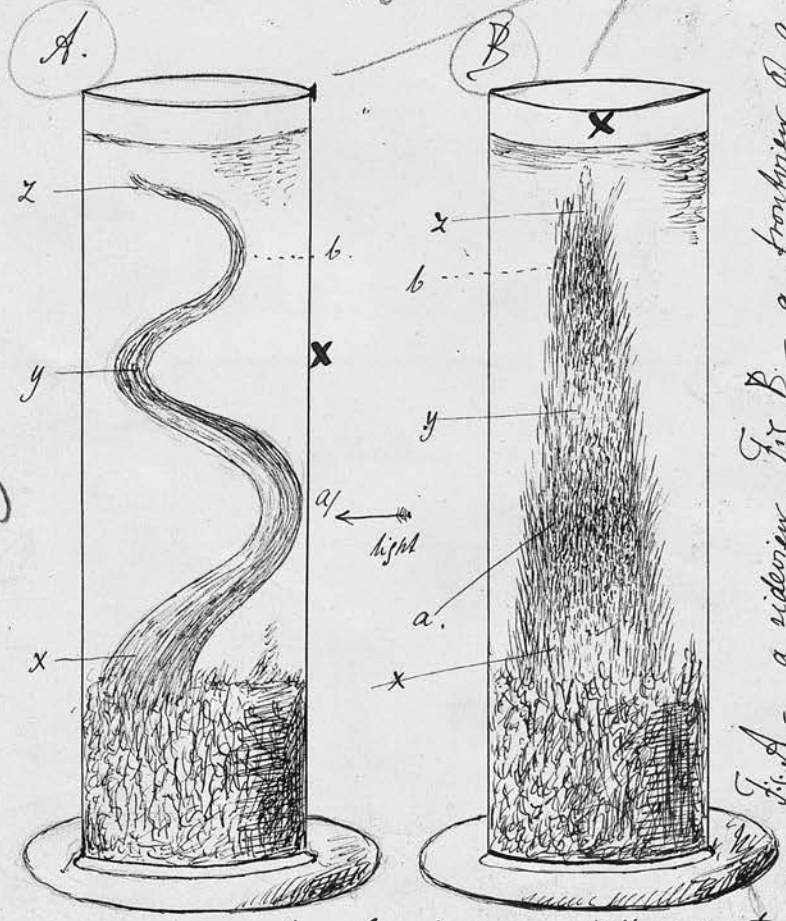


Fig. A - a sideview. Fig B - a frontview of a ribbon-shaped band of Spirogyra filament growing upwards and having its flat surface towards the side where the light comes from. a/ on the first day b/ on the second day x, y, z. changes during night.

Reduced 1/3

The diameters of the glassjars are: 4 1/2 inch transversely by two foot high.

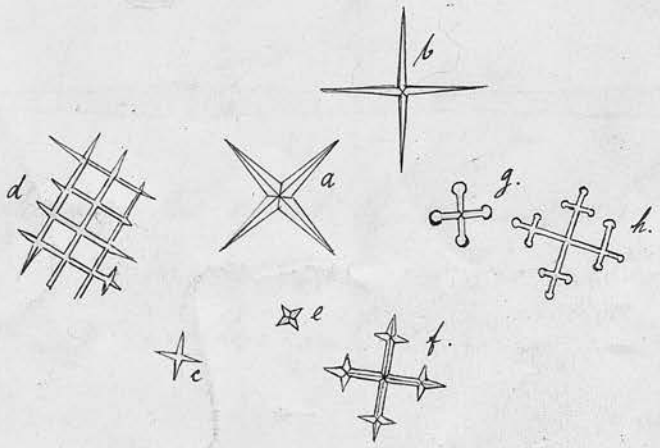
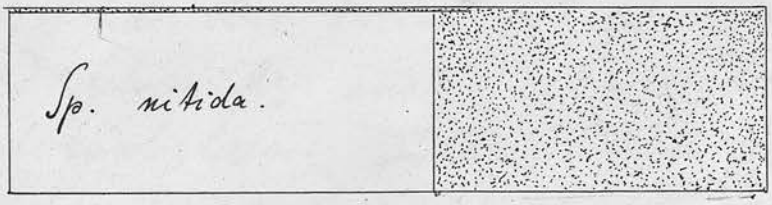


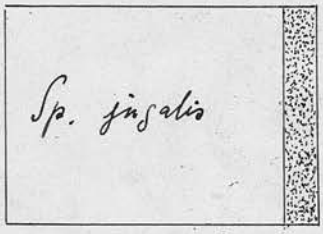
Fig. 1.
Fig. 8

Oxalate of calcium crystals.

a, b, c = common forms; b: the commonest
d = rare form in freshly gathered material
e = a short armed crystal
g. h = in quinolein stained filaments.

Fig: 1
Fig. 2.





Diagrams to illustrate increase in length of cells due to slow vegetation. The shaded part shows increase in length varying in different species. In Spirogyra nitida perhaps also some slight increase in thickness. The measurements were taken with an eyepiece micrometer and then calculated.

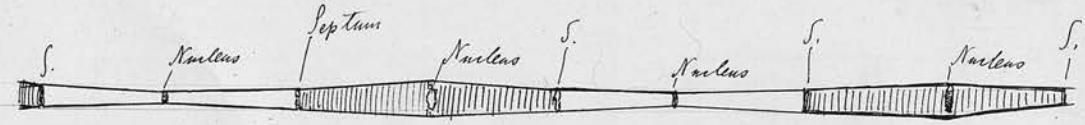


Fig. 1.

Diagram showing the effect of plasmolysis on Spirogyra. The cells are alternately constricted and swollen out.

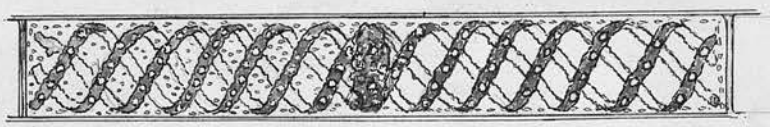
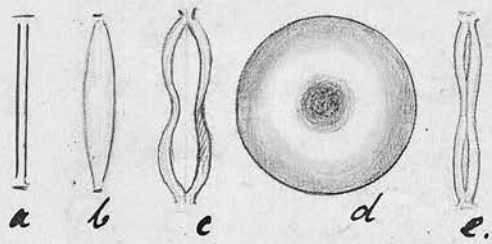


Fig. 2.
Fig 7.

A typical cell of Spir. taken from the middle of the thread, showing the chlorophyll bands enclosing the nucleus. On the left side of the nucleus microsomata are represented on surface view, and on the right side their distance from the cell wall is indicated, between them and the cell-wall lies the peripheral hyaloplasm. The septum is represented as it is just coming into focus.

Fig. 3.



a, b, c, represent the different appearances we get by focussing the septum in Sp. from above downward. d/ represents a diagram constructed to show the septum on surface view. e/ shows the septum in a normally growing cell, c/ the septum in a vegetating cell. —

Fig. 1.

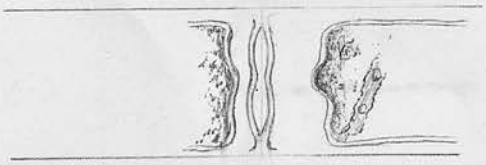
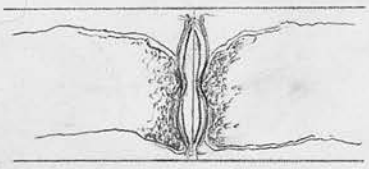


Fig. 2.



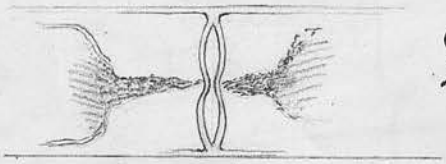
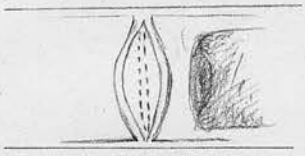


Fig. 3.

Fig. 4.



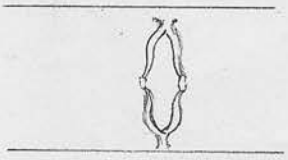


Fig. 5.

Figs. 1, 2, 3, showing plasmosis of the cells and beaklike projections of the protoplasm.

Fig. 4. shows the common form of septum consisting of 2 convex halves, that we are not just looking at the attachment of a single septum to the cell wall is shown 1/ by the attachment to the cell wall coming first in view in portion of the dots in the diagram, and 2/ by the cupshaped depression in the retracted protoplasm.

Fig. 5. Shows the replicate condition of the septum.

Fig. 4

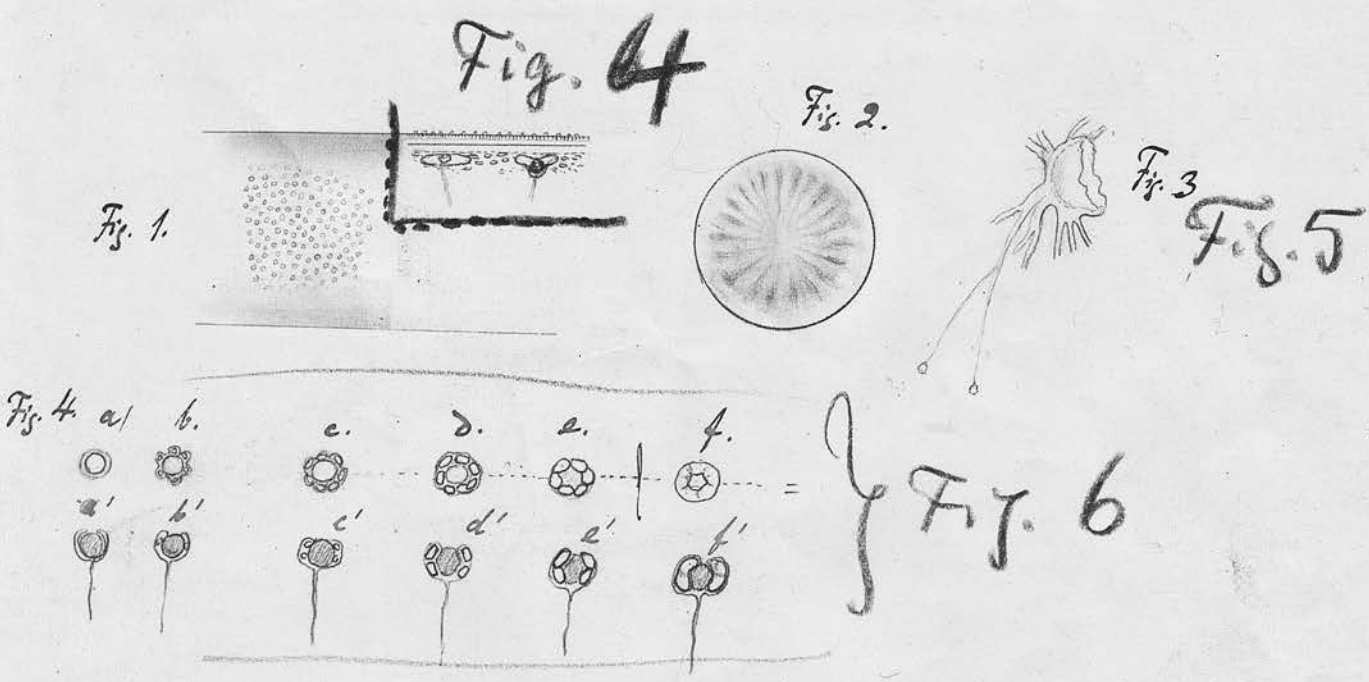


Fig. 1. Spirog. nitida, showing tubercular appearance of outer layer of the cell wall on the left side. and on the right side the appearance of the cell wall with expanded and contracted chlorophyll band, and microsomata, on side view.

Fig. 2. Surface view of septum of Sp. nitida

Fig. 3. Part of isolated mantle formed by the fusion of the thread round the nucleus with 2 thread going to 2 pyrenoïd. The chlorophyll band for diagramatic purposes is left out.

Fig. 4. a – f. successive stages in the formation of Starch.
d' is a sketch of an isolated pyrenoïd with piece of thread attached, the other figures are constructed after this and correspond to the actually observed condition of the pyrenoïd on surface view.
a' – f' c sideviews of the pyrenoïd.