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"STUDIES IN SPIROGYRA"

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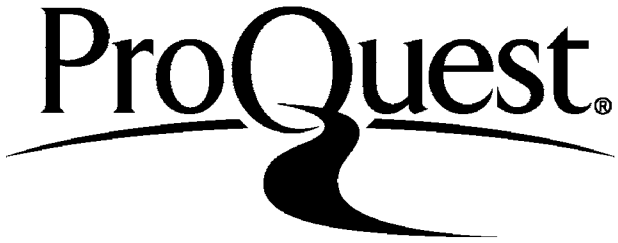

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Abstract.

Studies in Spirogyra.

Part I. Variation in S.cataeniformis.

S. cataeniformis has been investigated in order to find whether there is any relation between the range of variation it shows, and the season of the year.

Cell length. The range in variation of cell length, and an expression for the mean cell length, has been determined statistically at fortnightly intervals during twenty months. The method adopted was a s follows:- a standard length of filament was chosen and the number of cells in this length was determined for 200 filaments; and the maximum, minimum, and average length of cell, present in four cells of these 200 filaments, was recorded.

Chloroplast. Records have been made of the following:- (1) the range in variation of the number of chloroplast turns per cell; (2) the maximum, minimum, and average number of chloroplast turns per cell; (3) the relation between the number of chloroplast turns per cell, the length of cell, and the number of pyrenoids per cell.

Micro-chemical reactions. At various times of the year the starch and oil content of the cells and the nature and reaction of the cell wall have been recorded.

It has been found that with regard to all these characters S.cataeniformis shows no seasonal variation. In spite of a well marked periodicity the range in form and structure of the cells is approximately the same at all times of the year.

Part II Conjugation in Spirogyra.

An attempt has been made to discover the way in which the filaments of Spirogyra become laced together during conjugation. At the present time there exist two essentially different descriptions of the early stages of conjugation. In one, the two filaments are said to be parallel to each other, and the opposing cells to send out papillae which grow towards each

other till they meet and fuse. In the other, the two filaments are dais to lie in contact, and are pushed apart by the growth of papillae from opposing cells.

Three species of Spirogyra have been investigated: S.weberi S.varians, and S.cataeniformis. Special methods of culture were employed so that the conjugating filament pairs could be watched, under nearly natural conditions, from an early stage of conjugation to a later stage. Drawings of these stages have been made with camera lucida, and photographs have been taken.

In all three species it has been found that the filaments first lie in contact, and are later pushed apart by the growth of papillae from opposite cells; the first formed papilla may arise in either of the conjugating filaments and is independent of sex.

The position of the nucleus in the cells, the relative size of the conjugating cells, and the growth in length of the cells during conjugation have been described.

STUDIES IN SPIROGYRA.

PART I.

VARIATION IN SPIROGYRA CATAENIFORMIS.

INDEX TO PART I.

VARIATION IN SPIROGYRA CATAENIFORMIS.

INTRODUCTION.

A recent study of Trentepohlia has revealed the interesting fact that there is a relation between the range of variation in cell form and the season of the year. In an unpublished paper by Dr.W.H.Pearsall, which he has kindly lent, similar variations, not only in the size of cell but also in the nature of the all, are recorded for Ceratium hirundinella. In this species the form changes are considerable: the vegetative cells pass through a winter phase, a spring phase,and a summer phase, and in each of these phases they are very different in external appearance. This seasonal form variation has been explained in terms of known changes in the surrounding waters, and of the metabolic changes in the organism itself. In the present work S.cataeniformis has been investigated from a similar point of view,to discover whether there is any relation between the range of variation and the season. For this purpose the variation in cell width, cell length,and the number of turns of chloroplast per cell, starch content, oil content, and the structure of the wall, have been investigated. It has been found that with the exception of oil content the variation in these characters is not a seasonal one: the same range of variation is shown at all times of year when the species is to be found. In other words the investigation as planned has given a negative result. It is surprising that a species of Spirogyra with its periodicity, should not show a seasonal variation in size. The species worked on was selected because it grew in a pond close at hand,and not for any advantageous characters it posessed. Indeed it is a small species, and one that does not conjugate readily in this pond. It was observed closely from November 1927 to August 1929 but did not produce zygospores until July 1929. For this reason it could not be identified until the end of the investigation, and it was fortunate that this species, chosen at random, should turn out to be one,

described in the key of Borge and Pascher, as very variable in the length of its cells. One would expect such a species to show a greater seasonal variation than a species which is only slightly variable in cell length. Interesting results have been obtained, indicating that the range of variation in cell length, cell width, the number of turns of chloroplast in the cell, and the width of the chloroplast, is very different at a time when the Spirogyra is slowly disappearing from the pond. The majority of filaments at this time appear inactive and in a state which can only be described as senescent, The Spirogyra was only observed once in this senescent state and that was in the autumn of 1927, before which time its history in this pond was unknown. Since neither conjugation, nor senescence, were observed during the following year, 1928, it can be conjectured that possibly the two phenomena are in some way connected. In that year, when there were no zygotes to reproduce the species, it passed the winter by means of perennating filaments, which were very well hidden in the mud, and were by no means abundant, There was no time when the Spirogyra appeared to be in a senescent state; it gradually lessened in amount as the filaments passed into the mud, these filaments remaining active and vigorous in appearance. The perennating filaments were in function analagous to a resting spore, but they were in no obvious way different from ordinary vegetative filaments; they were very resistant, for the winter they endured was an abnormally cold one and the pond, which has a slow trickle of water through it, was frozen over fro a number of days. Perennating filaments were not observed when the Spirogyra disappeared after its period of senescence in 1927, possibly they only occur in the absence of conjugation and acquire some of the resistant characters of the zygote. In June 1929, conjugation was general in the whole mass of Spirogyra but only in a few cases was i t completed: the majority of filament pairs died. A senescent period was not observed in the autumn of 1929, very

little Spirogyra remained after the conjugation period, and by October it had all disappeared. The pond however was not under normal conditions for it was choked up with silt and grass. In connection with the periodicity of the species rough estimates of the composition of the water have been made, and the temperature of the water was recorded at fortnightly intervals during the investigation.

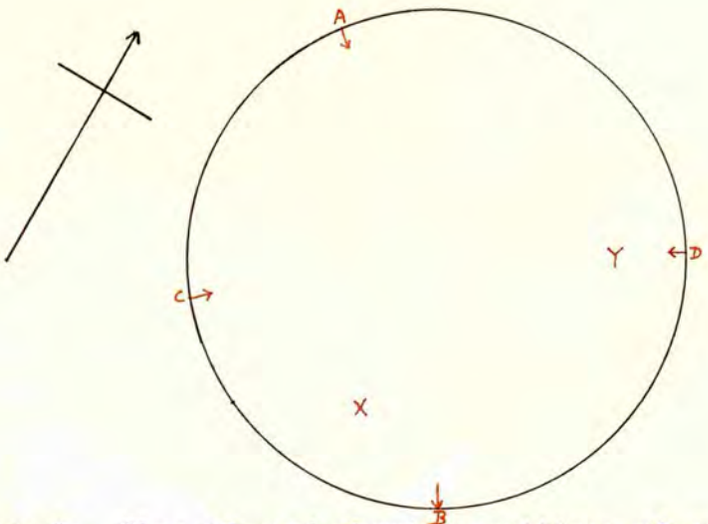
Although with regard to cell length, cell width, and the number of turns of chloroplast per cell, the range in variation shows no consistent alteration, except in the senescent stage, it must be recorded that certain filaments have been observed with two chloroplasts in some or all of the cells. Such a difference as this is often counted of specific value, but with these filaments this is not the case. They have been observed at all times of the year, in times of active growth, in perennating filaments, and in the senescent stage, and they are undoubtedly filaments of S.cataeniformis.

SOURCE OF MATERIAL.

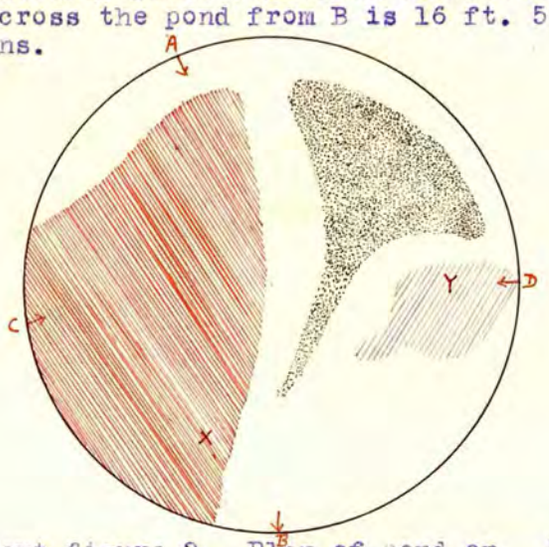
Fresh material of S.cataeniformis, was collected from a pond in the grounds of Royal Holloway College. The pond is roughly circular with a diameter varying from 16.4 to 19.2 ft. It is constantly replenished by drainage water from the surrounding higher ground, the water entering three pipes A, C, and D (Text fig. 1). The floor of the pond consists of silt deposited on a clay bottom, and at the edge, there is a foot or more of silt, and only a very small depth of clear water. The material used for measurements made between November 1927 and January 1928, was collected from a part marked X in text fig. 1. The material used for the later measurements, was collected from the opposite side of the pond, round about Y.

Text figs. 2 - 8 indicate diagrammatically, the vegetation of the pond as visible to the naked eye at various times of the year.

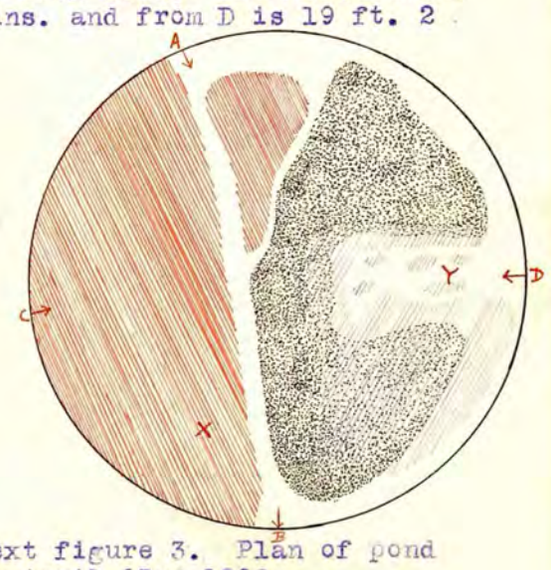
In the material collected between November 1927 and



Text figure 1. Plan of pond showing positions of supply pipes A, C, D, and of exit pipe B. X marks the position of material first collected on November 20th 1927. Y marks the position of material first collected on February 20th 1928. The measurement across the pond from B is 16 ft. 5 ins. and from D is 19 ft. 2 ins.



Text figure 2. Plan of pond on March 23rd 1928.



Text figure 3. Plan of pond on April 23rd 1928.
N.B. On May 3rd the drains at A and C were cleaned out and the pond disturbed in this region.

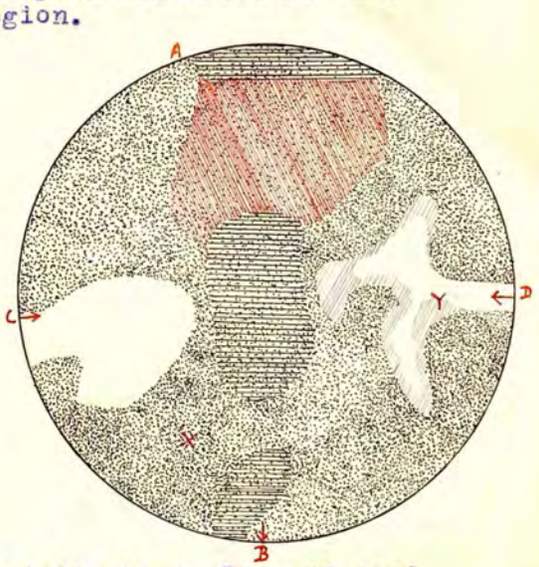
Key to text figs. 2 - 4.

Callitriche.

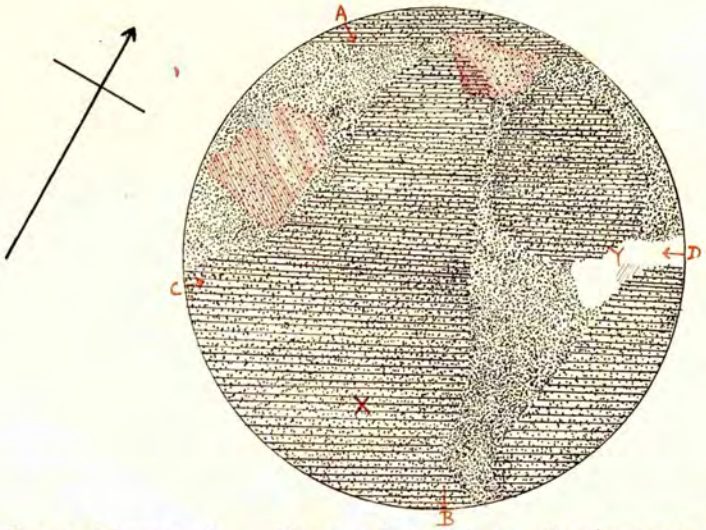
Poa fluitans.

Mougeotia and other algae.

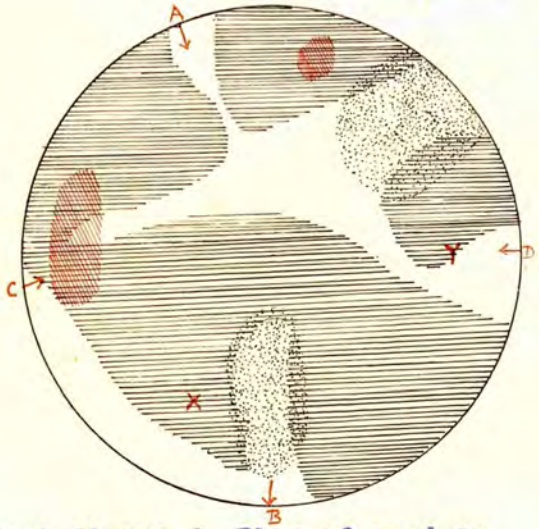
Spirogyra cataeniformis.



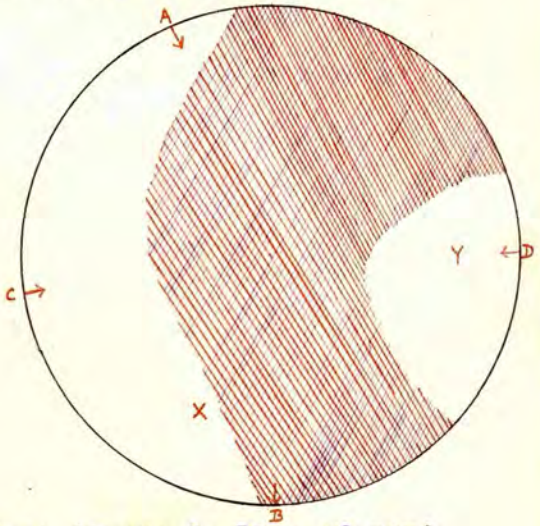
Text figure 4. Plan of pond on August 9th 1928.



Text figure 5. Plan of pond on September 4th 1928.



Text figure 6. Plan of pond on October 23rd 1928.
N.B. On Oct.23rd grass was uprooted from pond.
On Dec.4th the pond was cleaned out and accumulated silt removed except at X and Y.



Text figure 7. Plan of pond on April 26th 1929.
N.B. Spirogyra was generally distributed amongst the Mougeotia.

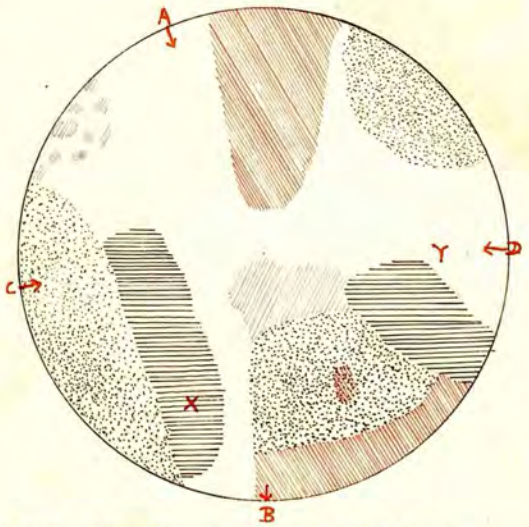
Key to text figs. 5 - 8.

Callitriche.

Poa fluitans.

Mougeotia and other algae.

Spirogyra cataeniformis.



Text figure 8. Plan of pond on August 9th 1929.
N.B. The drains were being cleaned out and much silt had collected in the pond

January 1928, there were filaments of various widths present, but in almost all the later collections there were filaments of two distinct widths, the difference between them being five microns. These two kinds of filament will be referred to as the wide and the narrow type, and they will be regarded as varieties of the one species S.cataeniformis.

The temperature of the water of the pond was taken when the material was collected, the following table gives the temperature in degrees Centigrade at about 9.a.m. on the first day of each fortnightly measurement. The square brackets indicate the periods when the Spirogyra was abundant in the pond; the dates and temperatures not included inthe brackets refer to times when no Spirogyra could be seen in the pond.

Date	Temperature in degrees centigrade.
December 7th 1927.	8.0 C.
December 22nd.	8.5.
January 6th 1928.	7.5.
February 2nd.	6.0.
February 6th.	6.25.
February 20th.	6.5.
March 6th.	8.0.
March 20th.	7.8.
April 2nd.	8.5.
April 18th.	7.8.
May 1st.	10.0.
May 15th.	10.0.
May 29th.	11.0.
June 13th.	11.75.
June 26th.	11.5.
July 10th.	12.0.
July 24th.	13.0.
August 7th.	12.75.
September 4th.	12.5.

September 18th.		12.25.
October 5th.		11.25.
November 7th.		9.5.
February 12th.	0.	0.
February 21st.		0.
March 21st.		7.5.
April 4th.		7.5.
April 22nd.		7.0.
June 12th.		13.0.

The water of the pond was analysed and its approximate composition was determined with respect to the following characters.

The concentration of the water was determined by evaporating 200 cc of the filtered water to dryness, weighing the residue, and calculating the total amount of mineral matter per 100,000 parts of water. The results are only of value comparatively, since the residue is hygroscopic, and moisture is absorbed during the weighing. A correct idea of the concentration could not be obtained by determining the fluctuations in the water level, because water was running through the pond.

The weight of chlorine per 100,000 parts of water was determined by titration of the pond water against silver nitrate using potassium chromate as an indicator.

The temporary and permanent hardness of the water was calculated by Herner's method. The temporary hardness was found to be negligible, but from the results for permanent hardness the weight of calcium per 100,000 parts of water was calculated.

The weight of nitrogen due to nitrates was determined by a colorimetric method using phenol sulphuric acid and standard sodium nitrate solution. It was assumed that no nitrates were present in the water since no coloration was given with metaphenyllenediamine.

The amount of iron in the water was not estimated quanti-

tatively, but its presence or absence was detected by the use of potassium ferricyanide and also by the coloration of the silt.

The following table gives the results of the water analysis of December 1st 1927, February 2nd 1928, March 19th 1928, and May 24th 1928. The same standard solutions were used in each estimation, and the water to be estimated was collected either from X or Y as indicated in the table.

	Dec. 1st. 1927.	Feb. 2nd. 1928.	Mar. 19th. 1928.	Mar. 19th. 1928.	Mar. 19th. 1928.	May. 24th. 1928.
Place of Collection.	X	X	X	Y	D	Y
Total minerals per 100,000 cc.	71.8	73.9	67.95	61.88	-	61.0
Wt. of Chlorine per 100,000 cc.	7.06	7.06	7.165	5.998	5.998	5.97
Wt. of Calcium per 100,000 cc.	28.95	28.56	24.9	21.73	21.9	22.12
Wt. of Nitrogen per 100,000 cc.	2.04	2.	2.	2.	-	2.
Relative amount of Iron present.	No Iron.	No Iron.	No Iron.	Much Iron.	-	Much Iron.

The table shows that the composition of the water at X was practically identical on December 1st and February 2nd, and that the composition of the water at Y was practically identical on March 19th and May 24th, but that on March 19th, the composition of the water at X and Y differed considerably. With regard to the previous estimation the water at X showed a very slight increase in the chlorine content, and a decrease in the calcium content. With regard to the water at Y, the chlorine and calcium contents were both higher, the chlorine content being considerably higher. This fact will be referred to later with regard to the periodicity of the Spirogyra. The composition of the water at Y and the water taken from pipe D before it entered the pond was practically identical.

PERIODICITY OF SPIROGYRA CATAENIFORMIS NOVEMBER 1927 - AUGUST 1929.

The pond was under observation from November 1927 until July 1929, and during this time there were three periods when the Spirogyra occurred in abundance, so that patches of it were visible to the naked eye. These periods were from November 20th 1927, (when the pond was first examined), to January 1928, from February 20th 1928 to October 6th 1928, and from March 1929 to August 1929 when there was still some of the Spirogyra visible. Between these periods a careful investigation of the pond showed that the Spirogyra was never completely absent but was present in minute quantities.

1st Period November 20th - January 6th 1928.

When the material was first collected from the pond on November 20th (Text fig. 1) it was bright green and a dipping brought up Spirogyra and Mougeotia in about equal quantities. From this time until January 6th 1928, the Spirogyra decreased in amount, so that for the "count" on January 6th there was only a very little Spirogyra, and this was in short filaments amongst the Mougeotia which showed no such decrease. On January 19th, more material was collected from the same spot for measurement, but no Spirogyra could be found. Material was then collected from various parts of the pond, but it consisted entirely of Mougeotia, and no Spirogyra could be seen. Mud was then collected and examined, but there was no trace of Spirogyra, and not until February 20th did it appear again in any quantity.

On January 19th, after a beaker full of material had been thoroughly searched for Spirogyra, it was thrown away. It was then noticed that a brownish deposit was left at the bottom of the beaker. This deposit was examined, it contained numerous diatoms, and amongst them Spirogyra in very short filaments or unicells. Forty seven of these short filaments were measured and they were of various types, corresponding to the various types of filament which had been seen. The longest of these short filaments had twelve cells, but the majority had one or

two cells. These short filaments of Spirogyra were by no means abundant, but with careful search they could usually be found in the deposits from each collection. They were found on the following days: January 20th, 24th, 27th; February 6th, 9th, 13th, 17th. Usually only one or two were found on a slide crowded with diatoms; occasionally they were more abundant; sometimes there were none at all. On January 24th and January 30th, material collected from the pond at B, near the exit, showed occasional quite long filaments of Spirogyra, amongst the Mougeotia, as well as short filaments in the deposit which adhered to the bottom of the beaker.

2nd Period February 20th - October 6th 1929.

The second appearance of the Spirogyra was noted on February 20th, when a small bright green clump was seen at the opposite side of the pond, in front of entrance pipe D. The pond here was quite brown due to the presence of an iron bacterium, and the water was considerably more shallow than at the other side, its temperature was 6.5 C (Text fig. 1). This clump consisted entirely of Spirogyra, except for a few individuals of a species of Closterium, and the threads were long, short-celled and appeared to be in active division. Since they occured in such a localised area it seemed probable that they had arisen by germination of zygotes, but although a thorough search of the mud was made no zygotes were seen. Single cells and very short filaments were seen in the mud collected with this material; they were similar to the long filaments, and had probably arisen by the breaking up of these, for their occurrence is general throughout the year. At the original place of collection (Text fig. 1. X) Mougeotia was still present but there was no Spirogyra. The Spirogyra at the Y increased in amount throughout the spring of 1928, and it was present in large quantity until July, when it began to decrease. It gradually decreased until October 9th when no Spirogyra could be seen by the unaided eye, although examination of the mud

showed that it was present there (Text figs. 2-6). After the Spirogyra had appeared at Y, it also appeared in other parts of the pond, though not to any large extent. At the original side of the pond, at X, Spirogyra was first noted on April 21st, there was still a large patch of green material here which consisted mainly of Mougeotia (Text fig. 3), but there was now with it a few Spirogyra filaments similar to those found at Y. On May 3rd the drains were cleaned out, silt was removed from around A and C so that the large Mougeotia patch was very much disturbed and temporarily disappeared. However by June 12th the Mougeotia patch had again appeared around X and short filaments of Spirogyra were found in it, On June 24th the patch consisted entirely of Mougeotia and Tribonema and no Spirogyra was seen. On August 7th there was no green patch visible at this side of the pond (Text fig. 4), but by September 4th it had appeared again (Text fig. 5), and consisted of Mougeotia amongst which were rare filaments of Spirogyra of the narrow type. This patch of Mougeotia was examined on September 18th, October 23rd, 31st, November 8th, and 16th, but no Spirogyra was found in it. At the S.E. of the pond, just to the right of the exit B, the Spirogyra was first noted to have reappeared on April 21st 1928, when a greenish patch, in water brown with iron, was examined, and found to contain both types of Spirogyra amongst abundant individuals of a species of Closterium; Spirogyra was also noted amongst Callitriche in the deeper water further from the bank. At the opposite side of the pond, to the N.E. of entrance A, Spirogyra was first noted on May 5th 1928 in a very small area of a green scum that appeared there, mainly of Mougeotia. This green patch increased in size: on August 7th (Text fig. 4) it consisted of Lemna, Mougeotia, Oedogonium, and Closterium, and in every examination about one filament of Spirogyra. On September 4th (Text fig. 5) in this comparatively small patch, Oedogonium and Mougeotia were still abundant; Cosmarium was locally abundant Spirogyra filaments of

the narrow type were rare. From September 4th to November 8th, occasional Spirogyra filaments of the wide and narrow types were seen in this patch, but on November 16th no Spirogyra was found, the patch consisting entirely of Oedogonium and Mougeotia

As has been stated, the patch of Spirogyra which lay around Y was visible until October 6th 1928. It had decreased in size from July 1928, and on September 4th, only a very minute green patch could be seen (Text fig. 5). This had increased in size by September 18th when it was large enough to cover the palm of one's hand. However on October 5th and 6th it was again very small, and after material had been collected for examination, no trace of the Spirogyra could be seen in the pond. From this time to the third appearance of the Spirogyra in any quantity, traces of it were found in the mud around Y. The following is a record of the rare filaments of Spirogyra found during its apparent disappearance ~~from~~ during the winter October 1928 - March 1929.

October 8th and 9th. Examination of the mud at Y revealed vigorous filaments of Spirogyra at the place where this was last seen.

October 23rd. No Spirogyra was found in the mud at Y, but while uprooting the grass which had grown in the pond, a very small tuft of green filaments was seen at Y. It was found to contain very long threads of actively dividing Spirogyra. After examination the tuft was replaced in the pond.

October 31st. No Spirogyra was found in mud collected from around Y.

November 7th. A minute tuft of green filaments was seen at place Y. It consisted of very vigorous Spirogyra; the cells were very short and the chloroplast so compact, that it could hardly be recognised as a spiral band except at the end of a filament. After examination this tuft was replaced in the pond.

November 12th. A small portion of mud was collected from the S.W. of Y. The mud seemed slightly darker in colour at this part, and on examination quite a number of vigorous filaments of Spirogyra were found in it. The mud collected was left in the tube till November 14th when quite a little tuft of Spirogyra, visible to the naked eye, had grown out of it.

November 14th. A small portion of mud was collected from Y and after a long search only two filaments of Spirogyra were found in it: one looked vigorous with a compact chloroplast, the other less so with a lax chloroplast. This mud was kept until November 22nd when two more filaments were found, and one of these had a number of small oil globules in the cells.

November 16th. A thorough examination of the mud

around Y was made but no Spirogyra could be found.

November 21st. A small portion of mud of a deeper colour than the rest was noted in place Y; a small amount was collected enough to cover a shilling, and on examination it was found to contain two filaments and a unicell of Spirogyra. One of the filaments looked very vigorous with short cells and a compact chloroplast, the other had longer cells and the chloroplast was not so compact. On December 4th, the College Authorities ordered the yearly cleaning of the pond and some of the accumulated silt was removed. The pond was however left undisturbed around X and Y, in case the removal of the silt should involve the removal of the rare filaments of Spirogyra which were still present. The greater amount of silt at X and Y naturally became dispersed over the the floor of the pond.

December 5th. A "stringy" bit of mud was collected from Y. The stringiness was caused by the presence of a dead filamentous alga probably Vaucheria but amongst it there were quite a number of Spirogyra filaments. These filaments were very vigorous with short cells andcompact chloroplasts. The mud was replaced in the pond.

January 19th 1929. The mud was thoroughly examined around Y but no Spirogyra was found.

January 28th. A "stringy" looking portion of floating material was collected from Y. It appeared to be slightly green and was found to contain abundant Closterium and some Mougeotia around the remains of Callitriche. After a long search one filament of Spirogyra was found in it; it had a very pale chloroplast which was packed with starch grains. The material was again examined on January 29th and a long vigorous filament of Spirogyra was found. The material was kept until February 4th when two more filaments of Spirogyra were found. One of these, a short filament had long cells and a lax chloroplast, the other was a long filament and had short cells with a compact chloroplast.

February 6th. A small portion of Callitriche covered with mud was collected from just S.E. of Y, at the edge of the area brown with the iron bacteria and green with Chlamydomonas. The rust brown mud was examined and as well as abundant Chlamydomonas there was found to be abundant Closterium. A few Mougeotia filaments were also seen, and after carefully teasing out the mud in which they were very well hidden, rare Spirogyra filaments were found. The material was kept in the laboratory in a specimen tube and frequently examined for Spirogyra filaments. A number of these were found, and round about February 19th they were found easily, one or two being present in each slide of material mounted. Either the Spirogyra had increased in amount, or else it was locally abundant even within so small an area of mud as had been collected in the tube. By March 1st there was not much Spirogyra left in the tube and so this was placed under constant illumination of 30 C.P. On March 4th, a number of Spirogyra threads could be seen in the water, and by March 16th these had greatly increased in number and a thick, long tuft of Spirogyra threads was present, visible to the naked eye. Sixty eight of these filaments were measured and a number of others were employed for various tests.

February 21st. A very small greenish patch of Callitriche and mud was collected from the edge of the iron patch to the S.E. of Y. The greeness was due to Mougeotia; for a long time no Spirogyra could be found, but after a long search two short filaments were found, one of these had a very pale chloroplast and both had chloroplasts

packed with starch.

February 21st and 24th. A few green floating patches had appeared in the middle of the pond, they consisted of Mougeotia and Tribonema, but no Spirogyra was found in them. A small green patch had appeared at B, it consisted of Mougeotia but after a long search one long filament of Spirogyra was found in it.

March 1st. A small amount of material was collected from one of the green patches in the middle of the pond S.W. of Y. It consisted mainly of Mougeotia, but some Tribonema and Closterium was present. Two Spirogyra filaments were found after a thorough search. Both looked very vigorous, having short cells with compact chloroplasts

March 1st. A very small portion of faintly green mud with Callitriche, was collected from the S.E. of Y. The green colour was due to Mougeotia which was abundant. In it were found two short filaments of Spirogyra. The material was kept in the laboratory and on March 5th, 7th, and 8th, filaments of Spirogyra were found looking very vigorous, with short cells and compact chloroplasts, probably in a state of division.

March 7th. Mud and some of the floating green material was collected from S.W. of Y and examined. No Spirogyra was found there. A green patch N.E. of A was also examined, it consisted of Mougeotia and Microspora but there was no Spirogyra.

March 12th. A small portion of stringy mud was collected from the S.E. of Y at the edge of the iron patch. It consisted of Mougeotia and Tribonema and Closterium: but Spirogyra was absent.

March 18th. A small portion of greenish "stringy" mud was collected from the S.E. of Y. There was abundant Tribonema and some Mougeotia and Closterium present, and each slide of material examined had one or two filaments of Spirogyra. The material was kept in the Laboratory and again examined on March 19th, when a whole clump of Spirogyra filaments was extracted. After being kept in the laboratory the mud sank to the bottom of the containing tube, and a thick tuft of long threads of Spirogyra could be seen growing from it. This collection made on March 18th was the first in which Spirogyra appeared in any quantity. The green algal patches floating in the middle of the pond had now become abundant. Some material was collected from them, it consisted mainly of Mougeotia but a few filaments of Spirogyra were present.

March 21st. Material was collected from floating green-brown "stringy" patches, and from the middle of the pond. In each case there was abundant Mougeotia with quite a lot of Closterium and some Spirogyra, two, three, or more filaments on each slide of material examined. The Spirogyra varied in abundance in each collection, some slides of material showed a number of Spirogyra filaments, others only two or three.

March 22nd and 23rd - 25th. In material collected from just to the S.W. of Y -(the green patches had not arisen at Y)-Mougeotia was abundant, there were about three filaments of Spirogyra on each slide examined, and sometimes many more as the Spirogyra occurred in little clumps. By March 25th some Spirogyra was present in all the patches of greeness in the pond.

April 4th. Darker green clumps could be seen among the green floating masses which were present over about half the pond. These clumps were found to consist purely of Spirogyra which had become once again visible to the naked eye.

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3rd Period April 1929 - August 1929 and subsequently.

The Spirogyra then had reappeared by April 1929. On April 26th the pond was examined, and the yellow green algal patches had spread so that they completely covered the whole of the central portion of the pond. They had not spread quite to Y, but only touched the former limits of the Spirogyra patch which previously occurred at this place; at X there was no greeness (Text fig. 7). It is curious that the Spirogyra should not reappear in either of the places where previously it had been abundant. The staling of the water, or the exhaustion of any growth factor from it in thesevtwo regions, would be prevented both by diffusion, and the slow but steady outflow of the water of the pond. On December 4th the pond was partly cleaned out and some of the silt which was constantly accumulating, so that there was very little free water was removed. The silt around Y and X was left untouched and it was expected that the Spirogyra would appear in these places. However no Spirogyra or Mougeotia appeared at X or Y, and moreover the area around Y free from Algae, corresponded to the area which was not cleaned out. Thus possibly the mud contained some staling substance which prevented growth. Examination of the green patches showed that Spirogyra was pretty generally distributed in them, and the edge of the patch nearest Y consisted purely of Spirogyra. The Spirogyra increased in amount and the Mougeotia decreased, the algal patches changing from yellow green to dark green. By May 13th the green area was almost pure Spirogyra, except at the N.W. of the pond where there was Mougeotia. The Spirogyra lay in thick dark green wads below the surface of the water. By June 6th the Spirogyra had grown in towards the entrance pipe D and covered Y; its appearance was somewhat different, very few of the thick dark green wads were present, but the surface of the pond was covered with dull green patches. To the S.W. of Y, where the Spirogyra first appeared in abundance in April 1929, there was a large

area covered with black material, this was found to consist of dead and decaying Spirogyra. To the S.E. of Y, Callitriche and grass had appeared, and amongst this there were dark green masses of Spirogyra which had occasional filaments in conjugation. By June 14th conjugation was fairly general in the pond, but at the same time there were a few dark green wads below the surface of the water which consisted of purely vegetative material. Conjugation continued throughout June and the amount of Spirogyra rapidly decreased. It is curious that throughout the conjugation period very few zygotes were found, although filament pairs laced together by conjugation tubes wrre abundant. Conjugating material was carefully watched from day to day, and it was found that many of the filament pairs died, giving rise to brownish black patches which sank into the mud. The pond was again examined on July 22nd, and there was no signs of conjugation in any part. At Y there were a few thick wads of Spirogyra; there were no wads elsewhere although the former green area still contained abundant Spirogyra. To the N.E. of A, there was abundant Mougeotia and a few Spirogyra filaments of various widths. At X, a bright green patch had now appeared, it consisted of Mougeotia amongst which occasional filaments of Spirogyra could be found. On August 9th the pond was examined and found to contain very little Spirogyra (Text fig. 8). At Y there was no Spirogyra, the pond was choked up with mud and there was no free water; this was partly due to the drainage pipes having been cleaned out, the pond itself being left untouched. To the S.E. of Y there was a small patch of Mougeotia and two unicells of Spirogyra were found in material collected from it; these unicells appeared to be dying. At X there was no Spirogyra but a little decaying Mougeotia was found. Between A and C (Text fig. 8) there was an area containing dark green patches consisting purely of Spirogyra. This was the first time since 1927, that Spirogyra had been noted in abundance at this side of the pond. A portion of green material

from near the middle of the pond was collected, it consisted purely of Spirogyra. Material from a large green patch to the N.E. of A was collected, it consisted mainly of Mougeotia but in each slideful of material examined, two or three filaments of Spirogyra were seen, and these were of various widths.

The above account of the periodicity of Spirogyra in the pond, indicates that with regard to the 2nd and 3rd periods of its abundance, the same species, S.cataeniformis was present. Between these two periods, when the Spirogyra had practically disaapeared from the pond, rare filaments which had survived from the 2nd period were found perennating in the mud; these filaments established the continuity of the Spirogyra, from the 2nd, to the 3rd period of its abundance. Particularly clear evidence of this continuity was shown by a culture, previously referred to, started from a collection made on February 6th, when no Spirogyra could be seen in the pond. The small portion of mud and Callitriche then collected,was taken from near Y where the Spirogyra of the 2nd period had been abundant. It was found to contain perennating filaments of Spirogyra which were well hidden in the mud,amd only found on carefully teasing this out on a slide. Between February 6th and March 1st,a culture of this material was kept in a specimen tube in the laboratory and during frequent examination of the mud,eighteen perennating filaments were found. By March 1st, the mud was becoming exhausted of its filaments and was placed under constant illumination of 20 C.P. On March 4th,a few filaments could be seen with the naked eye growing from the mud, and by March 16th,there was a thick long tuft of Spirogyra filaments curling round the specimen tube, and very easily visible to the naked eye. This tuft of Spirogyra had obviously arisen by growth of the perennating filaments and this development in culture, was paralleled by that in the pond, for a similar rapid appearance of the Spirogyra was observed a few days later. Without question the same species S.cataeniformis

accounted for the 2nd and 3rd periods of abunbance of the Spirogyra.

With regard to the 1st period of abundance of the Spirogyra there is no proof that it is the same species that was present: it occurred at the opposite side of the pond, it was never observed in conjugation, and its previous history was unknown. However there is no evidence to lead one to suppose that it was another species that was then present for it has never been seen since. Had the Spirogyra colonies of the 1st and 2nd periods appeared in the same part of the pond, there would have been no hesitation in accepting them as the same species; but, as has been stated, the Spirogyra of the 2nd period appeared at the opposite side of the pond. At first this seemed to indicate that they were different species, but later observations have shown that Spirogyra can grow and disappear with extreme rapidity. For instance, on July 22nd 1929, there was abundant Spirogyra around Y but none at all at the opposite side of the pomd. On August 9th, there was no Spirogyra at Y but a new patch had arisen at the opposite side of the pond, where there had been no Spirogyra in abundance since the 1st period. It is possible that the Spirogyra of the 2nd period arose from the unicells and short filaments of the Spirogyra of the 1st period, which havevbeen mentioned above. These unicells and filaments were only noted around X, and B, but it is possible that they also existed in the mud at Y. However, the presence of unicells and short filaments is by no means confined to a period when the Spirogyra is disappearing, they can be found at any time. The Spirogyra of the 1st period differs from that of the 2nd and 3rd periods in that it shows a greater range of variation with regard to cell length and breadth. It contained a number of types of the same species merging into each other, and two of these types were similar to two types of the 2nd and 3rd period. In the following account therefore it will be assumed

that the Spirogyra of the 1st, 2nd and 3rd period is S.cataeniformis.

The phenomenon of periodicity, although possibly largely due to an inherent tendency, is known to be influenced by a large number of factors such as changes in light intensity, changes in the temperature of the water, changes in the concentration of salts in the water, etc. The temperature of the pond water at various times of the year has already been given and the periodicity of Spirogyra cataeniformis with reference to this will now be discussed.

At the end of the 1st period on January 6th 1928, the temperature was 7.5 C, while at the beginning of the 2nd period on February 20th 1928, it was 6.5 C. Between these dates when no Spirogyra was visible in the pond, the temperature on February 2nd was recorded as 6 C. Thus from the time the Spirogyra disappeared, to the time when it reappeared, there was very little change in temperature, so that temperature was probably not acting as a limiting factor to the reappearance of the Spirogyra. At the end of the 2nd period, on October 6th 1928, the temperature was 11.25 C, by November 7th, it had sunk to 9.5 C, and between February 12th, and February 21st 1929, it was at 0 C and the pond was partially frozen over. On February 21st 1929, the temperature was considerably lower than the previous year and Spirogyra was still practically absent from the pond; whereas in the previous year, it had entered upon its Spring phase at this time. At the beginning of the 3rd period of abundance of the Spirogyra in April 1929, the temperature was 7 C. It had been 7 or 7.5 C since March 21st and it was about this time that the Spirogyra filaments were first found to be increasing in the pond. The temperature of the water at the beginning of the 2nd and 3rd periods was very nearly the same, and possibly the later appearance of the Spirogyra in 1929, was due to low temperatures acting asaa limiting factor to growth.

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The results of water analysis carried out during and between the 1st and 2nd periods of abundance of the Spirogyra, may bear some relation to periodicity. With regard to chlorine content it was found that at Y, when the Spirogyra reappeared, the chlorine content was considerably lower than it had previously been at X, when the Spirogyra was growing there. No Spirogyra had reappeared at X and the chlorine content had not decreased, but was very slightly higher than when the Spirogyra was present. This high chlorine content may have acted as an inhibiting factor to the spring growth of the Spirogyra.

Another factor which may bear some relation to the periodicity of the Spirogyra is the amount of iron in the pond, and this is correlated with the periodicity of iron bacteria. At X, when the Spirogyra was disappearing in December 1927, and after it had disappeared, there was no trace of iron in the water, which gave no reaction with potassium ferricyanide. On February 20th 1928, there was no iron at X but there was abundant iron at Y where the Spirogyra first appeared. Here the mud was a rusty-brown colour due to the presence of iron bacteria, and the water reacted with potassium ferricyanide indicating the presence of iron. The iron patch at Y spread S.E. to B and the Spirogyra also spread in this direction. A similar parallelism between the area of the pond occupied by iron bacteria, and the area covered by Spirogyra was shown in 1929. On December 4th 1928, after the 2nd period o f abundance of the Spirogyra, the pond was cleaned out, and a quantity of silt removed from all parts except X and Y. After the pond had been cleaned out, there was no visible sign of the presence of iron bacteria, the mud was not a rusty-brown colour in any portion of the pond. On January 17th there was a broad belt of a rusty-red colour spreading from B right across the centre of the pond. By January 28th this patch had spread towards Y and Closterium was abundant in parts of it. By February 6th the portion of the iron patch between B and Y was covered with

Chlamydomonas but the patch had not encroached upon X or Y where the pond had not been cleaned out. Green algal patches containing a little Spirogyra gradually arose all over the area formerly rich in iron bacteria. The patches spread, and the Spirogyra in them multiplied, so that by April 26th (Text fig. 7) a large area of the pond was covered with them and the Spirogyra was again present in abundance. Thus in both 1928 and 1929 the Spirogyra arose in areas which were rusty-red due to iron bacteria. This may merely indicate that the Spirogyra and the iron bacteria need the same conditions for growth, but it seems to suggest that the presence of iron is necessary, or favourable, to the rapid growth of Spirogyra.

VARIATION IN SIZE OF CELL NOVEMBER 1927 - JULY 1929.

Method of Collecting Data.

Gatherings of fresh material were examined at fortnightly intervals and the following vegetative characters were investigated: cell length, cell breadth, number of chloroplasts and turns of chloroplast per cell, width of chloroplast, number of pyrenoids per cell, starch content, oil content and nature of wall. Some of these characters were examined statistically and for this purpose a certain number of filaments were measured each fortnight by means of a micrometer eyepiece graduated in 1/10ths of a millimetre. To obtain an expression for the mean cell length in a filament, the number of cells in an arbitrary length was calculated for each filament. The length chosen was 960 microns which was the length of ten cells of the first filament examined. The first three or four cells were measured individually and the number of turns of the chloroplast in each of these was noted. Then, providing that the filament was fairly straight, the rest of the cells were measured in groups

chosen purely for convenience; thus if six cells happened to lie along the scale the total length of the six was noted down. The width in any one filament was found to be constant with but few exceptions, and so as a rule only one measurement of the width was made. All these measurements were made with the low power objective (magnification X 100); but the width of the chloroplast, which was only determined in one cell of each filament, was measured with the high power objective (magnification X 420). It was necessary that the filaments measured which were to represent the entire population, should be chosen at random, so that the personal factor should be eliminated as far as possible. A large number of slides of mounted material were examined, only a few filaments being measured on each slide. The microscope used for the work was fitted with a mechanical stage, so that chance measurements could more easily be made, and the possibility of counting the same cell twice was avoided. When the cells are short and the chloroplasts compact, it is often very difficult to see the transverse walls under low power, and in February 1928 it was found impossible to measure the first four cells individually. However, since that time, Ruthenium Red has been found to be an excellent stain for walls and to show up the transverse walls quite clearly, and since it causes no shrinkage, the material has been mounted in it and used for some of the measurements. For the majority of the measurements fresh material has been collected daily the fortnightly counts taking at least three and usually four whole days, for it was found that the appearance of the material altered when it was kept in the laboratory the cells as a whole becoming longer, presumably by growth and the absence of division. The pyrenoids frequently cannot be seen in fresh material, either with or without the addition of iodine, and thus a separate measurement of the number of pyrenoids per cell had to be made with fixed and stained material.

Therefore at every measurement of fresh material, two slides were prepared of material fixed with Bouin's or Hermann's fluid, and stained with iron alum haematoxylin. This stain was found to differentiate the pyrenoids clearly, and the two slides were used for the measurement of pyrenoid number, chloroplast turns, and cell length of 200 cells chosen at random. Time limited the number of measurements made from this fixed material; they will be referred to below.

The following tables 1, 2, and 3, summarise the results of the fortnightly counts, and show at a glance the range in variation of cell length, cell width, and the number of chloroplast turns per cell, throughout the 20 months. Unless otherwise indicated, fresh material was measured, and the date refers to the first day of the measurement. Some of the results from the fixed material, mentioned above, have been incorporated into table 3; in these cases 200 cells were measured but the number of filaments could not be counted. Where it is indicated that the results have been taken from a culture under artificial light it refers to the culture already mentioned, made from a small portion of mud and Callitriche, which was collected on February 6th 1928, placed under constant illumination on March 1st 1928, and measured on March 21st 1928. The total number of filaments from which the measurements were obtained, is indicated in the tables; when the measurements were first made, the wide and narrow types of filament were thought to represent two species, and thus their measurements were recorded separately. About 200 filaments of the wide type were usually measured, and as many of the narrow type as were found by chance during the measurement of the wide. In the tables the wide and narrow types have both been included and so the total number of filaments varies. In tables 1 and 2 this variation in number has been allowed for and the results calculated for 200 and 100 filaments respectively. All the measurements of cell length and width are given in microns. The three periods of abundance

of the Spirogyra referred to above, are indicated by large brackets.

Record and interpretation of data collected.

Table 1. In Table 1 the frequency of filaments made up of cells of different lengths, is shown in terms of the number of cells in the chosen length of 960 microns. On reference to the table it will be seen that the class interval is taken as one; for example, all filaments havong more than four cells and five or fewer cells in the given length,are included in the class 4-5 at the head of the table: Similarly all filaments with mare than five cells and six or fewer cells, are included in the class 5-6. The class interval could have been taken as $\frac{1}{4}$ since the number of cells in the given length was calculated to the nearest quarter of a cell. However the larger class interval has been used,since it is preferable for diagrams and small samples. The value of a series of graphs was considered in connection with the table but their irregularities were found to be great, and they were no clearer than the table, so they have been omitted. The table does not show a regular distribution of the filaments in each measurement, possibly this is largely due to the relatively small number of filaments measured, viz. about 200. This number was occasionally increased to 240 and 300 but the irregularities were just as great. Evidently in order to eliminate them,the number of filaments measured must be very greatly increased. The least time taken for a measurement was three whole days, it usually took four days and occasionally five days according to the ease with which the cross walls could be seen. A very large increase in the number of filaments measured would be almost physically impossible for one worker,and since the time taken would be considerably more than a week, fortnightly observations would be useless. In the table those filaments with very long cells will be found to the left since the given length will only contain a few cells. Conversely those filaments with very short

TABLE I FREQUENCY OF THE NUMBER OF CELLS FOUND IN A GIVEN LENGTH OF FILAMENT.

	DATE. of MEASUREMENT	No: of Fils Measured	NUMBER OF CELLS. 4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21	21-22	22-23	23-24	24-25	25-26	26-27	27-28	28-29	29-30	30-31	31-32	32-33	33-34	34-35	35-36	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50	50-51	51-52	52-53	53-54
1st PERIOD	Nov: 23rd 1927	200	1	3	7	20	31	40	30	22	12	6	3	1	1				1																															
	Dec: 7th "	200	8	23	23	42	21	26	20	8	9	6	2	1	1	1																																	
	Dec: 21st "	200	1	4	23	28	31	40	26	18	13	8	2	4	2																																		
	Jan: 6th 1928	200		2	4	6	17	26	33	29	21	23	10	11	6	6	2	3																															
2nd PERIOD.	Feb: 21st "	200					1	2	2	4	8	6	12	8	10	19	12	12	15	9	10	7	8	8	8	7	5	6	6	3	3	3		3	1	2													
	March 6th "	241			1	2	3	5	15	18	22	22	19	20	21	10	12	11	11	3	5			2			1																						
	March 20th "	200						1	4	11	6	20	25	21	14	19	22	18	14	2	7	4	5	6	1	1																						
	April 2nd "	220						11	18	14	27	22	24	27	15	8	10	4			1		1				1																						
	April 18th "	224		1	2	3	4	13	17	13	15	16	22	13	9	11	13	12	6	10	5	1	2	2	2	1			1			1	1															
	May 1st "	217		1	2	3	5	10	10	12	19	12	20	15	10	7	12	6	10	4	6	2	8	1	4		3	2			1																
	May 15th "	210								1	1	2	1	5	9	5	7	11	12	9	7	10	12	13	14	10	8	9	8	8	11	5	5	4	2	2	1	3	4			1	1	1				
	May 29th "	211								1	1	3	4	10	7	23	17	15	18	21	13	16	10	7	9	6	5	7	6	1	2																		
	June 13th "	211				5	9	9	10	9	10	23	14	18	12	9	12	14	12	5	9	5	5	2	1	4	1								1												
	June 28th "	204						1	1	3	4	5	5	8	13	12	16	23	17	13	15	12	11	6	16	7	4	3	2	3	1		1		1													
	July 10th "	204							1	3	5	7	14	14	16	16	21	21	14	17	15	13	9	6	6	3	2	1	1	1																		
	July 24th "	203							1				1	1	5	8	11	17	12	15	18	26	7	12	18	14	8	7	6	2	3	4	2														
	Aug: 7th "	203							1	5	8	4	7	13	18	12	17	11	15	11	12	14	10	7	14	6	4	3	3	2			1		2												
	Sept: 4th "	200						1				1	5	1	7	8	8	14	13	12	14	23	20	12	12	13	13	9	4	1	5	2		1												
	Sept: 18th "	203							1		1			1	3	5	6	3	9	14	9	14	21	15	16	14	18	21	5	11	6	2	4	3			1										
	Oct: 5th "	47														1	5	3	5	4	3	5	6	2	3	1	1	3	2	1		1															
3rd PERIOD	*March 21st 1929	63						1		1	1		7	11	5	5	8	8	8	3	4	1		3			1		1																		
	March 21st "	200				1		1	1			1		1	1	8	7	10	10	8	13	12	11	14	13	12	12	8	7	6	7	6	3	6	3	1	4	5	2	1	1			2		1	1	
	April 4th "	51												1	1	1	1	1	1	5		3	1	4	1	1	4	4																			
	April 22nd "	301													2	2	2	5	4	5	5	9	7	9	5	9	9	7	14	7	7	10	4	9	8	7	7	5	4	4	8	3	3	3	4	1	3	2		2	4
	June 12th "	200										3	1	2	1	5	6	9	4	11	10	9	12	12	10	20	13	10	9	6	5	6	5	12		2	2	2		1	1	1	1	2				1		

*Culture under artificial light.

cells will be found at the right of the table. The table shows that on the whole the filaments of the 1st period had longer cells than those of the 2nd and 3rd periods, and there were no filaments with very short cells. It also shows that the majority of filaments had shorter cells in the 2nd and 3rd period, for the shaded area moves to the right from the 1st period onwards. This area includes at each fortnightly measurement only the classes having 10 or more filaments. Thus the total range of the number of cells in the given length is considerably less in the 1st period than in either of the others. The minimum number of cells in the given length is only one less than the minimum number in the 2nd and 3rd periods, and this supports the view already taken that the same species was present in all three periods. In the 2nd and 3rd periods, the range of the number of cells in the given length is considerably greater and much more varied than in the 1st period. It differs for each measurement, but in spite of the small number of filaments measured, it can be seen that there is no seasonal variation in this range, which is as great in February 1928 as in June 1928. In the first measurement of the 2nd period the most frequent number of cells to the given length is 17-18. If only the freshly collected material is considered the most frequent number of cells in the given length is 17-18, and 20-21, and in all probability lies between these two maxima. The corresponding number of cells in the measurements at the beginning of the 3rd period, i.e. March 21st and April 22nd 1929, is considerably greater, 25-26, and 27-28, respectively, and the tables extend much further to the right showing that at these times shorter cells were present; this is also shown in table 3 for the minimum cell length is less at these times than at any other. The material of the 3rd period arose from perennating filaments, the origin of the material of the 2nd period is not known, possibly it arose from zygotes for its appearance was

very sudden and in a very small portion of the pond. From March 6th to May 1st inclusive, the most frequent number of cells to the given length is remarkably constant compared with the rest of the table; it lies between 12 and 15. On May 15th this suddenly changes to 26 and 27, the number of filaments with less than 15 cells to the given length is greatly decreased, and the table is extended much further to the right. This indicates that the cells on May 15th were considerably shorter, and that either more active division or less rapid growth, had occurred between May 1st and May 15th. There was no marked change in the meteorological conditions which would account for this, and it was probably dependent on some internal factor of cell development.

Thus table 1 indicates that with regard to the number of cells in a given length there is no seasonal variation in S. cataeniformis. Although the most frequent number and the range in number varies, the variation is occasioned by individual cell development.

Cell division. It was expected that the measurements from which Table 1 was drawn up, would furnish information about cell division, especially if this was restricted to certain periods. However the table gives no information about this except to indicate that active division probably occurred between May 1st and May 15th 1928. Several attempts have been made to determine the times at which cell division occurs, and to find whether it is confined to any period. On February 27th and 28th 1928, just after the Spirogyra had appeared in abundance, it was fixed in formalin, 70%, and 93%. alcohol, at two hourly intervals throughout each night. The material in alcohol was not well fixed, and that in formalin was not fixed well enough for staining, and so some of it was attached to slides by Land's slide fixative and a drop of Bouin's solution was added. The slides were then stained by the gentian violet

or iron alum haematixylin method and examined for evidence of nuclear division. Very little evidence was obtained but three cells in division were noted in material stained in gentian violet and fixed at 11 p.m., 12 midnight, and 2 a.m. respectively. Two cells in division were seen in filaments fixed at 11 p.m. and stained with iron alum haematoxylin. The material in alcohol was stained with chlor-zinc-iodine and examined for ingrowing walls, some of the filaments were quite well fixed and two ingrowing walls were seen in one filament. A similar sttempt to determine the time of division was made on March 23rd 1928 and examination of stained preparations showed division in one cell, of three filaments fixed at 11.10 p.m., and one filament fixed at 3.20 a.m. In 1929, during the 3rd period of abundance of the Spirogyra, other attempts were made to determine the time of division. On March 22nd, and 23rd, material was fixed at the pond in medium chrom-acetic and Bouin's fluid at 6 p.m., 11 p.m., 12 midnight, 1 a.m., 6 a.m. and 12 noon. The material fixed contained only a little Spirogyra, but examination of a number of filaments stained with iron alum haematoxylin gave no evidence of division. Later the material was stained with iodine and a large number of filaments examined. Two filaments fixed at 6 a.m. had a number of cells in which formation of the transverse walls was not quite complete for the blackened chloroplast had not been nipped in two. Evidently division had recently occurred in these two filaments, but they were the only two in a large number of filaments examined which had been fixed at the same time. In material fixed at 11 p.m., a large number of filaments were examined in iodine and four were seen showing division in one or two cells only. Some of the material fixed in February 1928 was then examined in iodine, but only one cell fixed at 6 a.m. showed division. Zimmerman's method of irrigating filaments on a slide was used to determine the frequency of division,

but without success. On March 21st, 22nd, and 23rd, slide cultures were set up containing an easily recognisable filament whose cells had been measured. The slide cultures were set up between 10 and 11 p.m., and placed in a damp atmosphere till the next morning when the cells were again measured and any division noted. Division occurred in four of the cultures, but only in one or two cells of very long filaments. During the fortnightly measurements made in 1929, a number of filaments were measured in iodine at various times of the day, and instance of division were seen at all times of the day. Material has been examined on March 21st, (freshly collected material and filaments from the culture in artificial light)-, April 22nd, May 3rd, and division has been noted at 9 a.m., 10.25 a.m., 12 noon, 3 p.m., 6 p.m., 9 p.m., 10 p.m., 12 midnight.

The results obtained dealing with cell division are for the most part negative, but they indicate that simultaneous division of the cells of a filament does not occur, and that division is not fixed to one time of the day or night. They also indicate that when the filaments are in an active condition (as in 1929) division may occur at any time of the day or night.

Table 2. In Table 2 the percentage distribution of filaments according to width is given. The table shows that, as has already been mentioned, the filaments of the 1st period were of various widths, whereas those of the 2nd and 3rd periods were of two distinct widths, 12.5 and 17.5μ, and were named the narrow and wide type respectively. These ~~different~~ two types could at once be distinguished under the microscope without measurement, but apart from their width there was no other difference between them. It can be seen from Table 2, that the narrow type was present throughout the measurements, it was never present in great abundance, its maximum abundance being between February and May 1928, when 27% of the filaments counted were the narrow

TABLE 2.

Percentage distribution of filaments according to width.

Date of Measurement	Number of filaments whose widths were measured	Width of Filaments in Microns. Widths estimated as varying by 2.5.						Summarised as Narrow & Wide type	
		10	10-12.5	12.5-15	15-17.5	17.5-20	20-22.5	10-15	15-20
1927.									
Nov: 23rd	193	3	8.2	31.6	49.2	8.1		42.8	57.3
Dec: 7th	200	16	34	17	25.5	7	.5	67.0	32.5
Dec: 21st	200	4	72.5	4.5	17.5	1		81.5	18.5
1928									
Jan: 6th	189	5.2	71.2	6.2	17.4			82.6	17.4
Feb: 28th	200		14.5	4.5	42.5	38.5		19.0	81.0
March 6th	193		19.4	.4	45.5	34.7		19.8	80.2
March 20th	200		24.5	1	52.5	22.0		25.5	74.5
April 2nd	214		27.1	.5	64.0	8.4		27.6	72.4
April 18th	224		10.3		62.5	27.2		10.3	89.7
May 1st	214		7.6		17.7	74.7		7.6	92.4
May 15th	208		3.8	1.5	15.8	78.4	.5	5.3	94.2
May 29th	211		1.5	3.8	31.1	63.5		5.3	94.6
June 13th	211		3.8	2.2	45.0	49.0		6.0	94.0
June 26th	203		1.5	1.5	61.1	35.4		3.0	97.0
July 10th	204		2.2	.6	58.9	38.3		2.8	97.2
July 24th	203		1.1	1.1	91.2	6.6		2.2	97.8
Aug: 7th	203		1.5	6.0	58.6	33.9		7.5	92.5
Sept: 4th	200			2.5	78.0	19.5		2.5	97.5
Sept: 18th	203		1.0	1.9	79.8	17.3		2.9	97.1
Oct: 5th	46		.5		82.2	17.3		.5	99.5
1929									
* March 21st	56			2	94	4		2.0	98.0
March 21st	178		6.7	8.4	82.1	2.8		15.1	84.9
April 4th	51		8	8	81.3	2.7		16.0	84.0
April 22nd	298			0.7	88.5	10.8		0.7	99.3
† June 12th	198		3.1		89.9	7.0		3.1	90.9
July 22nd	48		2	4.3	60.4	33.3		6.3	93.7

2nd PERIOD

3rd PERIOD

* Culture under artificial light.

† Measurement at time of conjugation.

type. The width of any one filament is usually constant, and only one measurement of width was made on each filament recorded in the table. However a few filaments, here described, were seen, in which the width appeared different in various regions and so more than one measurement was made. During the measurements on March 20th 1928, April 18th 1928, and March 21st 1929, four filaments had a width of 12.5μ at one point and 15μ at another. During the measurements of November 23rd 1927, one filament had a width of 15μ at one point and 17.5μ at another. In the same measurement one filament had a width of 17.5μ in one region and 20μ in another. During the measurement of March 21st 1929, one filament had a width of 12.5μ in one region and 17.5μ in another. This variation in width in the same filament, which, in the last recorded case, is a variation from the wide to the narrow type, justifies the inclusion of the two types as one species. At the same time the two are not proved to be the same species and their measurements have therefore always been kept separate. It was hoped that this question would be settled at the conjugation period but very few zygotes were observed, and none as the result of conjugation between filaments of the wide and narrow type. Nevertheless five filament pairs of the wide and narrow type, were observed and the conjugation process was apparently proceeding quite normally. In two of these the wide filaments had become differentiated as the female filament. Although these observations point to the fact that the wide and narrow types are the same species it is just possible that they are different species and that they were conjugating to form a hybrid. Table 2 shows that the filaments fall naturally into two classes as regards their width and moreover there are very few transitional filaments. The dividing line 1.5 between these classes is quite arbritary for from external appearances there is nothing to indicate that all those filaments with a width 15 should be included with

those of the narrow type. By far the majority of the filaments in the class 15-17.5 have a width of exactly 17.5μ and thus the division of the filaments into two classes is even more marked than the table shows. The range of width in the 2nd and 3rd periods is constant 10-20μ. As far as can be judged from 20 months of observation the distribution of the filaments of various widths is not seasonal, for although from February-May 1928 the narrow type of filament had its maximum abundance, there was no corresponding abundance in 1929. In the 1st period the filaments as a whole are narrower and the range in cell width is greater. There are very few filaments with a width greater than 17.5μ, some have a width 10μ, a width not otherwise recorded in the table. Although the table shows no filaments with a width 10μ in the 2nd and 3rd periods, such filaments were twice observed once at the end of the 2nd period, and once in the 3rd period. On March 7th 1929, when the pond was being frequently examined for the reappearance of Spirogyra, some green scum was collected. It was found to contain only Mougeotia and Microspora and was left in the laboratory in a specimen tube. On March 24th a small but distinctly deeper green tuft was seen growing from the mud, and it was found to consist largely of the narrow type of Spirogyra width 10μ and less frequently 15μ. Similarly the table indicates no filaments width 22.5μ in the 3rd period but occasional rare filaments of this width have been seen. In the 1st period the filaments do not so obviously fall into two classes as regards width. Although the majority of filaments in the class 15-17.5 have a width 17.5μ there are more transitional types in the class 12.5-15 . The narrower filaments are on the whole more abundant an exception being the count of November 23rd 1927. This exception can be explained: the measurement of November 23rd was the first measurement made, and it was thought that two species of Spirogyra were present, a narrow species with long

cells and a lax chloroplast and a wide species with shorter cells and a compact chloroplast. As the measurement went on these two "species" were found to have many transitions, and by the end of the measurement they were no longer considered as "species" but varieties of the same species. However at the beginning of the measurement there is no doubt that the wider filaments with shorter cells and more compact chloroplast, were chosen for measurement.

Table 3. Table 3 shows the range of variation in cell length and in the number of chloroplast turns during 20 months. The measurement of the wide and narrow types of filament have been given separately, and the total range collected from both. Reference to Table 2, will show that, apart from the 1st period, the number of filaments of the narrow type is never greater than 28% of the total number measured. Hence one would expect that this total range of variation would not be much affected by the filaments of the narrow type. Table 3 shows that this is the case, for, except with respect to the maximum number of chloroplast turns per cell, the total range of variation in cell length, and in number of chloroplast turns, is unaltered by inclusion of the narrow type of filaments. The table shows that in the 1st period, the range in cell length is greater than in the other two periods: the maximum cell length, 220-280 microns, is considerably greater, and the minimum cell length, 30-45 microns, is slightly greater, except in the case of the measurement on January 6th. The range in the number of chloroplast turns per cell is also greater the maximum varying from 8 to 9½ and the minimum from 2½ to 2½. The 2nd and 3rd periods are not marked off from each other by a different range of variation. The maximum cell length (excluding those measurement where only fifty or fewer filaments have been measured) varies from 80-190, and the minimum cell length from 15-35. The maximum number of chloroplast turns per cell varies from 4½ to 8

Table 3.

Range of variation in cell length and the

Date of measurement.	No. of filaments measured.	Cell length in microns. in "wide" form filament width 15-20 . maximum	minimum	in "narrow" form filament width 10-15 . maximum	minimum	total range in length.
1927						
Nov: 23rd.	200	220	180	50	45	220-45
Dec: 7th.	200	200	280	50	40	280-40
Dec: 21st.	200	200	230	50	40	230-40
Jan: 6th.	200	120	220	30	45	220-30
‖ Feb: 28th.		80		20		80-20
March 6th.	241	140	160	30	40	160-30
"		100		30		100-30
March 20th.	50	90	90	30	45	90-30
April 2nd.	220	130	150	35	50	150-35
April 18th.	224	170	160	20	30	170-20
‖ April 25th		100		30		100-30
May 1st.	217	190	150	20	60	190-20
May 15th.	210	100	75	20	40	100-20
May 29th.	211	105	75	25	25	105-25
‖ "		90		25		90-25
June 13th.	211	190	110	25	40	190-25
June 26th.	204	110	80	25	50	110-25
July 10th.	204	120	80	25	60	120-25
July 24th.	203	90	70	20	50	90-20
Aug: 7th.	203	120	110	20	45	120-20
‖ "		100		25		100-25
Sept: 4th.	200	140	90	20	30	140-20
Sept: 18th.	203	100	40	20	35	100-20
Oct: 5th.	47	70	50	25	50	70-25
"		135		20		135-20
* March 21st.	63	120		25		120-25
"	200	180	85	15	25	180-15
April 4th.	51	70	70	20	60	70-20
April 22nd.	301	80	50	15	50	80-15
‖ "		60		15		60-15
† June 12th.	200	100	75	15	35	100-15
# June 25th.	130	60		10		60-10
July 22nd.	48	140	120	30	50	140-30

‖ Fixed material measured. * Culture under artificial light.

Table 3 (continued)

number of chloroplast turns per cell during 20 months.

Most frequent length in each fortnightly measurement.	Number of chloroplast turns per cell. in "wide" form filament width 15-20 .		in "narrow" form filament width 10-15 .		total range in number.	most frequent number in each fortnightly measurement.
	maximum	minimum	maximum	minimum		
90-100	8	7½	2½	2½	8 -2½	4½
100-110	8	9½	2½	2½	9½-2½	4
70-80	7	9	2½	2½	9 -2½	5
80-90	7½	8½	2½	2½	8½-2½	5
30-40	6	7½	1½	2½	7½-1½	3
60-70	7	7½	2	2½	7½-2	3½
40-50	6½		2½		6½-2	3½ and 4
50-60	6½	7½	1½	2	7½-1½	2½
60-70	6½	7½	2	2½	7½-2	4
60-70	6½	7	1¼	2½	7 -1¼	3½ and 4
40-50	6½		2		6½-2	4
60-70	7	7½	2	3	7½-2	4
30-40	6½	6	1½	2½	6½-1½	2½
40-50	6	6½	2	2½	6½-2	3 and 3½
30-50	6½		2		6½-2	3
40-50	6	6½	2	2½	6½-2	3 and 3½
40-50	6½	5	1½	3½	6½-1½	3½
40-50	6	5½	1½	3½	6 -1½	3½
40-50	5	5	1½	2½	5 -1½	2½
40-50	5½	6	1½	3	6 -1½	3
30-40	6		2		6 -2	3
30-40	8½	4½	1½	2	8½-1½	2½
30-40	6	4½	1½	3½	6 -1½	2½
30-40	5½	4½	1½	4½	5½-1½	3½
30-40	6		2		6 -2	2½
40-50	8		2		8 -2	4½
30-40	6	5	1	1½	6 -1	2½
20-30	5	4	1½	2	5 -1½	2½
20-30	6	5½	1	4½	6 -1	2½
20-30	5½		1½		5½-1½	2
35-40	4½	4	1	1½	4½-1	2
30-40						
50-60	6	6	1½	3	6 -1½	3

)·Measurement at time of conjugation. #Conjugating filament pairs measured.

and the minimum number from 1-2½. It should be noted that the least value for the minimum number of chloroplast turns and the minimum cell length during the whole of the 2nd and 3rd period, is found at the beginning of the 3rd period. This agrees with the conclusion drawn from table 1, that the shortestcells at this time are shorter than at the corresponding time in the previous year or any other time in that ~~time~~ year. The table shows that the variation in the maximum cell length and the maximum number of chloroplast turns is not seasonal, but is probably largely due to the relatively small number of filaments measured. This is probably the case with respect to the variation in minimum cell length and number of chloroplast turns, but this variation is very small compared with the other.. The table shows the most frequent, or mean cell length and number of turns of chloroplast per cell at each measurement. This was obtained from the first four cells of each filament measured, these, but not necessarily all being measured individually.

The maximum cell length which was found during the twenty months is 280μ, and this cell occurred in a filament 10μ wide of the 1st period. The length of this cell was thus 28 times its width. The minimum cell length which was found during the twenty months is 15μ, and this cell occurred in a filament 17.5μ wide found in the 3rd period. The length of this cell was less than once its width. The corresponding range in the number of chloroplast turns per cell during the twenty months is 9½-1. S.æ taeniformis is stated to be a species very variable in the length of its cells and in the key arranged by Borge and Pascher the width is given as 20-27μ showing a range of 7μ; the cell length is given as 2-5½ times the width and the number of chloroplast turns per cell as 1½-6. Table 2 shows that the width of the filaments is 10-20μ showing a range of 10μ (in the case of 2 filaments the width was slightly greater than 20); that the cell length is 1-28 times the width and that the

number of chloroplast turns is 1-9½. It has already been stated that there is no proof that the Spirogyra of the 1st period, or even the narrow type of the 2nd and 3rd period, is S.cataeniformis. If these filaments are excluded the width of S.cataeniformis is 15-20 showing a variation of 5 ; the maximum cell length is 190 in a filament 17.5 wide, i.e. 11 times the width; the minimum cell length remains unaltered and the number of chloroplast turns varies from 1-8. Thus the cell length is considerably more varied than is reported by Borge and Bascher, and also the number of chloroplast turns per cell but this variation is not seasonal.

A necessary precaution. During the first of the fortnightly measurements of the 2nd period when the Spirogyra appeared to be very vigorous, the measurement of individual filaments was easier if the material had been kept over-night in the laboratory. The cross walls could be seen more easily, presumably because growth, unaccompanied by division, had occurred in the laboratory. During this first measurement, about half the material was collected daily, and the other half was taken from the material collected on the first day and kept in the laboratory. Table 4, constructed in the same way as Table 1, indicate the difference between records made from freshly collected and laboratory material: the freshly collected material had shorter cells.

Table 4.

DATE OF MEASUREMENT	No. of Fil. Measured	NUMBER OF CELLS IN A GIVEN LENGTH OF FILAMENT. 8-9 9-10 10-11 11-12 12-13 13-14 14-15 15-16 16-17 17-18 18-19 19-20 20-21 21-22 22-23 23-24 24-25 25-26 26-27 27-28 28-29 29-30 30-31 31-32 32-33 33-34 34-35 35-36 36-37 37-38
Material measured when collected	1 0 8	1 2 1 1 3 10 5 9 10 3 6 5 8 6 4 5 5 5 5 3 3 3 3 1 1
Material collected Feb 21st Measured Feb 23rd. 24th.	8 8	1 2 2 4 7 4 11 7 7 9 7 3 5 6 4 1 1 3 2 1 1

In all the subsequent measurements, material was collected daily to avoid the error due to growth. In some of the measurements the material of the first day of measurement was kept in the laboratory, and a few filaments were measured some days later; these were compared with the same number measured on the

first day. The material measured was insufficient to construct a table such as the above, but the maximum and mimimum number of cells to the given length was ascertained for the freshly collected and laboratory kept material. The results are given in table 5, and they show that in each case the maximum and minimum number of cells to the given length is greater in the freshly collected material. This clearly indicates that growth occurs in the laboratory and the material should be measured immediately after collection.

Table 5.

Date of collection in 1928.	Date of measurement.	Number of filaments measured.	Maximum no. of cells to standard length.	Minimum no. of cells to standard length.
Feb. 21st.	Feb. 21st.	21	35	16.
" "	Feb. 25th.	21	18½	11
March 6th.	March 6th.	14	23	11
" "	March 12th.	14	15	11
April 18th.	April 18th.	40	34	9½
" "	April 21st.	40	24	6
May 1st.	May 1st.	30	21	9
" "	May 8th.	30	21	7
May 15th.	May 15th.	24	46	17
" "	May 21st.	24	18	9
May 29th	May 29th.	25	30	17
" "	June 6th.	25	16	9

Summary of tables 1-5. It can be seen from these tables that in Spirogyra cataeniformis found between February 1928 and July 1929 there is a considerable range in cell length, cell width and the number of turns of the chloroplast per cell. This range shows no seasonal variation but is occasioned by individual cell development. In the Spirogyra found between November 1927 and January 1928 the range for all these values is increased. At this time it did not appear to be in so vigorous and actively dividing a state as subsequently. The increased range

of variation may have been occasioned by senescence, the appearance and description of some of the filaments indicate that the Spirogyra was entering on a senescent stage. There was no such senescent stage when the Spirogyra disappeared in October 1928 and this may be correlated with the absence of conjugation, and the production of perennating filaments as a means of reproduction. It is not known whether conjugation occurred in 1927, it occurred in 1929 but it was only completed in a few cases. A senescent period was not observed during the autumn ; only very few filaments survived the conjugation period. The pond had become choked up with silt and its condition was not normal.

VARIATION IN THE CHLOROPLAST.

Variation in number.

While S.cataeniformis was being measured for cell length and breadth, filaments were seen in which some or all of the cells had two chloroplasts, which turned within the cells in opposite directions. At first such filaments were not measured, as they were thought to belong to a different species, but, as table 4 shows, so many transitions have been seen between filaments having two chloroplasts in all the cells, and filaments having two chloroplasts in only one cell, that it is probable that all the filaments were S.cataeniformis. According to the key arranged by Borge and Pascher for the identification of species of Spirogyra, one would regard filaments having two chloroplasts in all the cells as belonging to a different group of species from the rest; but here the many transitional filaments justify their inclusion in the one species. Besides the filaments recorded in the table as having some cells with two chloroplasts, (and these include <u>all</u> that were seen during the fortnightly measurements, made during the examination of large amounts of material) only two other filaments were found with two chloroplasts in some of the cells. One was seen on February

TABLE 6.

Frequency of Filaments having cells with two chloroplasts per cell.

Date of Measurement	Number of filaments measured	Number of filaments seen having two chloroplasts per cell, and particulars of these,			
		No. of filaments.	Total no. of cells.	No. of cells with 2 chloroplasts.	No. of cells with 1 chloroplast.
1927.					
Nov: 23rd	200	4	∞	∞	∞
Dec: 21st	200	1	∞	∞	1
1928.					
Feb: 22nd	200	1	∞	∞	few
March 6th	241	2	13	12	1
			∞	half	half
March 20th	201	1	∞	∞	0
April 2nd	220	3	∞	∞	1
			∞	half	half
			∞	∞	0
April 18th	224	2	∞	∞	0
			∞	few	∞
May 1st	217	7	15	15	0
			64	50	14
			9	8	1
			∞	∞	0
			∞	∞	0
			24	1	23
			18	5	13
May 29th	211	1	∞	half	half
June 13th	211	2	∞	∞	few
			∞	∞	few
June 26th	204	2	∞	∞	few
			∞	few	∞
July 10th	204	1	∞	∞	few
July 24th	203	2	46	43	3
			44	26	18
Aug: 7th	203	1	18	2	16
Sept: 4th	200	2	∞	∞	∞
			∞	4	∞
Sept: 18th	203	2	∞	∞	∞
			∞	few	∞
Oct: 5th	47	3	∞	∞	few
			∞	∞	∞
			∞	few	∞
1929.					
March 21st	200	2	41	6	35
			∞	few	∞
*March 21st	63	2	27	1	26
			27	2	25
April 22nd	301	3	26	9	17
			36	3	33
			43	18	25

2nd PERIOD.

3rd PERIOD.

* Culture under artificial light.

22nd 1929 when Spirogyra filaments were very rare in the pond and then only occurred in the mud. In this filament about half the cells had two chloroplasts. The other was seen on March 16th 1929 in a small portion of mud which had been collected on February 6th 1929.

Table 6. Table 6 shows that occasional filaments having cells with two chloroplasts were found during every month when the Spirogyra was present. Thus their occurrence cannot be connected with external conditions, but is dependent on some internal condition of the cell. Those cells with two chloroplasts are nearly always adjacent in the filament, but occasionally they have been found in more than one part, separated by cells with one chloroplast.

Variation in character.

The range in the number of chloroplast turns, and the most frequent, or mean, number of chloroplast turns per cell, found at each measurement, is shown in Table 3. This table has already been discussed.

Table 7. The variation in width of the chloroplast is shown in Table 7, and the percentage of filaments of various chloroplast width is shown in this table. The width of the chloroplast was usually constant in any one filament and only one measurement of it was made; this was with the high power objective and in the table it is given in microns. The table is compiled in the same way, and from the same sets of measurements as Table 2, which has already been explained. The edge of the chloroplast is either smooth or serrated, and this is usually dependent on its width. Wide chloroplasts are usually serrated, whereas narrow ones are smooth in outline. Occasional filaments have been seen in which the character of the chloroplast is different at either end: one end having a comparatively wide chloroplast with a serrated edge, and the other a narrow chloroplast with a smooth edge. The table shows that in the 2nd and 3rd periods

TABLE 7

Percentage distribution of filaments with regard to the width of their chloroplast.

Date of measurement	Number of filaments measured.	Width of chloroplast in microns.					
		.24-.36	.36-.48	.48-.60	.60-.71	.71-.83	83-9
1927							
Dec: 7th	208		4	44	51	1	
Dec: 21st	189	.5	3	52	44.5		
1928							
Jan: 6th	189		.5	72	27.5		
March 6th	164			4	86	10	
March 20th	148			3	93	4	
April 2nd	149		.5	5	92	2.5	
April 18th	197		11	6.5	79	3.5	
May 1st	187		10	16.5	68	5.5	
May 15th	173		1.5	12	84	2.5	
May 29th	186			10	85	5	
June 13th	182		4	8	82	5	.5
June 26th	183			5.5	86	8	.5
July 10th	188			2	94	3.5	.5
July 24th	191			1.5	89.5	8	1.0
Aug: 7th	195		..5	8.5	84.0	3.5	1.0
Sept: 4th	196				98.5	1.5	
Sept:	199			1	90	6.5	2.0
Oct: 5th	46				87	13	
1929							
*March 21st	53		68	23	9		
March 21st.	177			.5	95	4	.5
April 22nd	199				90	10	
July 22nd	45			35.5	64.5		

*Culture under artificial light.

of abundance of the Spirogyra, the majority of filaments have a chloroplast width .6^{0}-.71 . Where there is a relatively high percentage of filaments with a narrower chloroplast .36-.60 (e.g. April 18th-May 15th 1928), tests with iodine have shown that thereis also relatively little starch in the filaments. In the culture kept under constant illumination from March 1st-March 21st, tests with iodine showed that most of the filaments had no starch at all,and the table shows that the majority had the narrow type of chloroplast. This culture clearly indicates that the filaments cannot be divided into types or varieties based on the width of the chloroplast. The filaments found in the culture before illumination had wider chloroplasts, as also had the filaments which appeared in the pond, in the region from which the mud for the culture had been extracted. During the 1st period the narrower type of chloroplast was more abundant than at any other time, as the table shows. From December 7th to January 6th 1928, when the Spirogyra was rapidly decreasing in amount, the percentage of filaments with the narrower chloroplast was greatly increased. Possibly the narrower chloroplast, and the absence of starch in it, are characters which follow on senescence. Filaments which appear to be dying have a narrow chloroplast with a smooth edge, and as has already been stated the Spirogyra of the 1st period seemed to be entering into a senescent stage. At the end of the 2nd period, there was no increase in the percentage of filaments with a narrow chloroplast, amd no signs of senescence in the filaments measured, which appeared to be actively dividing and vigorous.

Variations in relationships. Tables 8 and 9 show the relation between the number of chloroplast turns per cell, and the length of cell and number of pyrenoids. The data for these tables was obtained from measurements of the slides of fixed and stained material referred to on page 21. At each time of measurement 200 cells selected at random were investigated with

TABLE 8.

Relationship between length of cell and number of turns of chloroplast per cell.

Date of Fixation	Maximum, minimum f & average length of cell in microns.	Number of turns of chloroplast per cell.										
		1-1½	1½-2	2-2½	2½-3	3-3½	3½-4	4-4½	4½-5	5-5½	5½-6	6-6½
1928 Feb:28th	Maximum	25	30	40	50	55	80	60	65	60	80	
	Minimum	25	20	25	25	35	40	35	50	60	80	
	Average	25	25	30	35	42	46	50	56	60	80	
Mar:6th	Maximum			50	60	80	85	90	95	100	85	80
	Minimum			30	35	35	35	50	55	75	60	80
	Average			39	46	53.6	57.6	69.5	79.2	86.2	73.5	80
Apl:25th	Maximum		35	40	50	65	60	70	100	75		80
	Minimum		30	30	30	35	35	45	50	55		80
	Average		32.5	34.1	37.9	46.2	50	55.6	58	62.5		80
May 30th	Maximum		30	40	45	55	70	60	80	90		85
	Minimum		25	25	25	35	35	45	50	60		85
	Average		26.6	34.7	36.8	44.3	49.4	53.5	61.2	73		85
Aug:7th	Maximum		35	60	60	60	90	90	90	90	100	
	Minimum		25	25	30	40	45	50	45	70	90	
	Average		28.5	35.7	39.1	46.3	57.4	64.7	66.4	80	97.5	
Oct:8th	Maximum		30	45	45	55	55	55	125	65	135	
	Minimum		20	25	25	35	40	45	45	55	135	
	Average		26	29	34	41	46	40	62	59	135	
1929. Apl:26th	Maximum	20	30	35	40	45	60	45	50	50		
	Minimum	15	20	20	25	30	35	45	50	50		
	Average	19.4	22.7	26.4	32.3	37	40.9	45	50	50		

regard to the number of chloroplast turns, the cell length, and the number of pyrenoids. The number of filaments concerned is not known; it was probably less than 200, for only two slides of material were used at each time of measurement. The number of chloroplast turns was found to the nearest half turn, and for each value of this the maximum and minimum cell length and number of pyrenoids was found. The average value of all the cell lengths and pyrenoids numbers, was calculated for every value of the number of chloroplast turns. The results from these measurements are collected together in Tables 8 and 9 and Table 9 is illustrated by a graph.

Table 8. Table 8 shows that the maximum, minimum, and average cell lengths, and the number of chloroplast turns are directly proportional to each other. The relation is not so definite as the table indicates, for in some cases there are considerable differences in the lengths of cells having the same number of chloroplast turns; conversely, cells of the same length frequently have a varying number of chloroplast turns. The table also shows that the average cell length corresponding to each value of the chloroplast turns is not constant, but varies throughout the year from fortnightly measurement to fortnightly measurement. For every value of the chloroplast turns the averagecell length is shortest at the beginning of the 3rd period, in April 1929. A similar conclusion was obtained from the measurement of fresh material (cf. Tables 1 and 3).

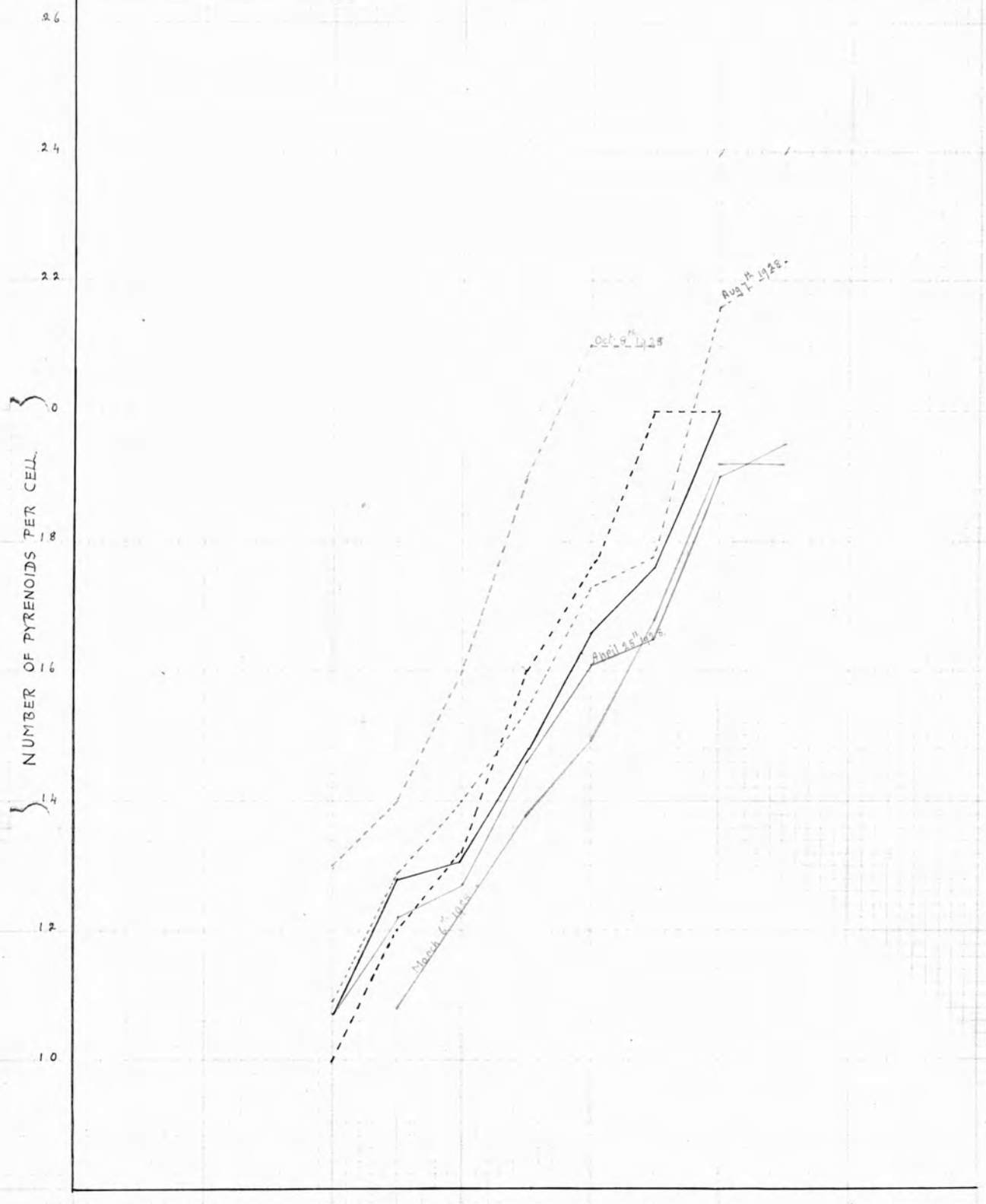
Table 9. Table 9 and the graph illustrating it show that the maximum, minimum, and average number of pyrenoids, and the number of chloroplast turns, are directly proportional to each other. As in the case of cell length, this relation does not always hold, for there are sometimes considerable differences in the number of pyrenoids in cells having the same number of chloroplast turns. The average pyrenoid number per cell, corresponding to the various values of the chloroplast turns,

TABLE 9.

Relationship between number of pyrenoids and number of turns of chloroplast per cell.

Date of Fixation	Maximum, minimum & average number of pyrenoids per cell.	Number of turns of chloroplast per cell										
		1-1½	1½-2	2-2½	2½-3	3-3½	3½-4	4-4½	4½-5	5-5½	5½-6	6-6½
1928. Feb:28th	Maximum	13	13	16	17	20	22	26	24	27	22	
	Minimum	13	9	8	9	11	11	13	14	19	22	
	Average	13	11	13	13	15	17	18	20	23	22	
Mar:6th	Maximum			12	19	18	18	23	25	24	21	24
	Minimum			9	8	10	10	11	16	14	19	24
	Average			11	12	14	15	17	19	19	20	24
Apl:25th	Maximum		12	15	16	20	21	21	22	21		18
	Minimum		8	8	10	12	11	12	16	18		18
	Average		11	12	13	15	16	17	19	20		18
May 30th	Maximum		14	15	17	20	25	26	24	23		24
	Minimum		7	9	8	11	14	14	15	18		24
	Average		10	12	13	16	18	20	20	21		24
Aug:7th	Maximum		14	17	18	21	25	24	28	25	24	
	Minimum		9	9	11	11	11	14	14	20	20	
	Average		11	13	14	15	17	18	22	22	22	
Oct:8th	Maximum		17	20	21	24	27	25	27	27	24	
	Minimum		11	10	12	14	15	16	21	22	24	
	Average		13	14	16	19	21	21	24	24	24	
Apl:26th	Maximum	13	14	16	17	23	22	19	27	27		
	Minimum	6	7	10	9	11	13	19	27	27		
	Average	9	10	11	14	17	17	19	27	27		

GRAPH SHOWING THE RELATIONSHIP BETWEEN THE NUMBER OF PYRENOIDS AND THE NUMBER OF TURNS OF CHLOROPLAST PER CELL.



does not alter appreciably from fortnightly measurement to fortnightly measurement throughout the year, except in the case of the measurements made on October 8th 1928, when for every value it is higher. This is clearly shown in the graph accompanying Table 9. The table shows that the minimum number of pyrenoids is also greater at that time, for every value of the chloroplast turns, and in most cases the maximum number is also greater. The material fixed on October 8th, was from the last minute tuft of Spirogyra visible in the pond at the end of the 2nd period. As has been stated, the filaments at the end of the 2nd period, disappeared into the mud, and perennating filaments were later found. Although these persisted all through the winter, and must have been very resistent, they were no different in external appearance from the ordinary vegetative filaments. Possibly the increased pyrenoid number on October 8th 1928 is a characteristic feature of perennating filaments as a whole, whereby a greater amount of starch can be formed: the filaments at this time were certainly packed with starch. In all the measurements some pyrenoids were seen in division, or still in contact after division, and a few were seen which were distinctly smaller than the rest, as though they had just been formed.

VARIATION IN NATURE AND AMOUNT OF RESERVE FOODS.

Starch Content.

When there is only a little starch in the cells it is confined to the pyrenoid sheath, but when there is abundant starch the whole of the chloroplast is also packed with starch grains. When the grains in the chloroplast are particularly abundant they can sometimes be seen without staining, for they give the chloroplast a granular appearance. At other times the individuak grains cannot be seen but the whole chloroplast appears glassy and replete with starch. Sometimes there are

only a few starch grains in the chloroplast: sometimes these are absent even from the pyrenoid sheath, and the filament have no starch. The amount of starch has been tested by the action of iodine on fresh material and on material preserved in formalin or medium chrom-acetic acid; in all three cases the re-action is similar and is constant throughout any one filament. Cotton blue has also been used to indicate the amount of starch present: after staining for two of three hours in cotton blue and de-staining in dilute glycerine, the starch grains round the pyrenoids and in the chloroplast can be seen with high magnification to be well differentiated, although they are quite unstained by the cotton blue. The following results have been collected from fresh or preserved material or both tested in one or both of the above ways.

1st Period The amount of starch was not ascertained until the end of this period, when the Spirogyra had almost disappeared from the pond. It was then found (in January 1928) that the short pieces of filaments which were then present had abundant starch, the chloroplasts appearing homogenously black when stained with iodine. These short pieces of filaments were only discivered on careful examination of the deposit left at the bottom of a vessel which had contained pond material.

2nd Period The Spirogyra was first noted to have reappeared on February 21st 1928 and it was tested for starch between February 21st and February 29th. Fresh and preserved material was tested, and it was found that starch was present in most of the filaments, and in a few of them the chloroplasts had a number of starch grains. Some of the filaments had no starch grains in the chloroplast, and some had no starch at all.

On March 23rd all the filaments were noted to have starch, in some of them the chloroplast was packed with starch and was stained completely black with iodine. The amount of starch present at these times was not so great as in the following autumn for it could not be seen in the chloroplasts without staining.

On April 4th, April 25th, May 5th, May 19th, in almost all the filaments there was no starch present, even the pyrenoid sheath was unstained by iodine. On April 4th and May 5th fresh material was tested, and on the other two dates preserved material was used and tested with iodine and cotton blue.

On May 30th and June 2nd the amount of starch varied, and a few filaments had abundant starch and had starch grains dispersed throughout the chloroplast, but the majority had only a little starch or no starch at all.

On June 18th - 23rd the majority of filaments had no starch.

On June 27th the amount of starch varied, in some

filaments there was no starch at all, in others there was a little starch,and in a few the whole chloroplast was turned black by iodine.

On July 13th and July 24th all the filaments had some starch and in a few the whole chloroplast was packed with starch, this was especially the case in those filaments showing the beginnings of conjugation.

On August 7th the majority of filaments only had a very little starch confined to a narrow pyrenoid sheath - a few had numerous starch grains in the chloroplast.

On September 4th the majority of filaments had abundant starch the chloroplast being packed with starch grains.

On September 18th all the filaments contained starch, in some only the pyrenoid sheath was stained, but in some the whole chloroplast was stained and contained numerous starch grains.

After October 5th the Spirogyra had almost completely disappeared from the pond, but careful search showed that from this time to its reappearance in bulk in April 1929 there were a few vigorous perennating filaments in the mud; these filaments had bright green compact chloroplasts,some of them were tested with iodine.

On November 12th a long filament was tested, but it contained no starch.

On December 1st a filament was taken from mud which had been kept in the laboratory from October 6th. The pyrenoidsheaths contained starch, but there were no starch grains in the chloroplasts.

On December 5th and February 4th 1929 two long filaments were tested with iodine and the pyrenoid sheaths contained starch.

On January 28th and February 22nd two long filaments were tested, and found to contain starch grains in the chloroplast as well as in the pyrenoid sheath.

On February 6th two filaments were tested, one had a chloroplast packed with starch, but the other had no starch at all and the pyrenoid sheath was unstained.

On February 21st two short filaments were found and both had abundant starch the chloroplast containing numerous starch grains. One of these filaments had a pale lax chloroplast and looked like a "resting filament".

On March 18th a filament was tested and found to contain abundant starch, the chloroplast being stained almost completely black.

On March 21st a number of filaments from the culture under artificial light were tested and starch was found to be almost completely absent. The culture was made from mud collected on February 6th and placed under constant illumination on March 1st.

In mud collected on March 1st and kept in the laboratory till March 24th four filaments were tested with iodine, two of them had very little starch, this being confined to a very narrow sheath round the pyrenoids, in another the pyrenoid sheaths were stained and there were very small starch grains in the chloroplast, while the third had abundant starch numerous grains being stained in the chloroplast.

On March 24th a filament was tested with iodine, the pyrenoid sheaths were wide, and there were numerous tiny starch grains in the chloroplast. This filament was found in mud which had been kept in the laboratory from March 7th,it did not appear vigorous like the other filaments described.

On March 25th the Spirogyra in the pond was beginning to become abundant again, and various filaments were tested

with iodine. The amount of starch varied greatly, some filaments had no starch, even the pyrenoids were unstained. Others had a little starch, while the majority had abundant starch, the chloroplast being packed with starch grains.

3rd Period. The Spirogyra had reappeared in quantity by April 1929 and on April 4th some material was tested with iodine. Most of the filaments had some starch, but a few were unstained. The majority contained a lot of starch, and numerous starch grains could be seen in the chloroplast.

April 16th, 19th, 22nd, 24th. On the whole the filaments contain a lot of starch, the number of starch grains in the chloroplast varies, but there are some in nearly all the filaments. A few filaments were seen which contained no starch.

On May 1st and May 13th all the filaments contained abundant starch, the chloroplasts being packed with starch grains..

On June 15th vegetative material had not so much starch as previously but the amount varied very greatly. Some filaments had very little starch and only a very narrow starch sheath was stained by iodine, others had a wider starch sheath but no starch grains in the chloroplast, while others had numerous starch grains in the chloroplast. In conjugating filaments, tested at the same time, there was abundant starch the chloroplast being packed with starch grains.

On July 22nd the amount of starch varied very greatly, and thw two extremes were filaments which had no starch at all (these were usually filaments with long cells and lax chloroplasts) and filaments whose chloroplasts were packed with starch.

Conclusions. The amount of starch present in the filaments varies, but the variation is by no means seasonal. Filaments with abundant starch, and filaments with little or no starch, are present at the same time and in the same collection of material. Thus the amount of starch in the filaments cannot be dependent on external conditions such as: temperature, concentration of the water of the pond, light intensity; but is rather determined by conditions of individual cell development. There is evidently some relation between the condition of the cells during conjugation, and their starch content; both in 1928 and 1929 all those filaments which had started to conjugate contained abundant starch. In vegetative material tested at the same time, the starch content varied very greatly. The amount of starch in the cells apparently does not influence cell division. In March 1929, in filaments stained with iodine, cell division was noted in cells which had the chloroplast packed

with starch, cells which had only a little starch, and cell of filaments from culture under artificial light which had no starch. The absence of starch in the filaments of this was probably due to exhaustion. Very rapid growth and division must have previously occurred in this culture for the filaments to have become so abundant.

Oil Content.

When there is a considerable amount of oil present in the cells, it takes the form of globules, which are dispersed superficially in the cell, and are usually on, or above, the chloroplast. These globules are stained red by Sudan III in about tem minutes, and they can then be seen with low magnification, sometimes additional tiny red globules can be seen in the cytoplasm. The superficial globules are highly refractive, and are stained a brownish grey colour by osmic acid, they are clearly globules of oil or fat. When they are particularly abundant, as at the time of conjugation, there is a visible difference in the appearance of the chloroplasts, the large oil globules can easily be seen without staining, and they give the chloroplast a granular appearance. This appearance arose in cultures set up during the conjugation period, in an attempt to induce conjugation. The culture solutions used were:- 3% cane sugar in distilled water renewed with the same strength of cane sugar; the same renewed with distilled water; calcium phosphate solution containing 1 mgm of phosphorous per litre of pond water; the same in distilled water; pond water containing a few filaments of Spirogyra. All these cultures were placed on the roof of the laboratory, and another culture, containing a few filaments of Spirogyra in pond water, was placed in the laboratory. On June 12th, when the filaments were placed in these cultures the chloroplasts appeared quite normal and no oil globules could be seen. When they were examined on June 21st, in only one culture was the previous normal appearance shown, this was

in the culture containing calcium phosphate in distilled water. In all the other cultures the filaments had very granular chloro plasts, and even with low magnification, they looked very different from their previous appearance. This difference in appearance was shown to be due to the production of large superficial oil globules, and also to the production of a number of starch grains in the chloroplasts.

At all times of the year a further "reaction" is given with sudan III, for after filaments have been left in this for some time, the whole chloroplast becomes stained a reddish colour of varying intensity, and in this red chloroplast small granules, also red, but not specially differentiated, can be seen. It is not known whether this "reaction" has any significance: if it indicates that the chloroplast matrix is permeated with fat or whether it is merely a general staining of the chloroplast. A similar "reaction" or general staining of the chloroplast is shown wil oil of alkanin after some hours.

It has already been stated that the large superficial oil globules, are stained a brownish grey colour by osmic acid, but an additional reaction is given, for in those filaments, and also in those without oil globules, a black precipitation is produced superficially in the cells. A corresponding blue precipitation is produced when filaments are treated with nile blue sulphate; this is a stain for unsaturated fatty acids as well as for fats, and so the blue precipitation with nile blue, and the black one with osmic acid, seem to indicate the presence of a fatty substance in a very finely divided state (Plate V.) When filaments are immersed for a long time in very dilute solutions of thesestains viz: dahlia, methylene blue and eosin, superficial precipitations the same colour as the solutions, are formed in the cells. Presumably these precipitations indicate the protoplasm, or inclusions in this, which have become coloured by the solutions. In the case of nile blue sulphate.

and osmic acid, the precipitations are not mere colourations of the protoplasm, for in the case of osmic acid, a definite colour change is produced, moreover neither of the precipitations is altered by dilute glycerine. It is not known whether these reactions indicate the presence of fatty substances or of tannin in the protoplasm. Osmic acid stains tannins as well as fats, and is found to react with tannic acid, giving a blue-black colouration. The precipitation produced in the filaments by osmic acid, has a bluish tinge, and is not like the brown black colour produced with the large oil globules. Whatever the nature of the reaction, it varies in intensity in different filaments, and it does not occur in material preserved in glycerine, formal, or spirit, or in material which has been treated with iodine. The reactions will be considered here with those for oil, and the following results have been obtained.

1st Period. Oil was certainly not abundant in this period, for no indication of its presence was noted in unstained material. The short pieces of filaments found in January 1929 gave no reaction with sudan III.

2nd Period. Reactions with sudan III.

From February - June 1928, only rare filaments were seen, which had a few small superficial oil globules.

On June 16th a few filaments with abundant large superficial oil globules were seen. The oil globules were similar to those which were always present in the rare filaments which attempted conjugation at this time and during July.

On June 26th and 29th a large number of the filaments examined during a measurement had abundant oil globules.

On July 12th Spirogyra collected from four of the places where it occurred in abundance, was tested with sudan III. Two of the places had filaments with abundant oil globules, and among them filaments attempting conjugation, also with abundant oil. The other two places had filaments which were mostly without oil, and among them there were no instances of conjugating filaments.

On July 18th filaments collected from the same places were all found to contain abundant oil.

On July 24th - 28th almost all the filaments had abundant oil.

On August 7th - 10th only a few filaments contained oil globules. The temperature of the air had been considerably higher since the previous test and apparently the Spirogyra had increased in amount for it covered a larger area of the pond.

On September 4th - 7th nearly all the filaments had abundant oil globules.

On September 18th and October 6th very few filaments had the large oil globules previously noted but the majority of them had a few quite small globules.

Between October 6th 1928 and April 1929 occasional

tests were made on the rare perennating filaments which were then present.

On November 8th and 9th a filament 12.5 in width and a filament 17.5 were both found to have small superficial oil globules.

On November 12th a vigorous filament with a compact chloroplast was found to have no oil.

On November 17th a short filament, which had been kept in culture in the laboratory since November 9th, was found to have a very large number of small superficial, oil globules.

On November 22nd two vigorous filaments with compact chloroplasts were tested. They had been kept in culture in the laboratory since November 14th. One of them had no oil globules but the other had a few.

On December 1st a vigorous filament was seen with a large number of oil globules.

On February 6th and March 1st 1929 filaments had no oil globules.

On March 18th two filaments were tested, one had oil, the other had a few globules.

On March 21st and 22nd several filaments were tested, but only rare filaments had oil globules.

Reactions with osmic acid and nile blue. From February to September 1928 various tests were made with osmic acid. In each case a black precipitation was produced in the cytoplasm, the amount varied in different filaments, but was usually quite abundant. On April 25th it was noted that very little precipitation was found in the long, i.e. the more mature cells.

From October 1928 to March 1929 some of the occasional perennating filaments were tested with osmic acid and nile blue.

On December 12th a filament, which was extracted from a culture of mud kept in the laboratory since October 6th, was tested with osmic acid. It contained oil in the form of large superficial globules, but these were masked by the very dense, black, precipitation which was formed. Apart from this filament, the majority of perennating filaments showed only a little precipitation, with osmic acid, or nile blue; less than that formed when the Spirogyra was abundant.

Between March 7th and 21st various tests were made with nile blue and osmic acid on filaments of the light culture. In the majority of filaments the precipitation was very sparse, and in two filaments tested with nile blue it was absent. (Plate V figs. 3 and 4).

Between March 18th and 24th, tests with nile blue and osmic acid on filaments from the pond, which were becoming much more abundant, showed copious precipitations in their cells (Plate V figs. 1, 2 and 5).

3rd Period. Reactions with sudan III.

On April 22nd 1929 a few tiny red globules could be seen with high magnification in most of the filaments.

On May 1st the majority of filaments had no oil, but occasionally a few tiny globules were seen.

On May 13th no oil globules were seen in any filaments

From June 12th - 24th the majority of vegetative filaments contained no oil. Conjugating filaments examined at the same time, and before and after, had abundant large superficial oil globules.

On July 22nd the majority of filaments had no oil globules, and there were no conjugating filaments. A few filaments were seen with cells containing abundant oil globules.

Filaments tested with nile blue and osmic acid on

April 4th 1929 showed a large amount of precipitation.

Conclusion. The oil content of the cells appears to be very definitely related to the process of conjugation. Both in 1928 and 1929 all filament pairs which were seen in the process of conjugation had a large number of oil globules placed superficially on the chloroplast. These globules were easily visible without staining. In 1928, at the conjugation period, almost all the filaments had very abundant oil, but conjugation was apparently arrested in this year, for only few filaments attained it. In 1929 conjugation occurred in a large number of filaments, and these had all developed abundant oil; the majority of the vegetative filaments, present at the same time, had no oil globules. The function of the oil is obscure; apparently its presence is not essential for the resistance of the perennating filaments, for in some of them no oil can be demonstrated. Both in 1928 and 1929 oil globules were only very rarely seen early in the season.

The precipitations which are developed with nile blue and osmic acid indicate the presence of a reserve, which may or may not be of a fatty nature. This reserve shows no seasonal variation in amount, but appears to be influenced by the age of the cells for it is less in the longer more mature cells. It was less in the perennating filaments and filaments from the culture under artificial light, indicating that the reserve is used up as the filaments approach exhaustion. This reserve will be discussed further in connection with wall formation.

THE CELL WALL.

Reactions of the Wall.

The cell wall of S.cataeniformis has been tested at various times of the year with a number of reagents. It has been found that there are very few substances that stain the cell wall, and it is therefore difficult to make out the structure of this in such a small species. Ruthenium red however stains the wall very clearly in fresh material at all times of the year. It

has been found very useful when measuring the cells, because it shows up the transverse walls very clearly, while causing no immediate shrinkage of the protoplasm. The significance of this reaction is not known, and the effect is not permanent, for the walls rapidly become colourless after the addition of dilute glycerine. Ruthenium red has little or no action on the walls of material preserved in formal or medium chrom-acetic acid, the walls are not then stained clearly. Nile blue sulphate is another stain which has been very useful in the elucidation of the wall structure: the walls are stained very clearly by it and transverse walls are clearly shown; the stain is useful for fixed material, for the walls are stained just as brightly as in fresh material. The colour rapidly fades in the walls and is at once removed by dilute glycerine. The reaction with iodine and sulphuric acid shows that the walls are composed of cellulose, for they swell and are stained blue. With chlor-zinc-iodine the behaviour of the walls varies at different times of the year (the same stock of chlor-zinc-iodine was used for every test). The following is a record of the tests made with chlor-zinc-iodine at various times of the year.

1st Period. During the first appearance of the Spirogyra in abundance no tests were made, but at the end of this period short pieces of filaments of various types, which were found in the deposits from the pond material, were tested, and reported to stain well with chlor-zinc-iodine.

2nd Period. The Spirogyra again appeared in abundance on February 21st 1928, and tests in February and March showed that the walls were stained very, very faintly blue by chlor-zinc-iodine; this was allowed to act for a long time, but the colour of the walls became no deeper. In April, May and June the walls were quite uncoloured even after leaving the filaments twenty-four hours in chlor-zinc-iodine. It was found that this same material after preservation in formalin, 93% spirit, or medium chrom-acetic acid, gave an immediate blue colouration with chlor-zinc-iodine, and fresh material was found to give a slight reaction after treatment with caustic potash. These facts suggest that during this period, there is either some difference in the physical or chemical condition of the cellulose, which renders it less reactive, or that the cellulose is mixed with some other substance, or substances that masks its reaction.

From October 1928 to March 1929 the Spirogyra was very scarce and only occasional perennating filaments were found in the mud. A few of these were tested with chlor-

zinc iodine, and the results are as follows:-

On December 1st and 5th very long filaments, which looked very vigorous, with short cells and a compact chloroplast, were tested, the walls were at once stained deeply blue, but apart from this they appeared no different from those of filaments which, tested in the previous April, had given no reaction.

On January 28th 1929 a filament with long cells and a filament with short cells were tested. The walls of both were at once stained faintly blue and after one hour they were deeply and brightly stained. It was found that the deeper colour was at once produced if the filaments were first treated with iodine.

On March 8th a filament was tested, it had been living in mud which had been kept in the laboratory from March 1st and was pale green and had very little starch. It contained long and short cells with compact chloroplasts. The walls werexquite unstained after 20 minutes but after six hours they were stained faintly blue.

On March 11th, 15th and 22nd, filaments from a culture which had been under constant illumination from March 1st, were tested: in one filament which had just been treated with iodine, the walls were at once stained blue; in the other two filaments the walls were stained very, very faintly blue, this at first could only be seen in the transverse walls, but after 15 minutes the whole filaments were very faintly blue.

On March 22nd fresh material, which was becoming much more abundant in the pond, was tested, a very, very faint blue colour could at once be seen in the transverse walls and after half an hour the whole filaments were very very faintly stained.

3rd Period. Filaments tested in April, June, and August, were at once stained faintly blue by chlor-zinc iodine, after a few minutes the colour deepened. The test in June was performed on conjugating filaments.

Wall formation.

It has already been mentioned that the walls are stained, or coloured, by nile blue sulphate. No importance is attached to this as a reaction, the colour soon fades, and is at once removed by dilute glycerine (Plate V fig. 3). The superficial blue precipitation which is formed with nile blue, and a similar black precipitation formed with osmic acid, is not removed by dilute glycerine. There is some evidence to show that these precipitations may be connected with wall formation. Between March 7th and 21st various tests with nile blue, and osmic acid, were made on filaments from the culture under artificial light; the precipitation produced was very sparse. In two of the filaments treated with nile blue there was no precipitation, but the plasma membrane was stained blue (Plate V

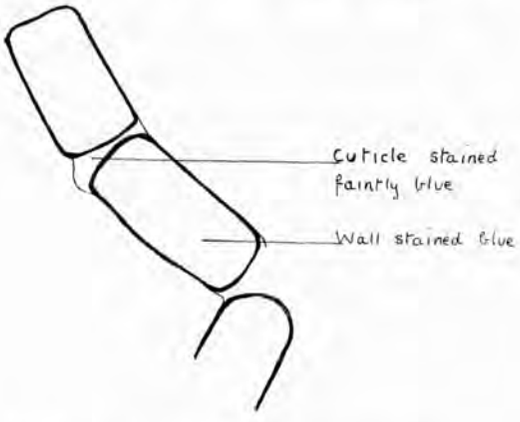
fig. 3); a similar filament treated with osmic acid showed only a very slight precipitation, while the plasma membrane was stained a greyish black colour. Tests made at about the same time on filaments from the pond showed copious precipitations with nile blue and osmic acid. The filaments from the culture under artificial light had recently undergone very rapid division for they had very rapidly increased. They appeared less vigorous than those from the pond having much longer cells, a paler chloroplast, and little or no starch; in fact they appeared to be in an etiolated condition, and were probably in a state of exhaustion. The very sparse precipitation which was produced in them suggested that during their active growth material had been used up from the protoplasts, and had not been replaced. The filaments which have little or no precipitation, and in which the plasma membrane is distinctly stained, suggest that the precipitations produced in the protoplasm, is connected with the materials used in wall formation, for where the plasma membrane is in contact with the wall there is a more deeply stained region. The more deeply stained regions appear in middle focus as large dots along the wall, while in top focus the plasma membrane appears to have a band like structure (Plate V fig. 3).

Conclusion. In the rare perennating filaments and the abundant vegetative filaments derived from them, the cellulose reaction with chlor-zinc iodine was given more readily than in the abundant vegetative filaments of the preceeding period. When no reaction is given, the cell wall is presumanly permeated by masking substances. The periods during which these masking substances are abundant, are not dependent on seasonal changes, for although tests on the filaments of 1928 indicate that the cellulose reactions are give more readily as the year advances, this was not confirmed in 1929.

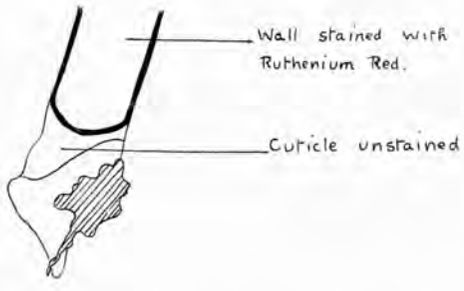
Structure of the Mature Wall.

The cell wall of Spirogyra cataeniformis, in common with other species, is composed of an inner cellulose layer and an outer sheath or "cuticle" continuous over all the cells. This "cuticle" is extremely thin and cannot be seen distinct from the inner cellulose layer in normal living vegetative filaments. Clear evidence of its presence can be seen in material preserved in formalin and stained in chlor-zinc iodine or nile blue sulphate; for in this material the transverse walls of adjacent cells are distinctly separated, and the cuticle can be seen passing over them. The "cuticle" was also clearly seen in living material in a culture of a few filaments which had been kept in the laboratory and aerated from April 30th to June 11th. On examination, these filaments appeared stale and in a few cases the "cuticle" could be seen quite distinct from the inner cellulose layer. The "cuticle" was demonstrated in the following way in normal filaments. The filaments were stained in ruthenium red, and then sulphuric acid was added, the cells were at once plasmolysed, and the transverse walls separated so that the cuticle could be seen passing over them. The "cuticle" has also been seen at various times in broken filaments, or filaments which are just breaking, and this state of affairs is particularly noticeable during the conjugation period. In a species with so thin a "cuticle" as S.cataeniformis, it is difficult to determine how far the reactions apparently due to the wall as a whole are also those of the"cuticle". Colour reactions of the "cuticle" cannot be seen in normal filaments, but convincing evidence of them has been obtained from one or two abnormal filaments, from parts where the "cuticle" has become separated from the inner cellulose layer. This evidence shows that the "cuticle" is faintly stained by chlor-zinc iodine whereas the inner cellulose layer is deeply stained. The difference in the depth of colour is due to the relative

thickness of the layers concerned. The "cuticle" is also stained by nile blue sulphate in common with the rest of the wall, but it is quite unstained by ruthenium red which stains the rest of the wall bright red (Text figs. 9 and 10).



Text fig. 9. Drawing with camera lucida of filament from material preserved in formal May 19th 1928 showing cuticle and inner wall layer stained with chlor-zinc iodine.



Text fig. 10. Drawing with camera lucida of filament from the culture kept under artificial light. Filament stained with ruthenium red on March 11th 1929. Only the inner wall layer was stained.

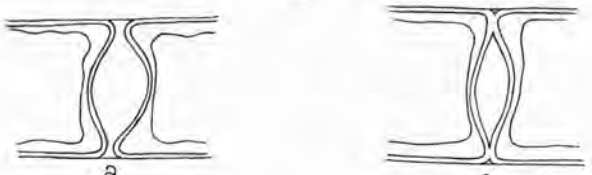
Transverse Walls. Some difficulty was at first experienced in elucidating the structure of the transverse walls, because no stain could be found which differentiated them clearly, and it was very difficult, especially in preserved material, to see their limits. Ruthenium red was found to be a good stain for fresh material but was of no value for preserved material. Chlor-zinc iodine, and iodine and sulphuric acid, both stain the walls of material preserved in formalin and sometimes of fresh material, but they also cause distortion of the walls and thus obscure the structure of the transverse walls Nile blue sulphate was found to be a useful stain for preserved material, it showed up the transverse walls very clearly and their structure could be made out. It appears that the middle lamella between the transverse wall dissolves, so that at this point the cells are held together solely by the "cuticle". In living material, the turgidity of the cells usually keeps the transverse walls distended against each other, so that they

appear to be joined when stained with ruthenium red and nile blue sulphate. However, sometimes in cells which are dying, the transverse walls cah be seen separated from each other and sunk into the cell cavity, so that their outlines form a bi-convex lense-like structure, (text fig. 11). Such cells must be much reduced in turgor. This change in the shape of the walls was indicated as follows. A vigorous looking filament was placed in a hanging drop culture on February 16th 1929, the transverse walls appeared distended against each other; the filament was again observed on February 18th, it was dying, and in adjacent cells one pairnof transverse walls was separated while another pair was joined. It appears that when thecells of a filament are plasmolysed their turgidity is reduced, thus the transverse walls separate, and the biconvex lense-like structure is produced.

Text fig. 11 show the same pair of transverse walls drawn in ruthenium red both when the wall structure is normal, and after the addition of iodine and sulphuric acid and when plasmolysis occurs.

Text fig. 11. Drawings with camera lucida of a filament in ruthenium red (a) showing normal appearance of transverse walls (b) appearance of the same walls after addition of sulphuric acid. The transverse walls are shown in middle focus.

Plasmolysis is brought about by formalin, by 93% spirit, and by medium chrom-acetic acid, but with the last two named fixatives it does not always occur, and all stages can be seen between those where the transverse walls are completely joined and those where they are completely separated. Frequently the transverse walls become partially separated by plasmolysis and they still remain touching at their outer edges. Text fig. 12 indicates the difference invthese cases.



Text fig. 12. Filaments from material fixed July 13th 1928 in formal and stained with nile blue sulphate, (a) transverse walls completely separated (b) transverse walls joined at the edges.

The solution of the middle lamella must take place very soon after the transverse walls are formed, for material preserved in formal just after its first appearance in February 1928 shows that it had already taken place, the transverse walls appearing separated when treated with nile blue sulphate. The cells of these filaments must have been recently formed for the Spirogyra was multiplying rapidly and hence dividing.

CONJUGATION.

In 1927 no conjugation was noted in the pond, this was not under observation until November, so that it is quite possible that conjugation occurred earlier in the year. In 1928 the Spirogyra was under continual observation, but no true conjugation was seen. Rare attempts at conjugation were seen at the end of June, and these attempts were more frequent during July, when two or three could be observed in looking through one slide of material. However, in no filament was a mature zygote seen, and only in one filament pair was the beginning of protoplasmic fusion shown. Evidently some condition was inhibiting conjugation, or possibly, in S.cataeniformis, it does not occur every year. The various attempts at conjugation were all shown in very short filament pairs, which could easily be distinguished because they were very thickly coated with detritus. Some of the filament pairs were widely separated, and had moderately long conjugation tubes; others were only slightly separated, and had very short conjugation tubes. No change was observed in any of these filaments when they were kept wet and observed later in the day. One of the earliest signs of conjugation was shown in a flask containing 90 ccs of pond water and in which

on April 30th, about eight long Spirogyra filaments were introduced. The flask was left in the laboratory as a control to a similar flask which was constantly aerated. Both flasks were examined on June 4th, In the aerated flask the filaments, which had broken up into shorter pieces and were scattered in the flask, were mostly dead, but one or two quite healthy filaments were seen. In the unaerated flask, the filaments were in a loose colourless tangle, and on examination of this, it was seen that all the filaments were dead, but that many of them had attempted conjugation, and were united by conjugation tubes while various unsatisfied conjugation tubes had been produced.

During May, June and July the Spirogyra was placed in various culture solutions in an attempt to induce conjugation, viz: Bristol's solution, Knop's solution, various strengths of cane sugar solution, calcium phosphate solution (1 mmg P per litre) distilled water. These cultures were set up in the laboratory under ordinary conditions, and under artificial illumination of 30 C.P.; they were also set up in the open air, in the shade, and in the sun, in which case excessive heat was prevented by surrounding the cultures with water. It was interesting to note that no attempts at conjugation were found in these cultures until after the conjugation period had set in and attempts at conjugation were found in the pond. Then it was noted that cane sugar solution acted as a stimulus to conjugation and in 3% solutions of this, one or two filaments with zygotes were obtained.

On August 7th there were no signs of conjugation and no filaments could be found which had attempted.it. Probably they had all died, for in July, when these attempts were fairly frequent, small grey patches could be seen in the pond, and they consisted of dead filament pairs which had attempted conjugation

In 1929 conjugation was first noted at the beginning of June and it was constantly observed until the end of the month

when conjugating filament pairs were observed in all parts of the pond covered by Spirogyra. On July 22nd the pond was again observed, and there were no signs of conjugation although there was still quite a lot of Spirogyra in the pond. Details of the method of conjugation and drawings are given in the second part of this paper. Although large masses of material entered upon conjugation, zygotes were very rare, and the following observation indicates how very scarce they were. In an hour and a half six slides of material were examined; each slide consisted of filaments united in pairs by conjugation tubes, but only 7 filament pairs were seen with zygotes and 3 with parthenospores. The material was not too young to have formed zygotes because conjugating filaments had been observed in the same place for several days.. Masses of material which had attempted conjugation, and showed conjugation tubes, died, and there was a large black patch at D (text fig. 1) which consisted of decaying conjugating filaments. This patch of decaying filaments appeared at the place where the Spirogyra was first abundant in 1929.

STUDIES IN SPIROGYRA.

PART II.

CONJUGATION IN SPIROGYRA.

INDEX TO PART II.

INTRODUCTION.

The lacing together of the filaments of Spirogyra during conjugation has been described in the following way: "two threads become ranged parallel to one another, and their opposing cells develop finger like protrusioms, which grow towards each other till they meet and fuse; after this the separating wall breaks down so that an open tube (the conjugation canal) is established." This quotation is an extract from one of our well known and much used text books.[1] (4)

Descriptions such as the above are ultimately derived from the records of De Bary, Haberlandt, Klebs, and other early workers. Now if reference be made to the original papers of these authors, it will be noted that their descriptions of the early stages of conjugation are very scanty. It is true that each of them quite definitely states that the papillae from opposite cells grow out and meet each other, but details as to the time taken for this process, and the distance apart of the filaments at the beginning of the process, are completely absent. The following quotation is from De Bary's classic paper, and is the only reference he makes to what even then he describes as the "well known way" in which the filament pairs become laced together. "Die paarweise Verbindung der Zellen, welche copuliren, durch kurze, gegeneinander wachsende und mit einander verwachsende Austülpungen ist allgemein bekannt. In den meisten Fällen findet zwischen den Zellen von zwei nebeneinanderliegenden Fäden eine leiterförmige Vereinigung statt"(3) Overton (10), Haberlandt (5), and Klebs (7), all remark on the certainty with which the papillae meet and are formed on the sides of the filaments which are turned towards each other; that this fact puzzled them, shows that they had no conception of the filaments first lying in contact, when the mutual chemical attraction (which they assume) could so easily be accounted for at corresponding places of the filaments.

Czurda has recently revived and supported certain obser-

[1] Footnote

In a later edition of this book, published (after the present work was started) in 1927, this quotation runs as follows:- ".....two threads become ranged parallel to one another, and their opposing cells develop finger like protrusions, the tips of which are in contact with one another; after this the separating wall breaks down so that an open tube (the conjugation canal) is established.

vations of Hemleben, and describes (a) an essentially different method for the lacing together of the filaments. These observers hold that in the earliest stages of conjugation the filaments are united in pairs or bundles; that opposing protuberances are later put out from the sides of the filaments which are in contact; that these protuberances are thus in contact from the first moment of their formation, and that by their growth in length, the filaments are pushed apart.

The present account of conjugation in S. varians, S. weberi, and S. cataeniformis, is given as further evidence for this latter method. It may indeed be the usual method for all species of Spirogyra.

SOURCES OF MATERIAL.

Observations on the process of conjugation in Spirogyra are of necessity limited to the season at which this occurs. In the majority of species conjugation is confined to the spring months, occurring between April and June, but it is not necessarily an annual event. In 1928 conjugation was very scarce, and although two species of Spirogyra were examined fortnightly throughout that year, no observations on conjugation were made, as this only rarely occurred. In both cases the species shewed a tendency to conjugate, but this was not realised. Conjugation could not be induced in culture by the recognised methods, though evidence has since been obtained indicating that once it has begun in nature, it may be stimulated and hastened in culture. In most species of Spirogyra the period of conjugation is very short, and thus not only must every moment of this period be used for recording observations, but a sharp look out must be kept for the first signs of conjugation. It is essential that the species used should be near at hand because fresh material must frequently be collected. While the observations here given were being recorded, collections were made at least once a day, and sometimes twice a day. In 1929 a number of localities with Spirogyra were observed, and during

the season of conjugation, three species were found whose conjugation followed conveniently one after the other.

S.weberi Kutz. was the first met with in conjugation. It was found in conspicuous green patches in a very narrow ditch near Holloway Sanatorium, about two and a half miles from Royal Holloway College. The ditch had been kept under observation since the previous autumn, and the Spirogyra seen to have disappeared without conjugation in November. It was found in a different part of the ditch on April 19th 1929 when it was already in conjugation. Conjugation had apparently only just set in, since no zygotes were to be found, and nearly all the conjugating filaments were of the same width, and had not developed the characteristic swollen female cells which in culture are formed sixty hours at the most after the filament pairs are in contact. Early stages in conjugation were found until May 8th, but after this only vegetative filaments and filaments in the later stages of conjugation. The ditch was observed about a fortnight later and there was no trace of any Spirogyra. This is one instance of many that have shown the rapidy with which Spirogyra can disappear from a pond.

S.varians (Hass.) Kutz. var. scrobiculata Stockm. was next found in a few bright green floating patches among abundant Vaucheria, in a much larger ditch through which there was a slow movement of the water; the ditch was about one and a half miles from Royal Holloway College. Before zygote formation these patches could easily be distinguished from the Vaucheria with the naked eye, because of their bright green colour, and after a little experience, those portions of them containing early stages of conjugation, could be distinguished from those containing only later stages, i.e. filament pairs with mature tubes, by the fact that the former portions were denser and of a deeper green colour. The Spirogyra was quite out of reach by hand, but small portions could be withdrawn on the end of a long stick without any disturbance of the main mass. The ease with which the Spirogyra could thus be lifted out of the ditch, suggested that

it was in relatively short filaments which was found to be the case. The material was first found conjugating on May 16th, and early stages were found until June 4th, when there was only a very small green patch of Spirogyra in the dyke.

S. cataeniformis (Han.) Kutz. had been under observation in a small pond in the grounds of Royal Holloway College since autumn 1927. A few attempts at conjugation were made in June 1928, but no zygotes were found, and only one filament pair showing protoplasmic fusion. This almost complete absence of conjugation may have been connected with the heavy rain of the previous autumn and winter; conjugation was known to be scarce in 1928. On June 6th 1929 a collection of this material showed rare filament pairs with conjugation tubes, and a very thorough search was made in all portions of the pond for further instance of conjugation. Although the pond was very small conjugation was at first very much localised, and was only found to occur with any frequency in one small area. The pond was thoroughly examined each day and by June 11th conjugation stages were found in all parts where the Spirogyra was present,

It is worthy of note that these three species showed conjugation first in shallow water, in a part of the pond or ditch that was tending to dry up. In the case of S.weberi one part of the ditch had very little water, and in places the Spirogyra was lying on damp mud; another part of the ditch had quite a lot of water, and here conjugation did not set in till later: four days after it had been observed in the first part, there were only rare filaments here showing conjugation. In the case of S.varians conjugation started earlier in a part of the ditch where the water was not more than an inch or so deep. It was completely over in this part when only beginning in the floating masses in the deeper parts of the ditch, where there was over two feet of water. In the case of S.cataeniformis conjugation stages were at first almost completely confined to a portion of the pond where the silt came to the surface of the

water and supported Poa fluitans and Callitriche. Thus again the conjugating Spirogyra was in very shallow water, in fact almost on marsh, although there was plenty of water elsewhere in the pond. In such shallow areas the Spirogyra would become subjected to greater variations in temperature and in concentration of the water.

Czurda has stated that from the earliest stages of conjugation the filaments become covered with detritus which is richly deposited on them. This messy appearance of the filaments makes observations on early stages of conjugation very difficult, as the small papillae (protuberances) and even the limits of the wall are often quite obscured. It was fortunate for the present observations that most of the material of S. weberi and S.varians examined, did not pass through this messy condition, as the water in which they were growing was free from detritus. A small patch of S.varians was however once observed in a shallow and very muddy area at the side of the ditch, this was collected, and on examination it was found that some of the filaments were showing early stages of conjugation, and these moreover were richly coated with detritus. Cultures were set up from this material and it was found that whereas the larger pieces of detritus proved useful for the recognition of selected parts of the filaments, as a whole they obscured the small papillae and the cell outline. Amongst this material portions of vegetative or non-conjugating filaments were also seen covered with detritus, suggesting that the changes which occur in the mucilage sheath, (referred to below) and which account for the sticking together of the filament pairs, may occur before they come into contact. These particular filaments so much coated with detritus are again referred to in the paragraph on the cell wall, towards the elucidation of which they contributed. In S.cataeniformis the conjugating material nearly always showed this messy appearance, which increased from the time the filament pairs were lying in contact to the time when mature

tubes were found and female cells differentiated. This was noted in June 1928, the rare filaments attempting conjugation could at once be distinguished even with a low power objective, though the individual cells and conjugation tubes were quite obscured and could only be seen with high magnification. This accumulation of detritus finally leads to a visible difference in the macroscopic appearance of the alga: the dark green wads changing to yellow green masses. Where the detritus is particularly richly deposited it is very difficult, even with high magnification, to see whether the filament pairs have any papillae or conjugation tubes or even to see whether zygotes have been formed. As was suggested in the case of S,varians the messy appearance of the conjugating filaments depends on the amount of detritus in the water. The pond in which S.cataeniformis was growing contained a particularly large amount of silt, and this was responsible for the richly coated filaments. Some filaments pairs showing early stages of conjugation were found among a deep green wad of material which was almost entirely vegetative, they were quite free from detritus, presumably because the wad had not yet broken up and so they had not come into intimate contact with the silty water.

METHOD OF TAKING AND RECORDING OBSERVATIONS.

General. In the three species investigated for early stages of conjugation, fresh material has been used entirely, since it is essential that conjugation should be followed through. At the same time some of the material was fixed in medium chrom-acetic acid, and in formalin. Before fixation the material was known to contain early stages of conjugation, i.e. filament pairs in close contact, or slightly separated by unpaired papillae, or by paired papillae, and it was hoped that stained preparations of these stages would be obtained. However, in all cases, examination of the material fixed in medium chrom-acetic acid showed that there were no filament pairs in contact, or separated by unpaired or paired papillae. The fixative had therefore

destroyed all early stages of conjugation by dissolving, or destroying, an adhesive, (a "mucilage sheath"), which firmly unites the filament pairs. In the case of S.varians, sometimes even the conjugation tubes were found broken at the former limits of the two papillae, but this was not the case with the other two species. An even more convincing proof that material fixed in medium-chrom-acetic acid is of no use for the observation of early stages of conjugation, was shown by means of S. cataeniformis. A long filament pair closely in contact all the way, was treated with medium chrom-acetic acid and observed under the microscope. Before and immediately after the addition of the fixative the filaments were firmly united and could not be separated by teasing with needles. The slide containing them was placed overnight in a damp atmosphere and examined in the morning, when it was hoped that fixation being complete the filament pair could be stained. However by morning the two filaments had become completely separated, and none of the filaments on the slide showed any trace of having been united. Material of S.varians and S.cataeniformis has also been preserved in formalin, and a similar separation of the filaments has been noted; even if some of the early stages remain, it would be very difficult to see them because of the shrinkage and tangling of the filaments. The only fixative found in which early stages were preserved was picronigrosin, a fixative not universally employed; evidence as to the occurrence of filaments in contact, or slightly separated by papillae, can certainly be obtained from such material, but it is not nearly so clear as in living material.

Czurda has drawn attention to the rapidity with which early stages are passed through, and his observations on the conjugation process were made on the spot. He was fortunate enough to find wads of Spirogyra in which conjugation started simultaneously, so that anyone filament pair represented the stage reached by the whole wad. Thus he made observations and

took photographs of portions of a wad at a certain time, and later made further observations and took further photographs of different filaments from the same wad. In this way he followed conjugation through. With the three species investigated here such a method was not possible. Conjugation was not simultaneous in any of the wads and the localities were too thickly populated for observations to be made on the spot. Observations and photographs were therefore confined to the laboratory where they were made as soon after collection as possible.

The necessity for frequent collection of material was very soon realised; the earliest stage of conjugation, viz: the arrangement of the filaments in pairs or bundles, was only found in material examined immediately after collection, and did not occur after the material had been removed from its native habitat, although the development of this stage continued. After one day in the laboratory no early stages were found in material which, immediately after collection, had shown a number of these. An exception to this was found in S.weberi, in which species the earliest stage of conjugation was initiated in cultures kept under artificial light. This illumination necessarily involves the raising of the temperature to between 17° and 20° C, and temperature may be a factor to be taken account of. When thus illuminated, cultures which have been in the laboratory for a day or so, and therefore no longer show early stages, begin to develop them afresh. Even in this species the necessity for the frequent collection of material is not however eliminated, because early stages are not to be found after a day or so under artificial light; the filaments have all by that time reached some stage of conjugation, or else in unpaired filaments have developed irregular papillae in all directions, from some or all of the cells. Obviously then observations on the early stages of conjugation can only be made by a worker who has near to his laboratory, localities in

which Spirogyra flourishes, so that daily collections can be made and examined at once.

Special. A small portion of the conjugating material was snipped out of the wad or drawn out on a needle. Glass needles were used as likely to be less injurious than metal ones to such highly sensitive material. The material was gently teased out on a slide in a drop of its natural water and observed under the microscope. When two filaments lay in contact, and there were no papillae from either, they may have so come together either through chance or because they were "glued" together preparatory to conjugation. This could be ascertained by agitating the water round them, or by moving a projecting end of one of the filaments. Treated thus, vegetative filaments in contact by chance immediately separate or slide along one another: when the filaments are "glued" together they cannot be separated at all. By applying this test to the filament pair, the conjugating ones can at once be identified, and in this investigation such identification has been infallible. A filament pair A and B was then selected which could be recognised later by some special character, e.g. by the length of A and B, by the ends of the filaments with reference to each other, whether ending naturally or through a break, by the position of a dead cell or cells, by the position of the filament pair on the slide: this last was only of use if they were near the outside of the drop. An easily recognisable portion of the filament pair was selected for observation, and each cell of filament A was numbered and described, and the changes in A with regard to the cells of the corresponding filament B were noted down at each observation.

As early stages in conjugation were not very frequently found, the selection of conjugating pairs, and setting up of the cultures, took considerable time. Outline drawings with camera lucida were made as far as time allowed, but as it was thought necessary to make as many observations as possible, these had

frequently to be made in note form, Where drawings had been made however, any subsequent division of the cells in the conjugating filaments, was more readily observed.

After the first observation the slide was transferred to a petri dish lined with wet filter paper, a constantly moist atmosphere being thus assured. By this means the cultures could be kept over night without drying up. In the case of S. weberi and S.cataeniformis these dishes were placed under constant artificial illumination of 30 C.P. as this seemed to hasten papilla formation. In the case of S.varians artificial light was of no help but rather seemed to hinder the process. Under these conditions the filament pairs are found to remain normal and vigorous, and development proceeds until the production of female cells, about sixty hours after contact in S.weberi and thirty-six in S.varians. In S.cataeniformis the formation of conjugation tubes on the rare occasions when it occurs in culture, is extremely slow. More often than not the cultures show no growth. In the case of this species the cultures are much more difficult to set up; the conjugating filaments are relatively short and tend to break up during conjugation, and thus unless all other filament pairs are removed from the culture they cannot be recognised with certainty. This is a difficult matter, for the filaments are short and very small, and are shifted about with the rest of the material on the slide. However with the aid of a dissecting microscope several cultures containing isolated filament pairs were set up, and further development proceeded in a few of them.

After it was realised that papilla formation and growth occurs comparatively rapidly in S.varians, cultures of this species were permamently kept on the stage of a microscope and observed from time to time. This saved all the time necessarily spent in again finding the selected filaments, and ensured that they were not lost sight of. Between each observation a watch glass was placed over the slide, and the culture was

frequently replenished with distilled water. A number of such cultures was set up on the stages of different microscopes, and frequent drawings with camera lucida were made. Reference to the drawings accompanying the tables will show that the size is not always the same, because the magnification of the lenses differed. It was found that this method could not be applied successfully to S.weberi and S.cataeniformis, as the process of papilla formation was so slow.

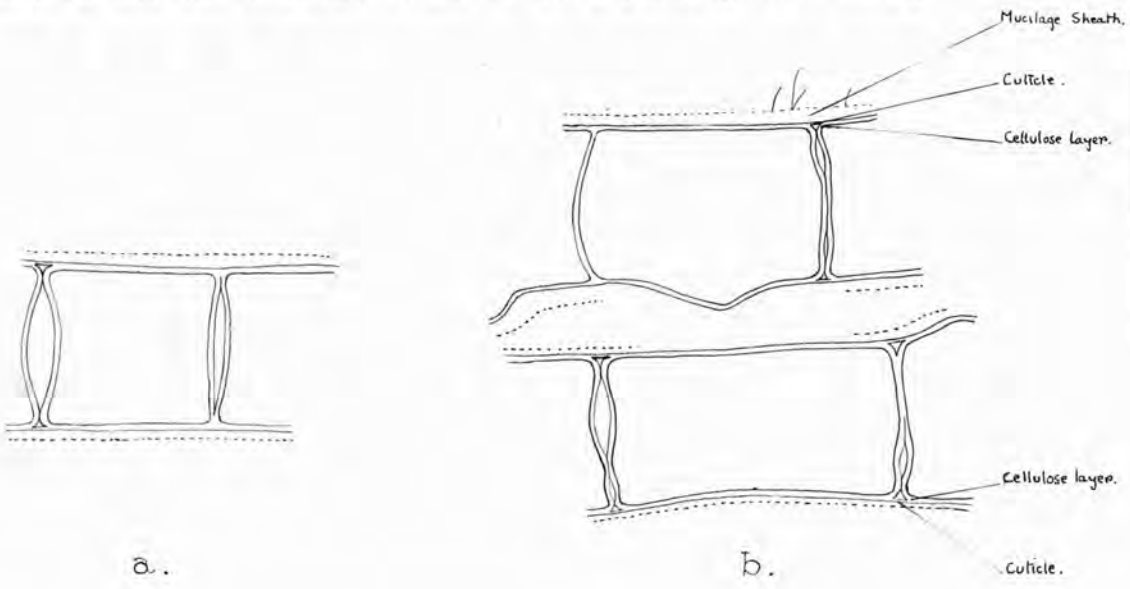
In proof of the statements and drawings here presented of the early stages of conjugation, photographs of the filament pairs were taken. Here again was felt the necessity for following through the conjugation process, and this involved the taking of a photograph of an early stage of conjugation, and a second photograph of the same portion of the filament pair at a later stage. The difficulties encountered in obtaining such a pair of photographs were great. After the first photograph had been taken the filament pair frequently died, or was lost to view, or the filaments of the pair were overlapping in the part previously photographed, so that a second photograph could not be taken. No coverslip could be placed over the filament pair since this inhibited further growth, and thus the photographs had to be taken through a drop of water which varied in depth. The time exposure necessary varied with the depth of the water, and with the position of the filament pair whether at the surface or the bottom of the drop. During the exposure which was reduced as far as possible by using strong illumination from a point o' lite lamp the filament pairs frequently moved, being pushed by swimming uni-cellular and other living organisms necessarily included in the drop for the well being of the Spirogyra. Thus the achievment of a pair of photographs was largely a matter of chance, and a good pair well nigh impossible. The photographs have little value as photographs, but they serve as evidence for the early stages of the conjugation process.

OBSERVATIONS AND RECORDS.

The Wall of Spirogyra.

Before proceeding to a description of the process of conjugation, something must be said with regard to the structure of the cell wall. The wall of Spirogyra is said to consist of an inner wall of cellulose around each cell, and an outer wall continuous over the whole filament. This outer wall has been referred to under various names: viz. Hüllhaut (de Bary); Cuticula or Cuticularschicht (Strassburger, Oltmanns); Gallertscheide (Klebs). S.varians when treated with indian ink, methyl violet, or methylene blue, shows that this outer wall is composed of two distinct layers viz: a firm layer directly in contact with the cellulose wall, which layer marks the visible limit of the wall in unstained filaments, and an outer mucilage sheath of varying width, which is stained blue and violet by methylene blue and methyl violet respectively, and which is quite invisible in unstained filaments. These two layers of the outer wall correspond to the cuticle (Cuticula) and mucilage layer (Schleimschicht) described by Benecke(). Although not demonstrable, there is indirect evidence to show that a very narrow mucilage sheath is present in the two narrow species S. weberi and S.cataeniformis; moreover these filaments shine up clearly in indian ink. Since the mucilage sheath is of varying thickness, Kleb's idea that the "Gallertscheide" (or in this case part of it) is probably a secretion of the cell is very acceptable. In S.varians and presumably the other species mentioned above, the mucilage sheath is present in both vegetative and conjugating filaments and the structure of the cell wall appears to remain unchanged. Text fig: 1 is an outline drawing with camera lucida of the wall of S.varians in a vegetative filament and in a conjugating pair. The filaments had been treated with methylene blue which after some time caused death of the cells, and hence the wall structure became more easily visible. In some cases the transverse walls had sep-

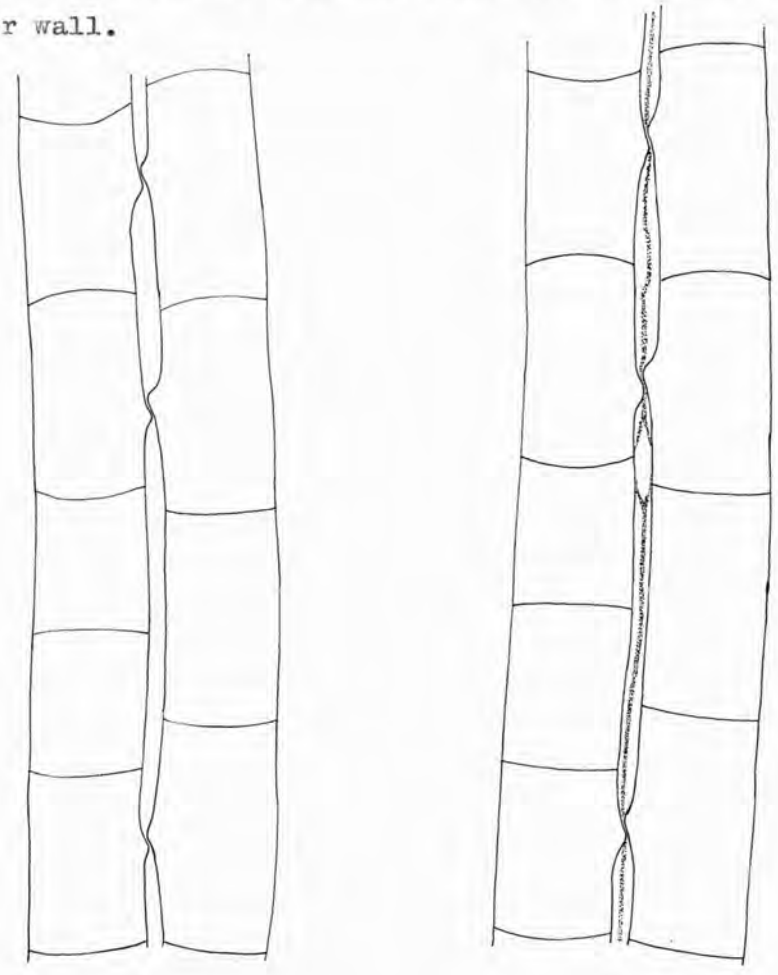
arated and the cuticle could be seen passing over them.



Text fig. 1. Spirogyra varians (a) vegetative filament (b) conjugating filament pair in solution of methylene blue.

Czurda has stated with regard to the early stages of conjugation in S.setiformis, that the filaments are cemented together by means of the swollen indistinct "primary cell wall layer". "Es ist jedoch nicht nur ein scheinbares Verklebtsein sondern eine ausgesprochene Verkittung der Fäden mittels der verquollenen, undeutlich geschichteten primären Mandschicht (Hüllhaut, de Bary; Cuticula oder Cutieularschicht, Strassburger, Oltmanns; Gallertschide Klebs). With regard to the species here investigated this statement must be modified. By the term "primary cell wall layer" Czurda is denoting a layer which he says is identical with the "hüllhaut" of de Bary i.e. with the whole of the outer wall, including the cuticle. In S. varians, S.weberi, and S.cataeniformis, the filaments are cemented together by the mucilage sheath, and this is an invisible part of the outer wall, which moreover is very easily removed. No change can be observed in the "cuticle"-in these species the visible limit of the outer wall-during conjugation. In S.varians where the filaments are lying in very close contact, no swelling of the wall as reported by Czurda has been noted, and a mucilage sheath cannot be demonstrated between the filaments. On the

other hand where they appear to be slightly apart, it can be shown that they are bound together by mucilage, the mucilage sheath presumably altered. Immersion in indian ink shows that when the filament pairs are very slightly pushed apart by recently formed papillae, the "space" between them is occupied by mucilage, but this is not the case when the filaments are more widely separated through the growth of the tubes. It is difficult to prove that this "mucilage" is not the altered "primary cell wall layer" as Czurda claims but only part of it. The following case indicates the difficulty of interpretation, but at the same time it supplies proof of the double nature of the outer wall.



а. б.

Text fig. 2. Spirogyra varians (a) filament pair in 0.1 % Ruthenium Red (b) the same pair after addition of Picronigrosin.

Text fig. 2 is the outline sketch of an early stage of conjugation in S.varians treated with ruthenium red. This

stains the walls very clearly, although it does not conclusively differentiate the wall layers, and there was no indication that these had been altered in any way. While this filament pair was watched beneath the microscope, picronigrosin was added to kill and fix it; the visible limits of the filaments were at once stained a greenish black, and between the filaments there appeared a greenish black line (see fig.). This line must indicate the common surface of the two filaments, and it could be interpreted as the outer limit either of the cuticle, or of the mucilage sheath. That the latter interpretation is correct was shown as follows. A filament pair A and B in an early stage of conjugation was treated with methylene blue. A had small papillae which in most cases were not touching the wall of B. B had only one cell with a papilla, and this was united with the corresponding papilla from A, forming a conjugation tube. The filaments were firmly glued together and could not be moved apart; this and the additional fact that methylene blue stained the debris between them indicated the presence of mucilage. The filaments were observed under the microscope and chlor zinc iodine was drawn beneath the coverslip. The debris immediately disappeared, the filaments at first shrank and then regained their normal size, but they were no longer connected except by the one conjugation tube, and at the slightest touch of the coverslip they moved apart. Thus the connecting mucilage had been destroyed. After some time the filaments were stained faintly blue, and after the additionof glycerine the "cuticle" could be clearly seen across the now separated transervse walls. The method was thereupon applied to zygote material of S.varians and S.weberi. On treatment with chlor zinc iodine the cell walls were stained and the cuticle could be clearly seen above the separated transverse walls.

The small portion of S.varians previously referred to, which contained conjugating filaments very richly coated with detritus, seemed at first to support Czurda's view that the

primary cell wall layer or cuticle is greatly swollen and indefinite in contour. This was also the case in the richly coated conjugating filaments of S.cataeniformis,and seemed to be the case when such filaments were treated with indian ink, as the limits of the wall were hidden. Ruthenium red showed up these limits quite clearly within a mucilage sheath covered with detritus, but without the above evidence it could not have been conclusively shown that this mucilage sheath was not the altered swollen primary cell wall layer or cuticle. Filaments of S. cataeniformis which were firmly glued together were treated with chlor zinc iodine in the same way as those of S.varians; as in the latter species they at once became free but the cuticle remained unchanged. Very clear proof of the presence of the cuticle can be seen when, as frequently happens in this species, the conjugationg filaments break up; the cuticle, apparently unaltered, can be seen at the broken ends (see case 1.).

Method of Pairing of Filaments.

Examination of material of S.weberi, S.varians and S. cataeniformis,shows, in accordance with Czurda's observations that at the beginning of conjugation the filaments lie in contact in parallel pairs or bundles. In S.cataeniformis bundles of filaments are more frequently met with than pairs of filaments. Such pairs or bundles of filaments are firmly stuck together and cannot be separated by teasing with needles. As has been already mentioned the presence of mucilage can be shown in all three species by the indian ink method: when immersed in indian ink the filaments shine up clearly. Mucilage can also be demonstrated in vegetative filaments, and in S.varians some of the vegetative and conjugating filaments show quite a wide mucilage sheath. It is probably due to some alteration in this mucilage sheath that the filaments are so firmly stuck together; the cuticle is evident as something distinct,both from the mucilage sheath without,and the cellulose

layer within; except at the place where the conjugation tube forms it apparently remains unchanged during conjugation.

Possibly this sticking together of the filaments is the same nature as the adhesions, which, together with geniculations, were interpreted by de Bary as early stages in conjugation in Sirogonium and Craterospermum. He noted these phenomena in Mougeotia and Mesocarpus as well, and that in these genera they had no connection with conjugation. De Bary regarded the adhesive as a strongly developed portion of the "Hüllhaut": his comprehensive term for the sheath outside the cellulose layer. In a short paper entitled "Adhesions and Geniculations", Lloyd enters fully into the subject, and as a result of his investigations in S.longata he concludes that the adhesions and geniculations found at the time of conjugation have no direct relation to this process. He states that in S.longata it is oftem impossible to see any definite adhesive material without staining, but that after staining it may be seen to be "quite distinct from the cuticle, and does not in any degree pass over into it". He therefore regards the adhesive as the material of the sheath modified in some way though he adds "the possibility of a secretion naturally suggests itself". As has been already stated the adhesions which occur during conjugation in the three species here investigated are probably due to an alteration in the mucilage sheath which is quite distinct from the cuticle, and with regard to this sheath Lloyd's idea of a secretion is acceptable.

In S.weberi, S.varians, and S.cataeniformis, the filaments which are already in contact, put out slight papillae against, rather than towards each other. The papilla from one of the opposite cells arises first, and later the corresponding papilla is produced at the place of contact of the first. Thus, as Czurda has stated, the papillae are in contact from the first moment of their formation. By the growth of these papillae the paired filaments or filament bundles become separated, and the

familiar appearance of filaments laced together by conjugation tubes is seen. Proof for these statements will be found in tables and drawings at the end of this paper which summarise consecutive observations on filament pairs or bundles, from the time they were in contact, to the time that death occurred or some other misadventure befell them.

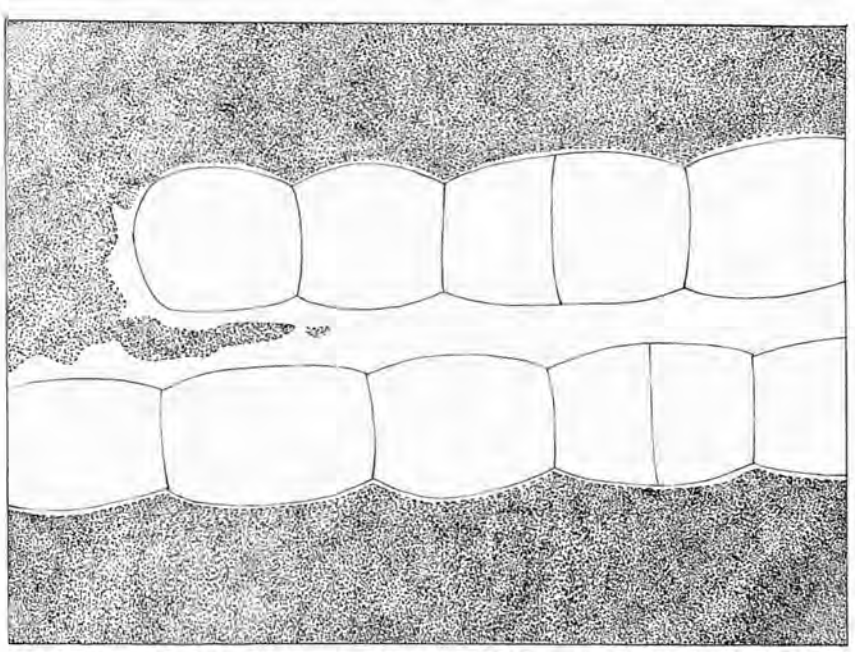
S.weberi was the first species to be found in conjugation and fresh material was critically examined for early stages. At first the method of observation was merely statistical, abundaht material was mounted and examined for early stages, i.e. stages where the filaments of a pair were not separated by a wide space as at maturity. These stages were rare but several were found and drawn, (e.g. cases 27, 28, 29.) and they conclusively showed that if the filaments are not in contact when papillae are first put out, they are very close together, and that from the time when slight opposite papillae are touching to the time when they have developed mature conjugation tubes, the distance apart of the filaments increases. These conclusions were deduced from a number of cases observed but not followed through. No development was noted when such stages were watched throughout the day, in spite of the fact that the slide was continually irrigated. It was not until it was found that in this spec ies artificial light acts as a stimulus to conjugation, and the special methods of culture described above were employed, that the development of early stages could be followed. The tables and drawings of this species, show the development of twenty-six filament pairs from an early stage of conjugation where the filaments were in contact or were separated by very slight papillae, to a later stage where they were further apart and had developed large opposite papillae or mature tubes.

In each slide culture of the material examined, and the number of these examinations greatly exceeded the cases here reported, search was made for evidence of the usually described

method of conjugation. In slide cultures of S.weberi such evidence was never found. One very suggestive case was observed where on a slide which had been under atificial light for two days, two filaments A and B were parallel for a short distance, and A had three cells with wide papillae and B had one cell with a wide papilla opposite one of the papillae from A. The slide was again placed under artificial light and observed the following day, but the filaments had both turned over so that the papillae were on their outer sides. A number of other filaments showed irregular papillae, probably a pathological phenomenon, and possibly due to the effect of artificial light. With but two exceptions, the material collected from the part of the ditch with plenty of water, where conjugation was observed from its first appearance, showed no papillae from unpaired filaments, such papillae if found would have served as evidence, though not as proof, for the usual view of conjugation, since the absence of the fellow filament could have been explained as due to its loss during mounting. The exceptions referred to were both seen in the projecting end of one of the filaments of a conjugation pair, many of the cells in this unpaired region having formed papillae. Cultures of this material when kept for two or three days under artificial light, showed numerous irregular papillae from unpaired filaments, thus again suggesting that this is a pathological phenomenon. One or two cultures were made in the following way so that the material was not disturbed during mounting. A coverslip was inserted beneath the filaments and they were snipped round the edge of the coverslip so that this could be lifted out. It was then laid on a slide and kept moist as described above. No filaments were observed to conjugate unless they were previously in contact. Thus in S.weberi no evidence has been obtained in support of the usual description of conjugation.

S.varians was next found in conjugation and a large amount of material was available; thus, as the tables and drawings

show, it was possible to follow a large number of filament pairs through the conjugation process. Besides the one hundred and eleven cases followed through from early stages of conjugation when the filaments were in contact or separated by slight papillae to the time when they were separated by large papillae or mature tubes, a large number were seen which have not been reported; several early stages were usually observed in the setting up of one culture but only one was specially noted and followed through. In S.varians besides those filament pairs which are obviously in contact (e.g. cases 42, 43.) there are pairs which appear to be slightly separated so that the small papillae from one filament do not reach the visible limit of the other filament (e.g. cases 1; 15.) That this separation is not real was shown as follows. Tests with indian ink on several such pairs have always shown that the area between them is occupied by a mucilaginous substance and that they are therefore in contact by the outer limit of their mucilage sheath (see text fig. 3.)



Text fig. 3 Spirogyra varians Filament pair immersed in Indian Ink.

In the cases followed through conjugation and therefore not tested with indian ink, the presence of an adhesive was indicated by the fact that the filaments would not move apart. In this connection it is significant that the mucilage sheath in vegetative filaments varies in width, some filaments when treated with indian ink or stained with methylene blue show a wide sheath, whereas in others it is sometimes so narrow that its presence can only be inferred by the fact that the filaments shine up clearly when immersed in indian ink.

Although in S.varians the normal process of tube formation is essentially as has been described i.e. the filaments are first in contact, some evidence has been obtained showing that tubes can grow together from cells which are not in contact according to the usual description of conjugation. With but one apparent exception, to be mentioned later, this method of conjugation has only been found in free portions of filaments which have already conjugated elsewhere, in the way described above. It is in these portions that the usually described method of tube formation takes place and after this is complete such portions can be recognised by the greater length of the tubes (e.g. cases 70, 100.). In the numerous cultures examined, and in these the filaments pairs far exceeded the number here reported, only nine cases were seen where tubes had grown together and met, and in three of these cases only one cell was concerned. Occasionally in looking through fixed material a small portion of a filament pair can be seen in which the filaments are separated by abnormally long tubes. As has been stated these tubes point to the previous occurrence of the usual methods of conjugation. Possibly such instances that have been observed where the papillae grow together and meet, are abnormal, because they are only found in filaments that have elsewhere conjugated normally. Moreover when the main mass of material is in conjugation the filaments being separated by tubes, the production of abnormal papillae and the swelling of

cells of unpaired filaments is very frequent. This phenomenon is further found in the diverging ends of conjugating filaments and in those cells (the odd cells) which have no partners. By the time that conjugation tubes are fully formed in the paired filaments or paired parts of filaments, these unsatisfied papillae have changed into wide bulges. The one apparent exception referred to was case 49. Two filaments which were curved lay near together in the form of an X. In the middle region of the X where the filaments were nearly touching there were three pairs of cells whose papillae had not yet met (see case 49.) The filaments were apparently not in contact in any part and yet these three cells formed conjugation tubes and later another pair of cells quite widely separated formed a conjugation tube. However this case too can be claimed as one in which the filaments were in contact through the outer limit of a wide mucilage sheath. The presence of an adhesive was indicated since no special care was taken in mounting the culture and they remained together. It seems inconceivable that the three pairs of cells with opposite papillae would have remained in position unless the filaments had been bound together in this region by mucilage.

S.cataeniformis was the third species found in conjugation but it proved very difficult of observation. Many cultures with early stages were set up and watched but development was very slow and only took place in those cultures which were artificially illuminated. The tables and their accompanying drawings are of seventeen filament pairs observed from the time they were in contact or slightly separated by small papillae, to the time when some of their cells were widely separated by large papillae or by tubes. Time limited the number of observations made, for as has already been stated cultures of this species took much longer to set up and the development of the early stages was very slow and very rare;

thus in following the development of the seventeen filament pairs over forty cultures were set up. All the unsuccessful cultures give evidence for the method of conjugation described here, each of them contained one or more filament pairs which were in contact or slightly separated by small papillae. In setting up the later cultures a large number of filament pairs were seen in which the filaments firmly adhered together and could not be separated by teasing with needles; these were not used for the cultures since it was found that development was more likely to proceed in filament pairs which showed papillae.

Observations on this species having given no evidence in support of the generally accepted view of tube formation. In living material unpaired filaments with papillae have never been seen, ewen when the cultures have been kept under artificial light. This evidence, although negative, gives strong support to the method of the lacing together of the filament pairs here described.

Papilla Formation.

With regard to papilla formation, Czurda has stated that in Spirogyra species in general, the papillae are first produced by the male cells, while in those Zygnemales which form zygotes in the conjugation canal, papilla formation is simultaneous on either side: "der Beginn der Papillenbildung an den beiden Partnerzellen nicht gleichzeitig erfolgt, sondern das an den männlichen Zellen die Vorstülpungen zuerst zu beobachten sind. Bei anderen Zygnemalen, bei denen sich die Zygoten im Copulationskanal bilden erfolgt die Papillenanlage gleichzeitig".

S.varians. Observations on the earliest stages of papilla formation, show that in S.varians the opposite papillae which are in contact from the first moment of their formation, are not produced.simultaneously, but that one of the pairing cells produces them before the other. The cells which put out the first papillae are usually restricted to one filament of a pair,

this has been found to be the case in fifty-one filament pairs observed. In eight filament pairs observed, papillae appeared indiferently from the cells of either filament. Of the fifty-one cases mentioned sex was clearly differentiated in twenty-five, either by bulging of the female cells on the side of the conjugation canal, or by zygote formation, the rest died without showing such differentiation. In eleven of these twenty-five cases, the male cells produced the first papillae, and in fourteen, the female cells produced them. Thus although papilla formation in paired cells is not simultaneous, the first formed papilla is not determined by the sex of the filament. Since no instances of cross-conjugation have been observed the filaments are presumably unisexual, the sex having been determined in the vegetative period before conjugation set in.

S.weberi. In S.weberi sililar conclusions can be drawn as to the formation of papillae, but few cases have been followed through to the time of differentiation of female cells; the majority of filament pairs in culture died before this occurred, since the production and growth of papillae was so much slower than in S.varians. Six cases have been observed where the papillae were produced indifferently from the cells of either filament of a pair, thus again clearly showing that the first formed papilla in paired cells is not determined by the sex of the filament. Eighteen cases have been observed suggesting that the cells forming the first papillae are restricted to one of the filaments of a pair; in one of these cases this filament was the female filament. It is presumed that the filaments are unisexual since no instances of cross-conjugation have been seen.

S.cataeniformis. In S.cataeniformis a number of observations have shown that papilla formation is not simultaneous in the paired cells. Sometimes the first formed papillae grow to a considerable size, almost the mature size, before the opposite papillae are produced; sometimes they are very small.

(cf. cases 3 and 5.) In S.varians and S.weberi, the first formed papillae are usually very small when the opposite papillae are produced, an exception is shown for S.varians in case 18. In this sensitive species, so difficult to culture, production of papillae and differentiation of female cells only rarely occur in culture, and in any case development is very slow, thus very few filament pairs have been observed from the time of production of unpaired papillae, to the time of differentiation of female cells. Three filament pairs have been noted in which the first formed papillae were produced indifferently from the cells of either filament, and one of these, (case 1.), was observed until the differentiation of swollen female cells throughout one of the filaments. This one case shows that the first formed papillae may occur in either male or female filaments. Presumably the other two cases would have shown the same thing, since in all cases where female cells have been recognised they have been restricted to one of the filaments of a pair. Of the numerous cases observed where the cells forming the first papillae are restricted to one of the filaments of a pair, only four have been followed through until the differentiation of female cells. In one of these the first papillae were produced by the female cells, and in three they were produced by the male cells. Thus even from these limited observations it may be concluded that the production of the first papillae is not determined by the sex of the cell.

These results are not in agreement with those of Czurda, nor with those of Lloyd who states, with regard to the number of species including S.weberi and S.varians, that "the tubes grow out in sequence and it seems certain that the male is always the first and the female tube is consequently always the shorter"(8) However they conform with Haberlandt's observations which point to the fact that the papillae of corresponding cells are not produced simultaneously, but that sometimes the male, sometimes the female,"fruher einen Copulationsschlauch treibt"(5). As Czurda stated these observations may be passed over since Haberlandt did not observe the beginnings of papillae formation,

but deduced them from the lengths of the male and female portions of the conjugation canals, assuming that these grew equally in length in a given time. That this is not the case however, has been shown in S.varians, where the conjugation canal is often longer from the female cell, in those cases where the male cell has been observed to produce the first papilla; and again in S.weberi and S.cataeniformis where the component parts of the conjugation canals have not been found to be unequal in length, in those cases where papilla formation has been observed earlier in one of the conjugating cells.

Rate of Papilla and Tube Formation.

In S.varians both production and growth of papillae are considerably more rapid than in the other two species. Filament pairs which between 9.0.am. and 12.0.pm. were in very close contact or showed very slight papillae, were already separated by tubes when observed between 7.0.am. and 11.0.am. the following morning. Frequent observations on slide cultures kept on the stage of a microscope have shown that in less than three hours a filament pair showing very slight papillae from one of the filaments may have developed considerably larger united papillae from both, and within twenty-four hours may have differentiated female cells (see case 5.) Within an hour and a quarter slight papillae were observed to have developed from filaments which previously lay in contact and showed no papillae. In this case artificial illumination does not hasten the production or growth of papillae. Observations on the material in its natural habitat have shown that, as in culture, the formation of tubes occurs very rapidly. Material was collected at 6.0.pm. from a small bright green patch; the position of the remainder was carefully noted, this being possible since there were no other really bright green patches. The material was examined as soon as possible and showed a number of filament pairs in early stages of conjugation, i.e. in contact or with very slight papillae, one or two of these

stages were observed in each portion of material mounted. Material was collected from the same wad at 7.30.am. the next morning, and on examination no early stages of conjugation were found but abundant filament pairs united by tubes, none of these filament pairs showed protoplasmic fusion. In this species the production of papillae has been observed to occur at any time during the day, and thus, unlike S.setiformis as reported by Czurda, early stages are not confined to the evening hours.

In S.weberi the process is on the whole much slower. The shortest time taken for the production of mature tubes and the differentiation of female cells was twenty-six and a half hours from the time that the filaments of the pair were observed in contact, this was in a culture kept under artificial light of 30 C.P. which seems to stimulate the process. Three other filament pairs were observed to the same stage and they took considerably more time, thirty-four to fourty-four hours. In cultures under artificial light, the time taken for the production of slight papillae from fil aments in contact, ranges from four to nineteen hours, thus indicating that the filaments may probably lie in contact for some hours before papillae are produced. The corresponding time for the formation of tubes ranges from thirty-nine to sixty-four hours, and is thus considerably longer than in S.varians. No indication of the time taken for these processes in nature has been obtained, as conjugation was more or less continuous in the ditch, and there were no easily recognisable wads.

In S.cataeniformis, if and when production of papillae and further growth occur in culture, these processes are very slow. The time for the production of papillae from filaments lying in contact, ranges from nine to twenty-three hours, and the corresponding time for the formation of tubes, ranges from thirty-nine to seventy-four hours. Even when one of the filaments has produced papillae, a long time elapses before the opposite papillae are produced, and again before tubes are

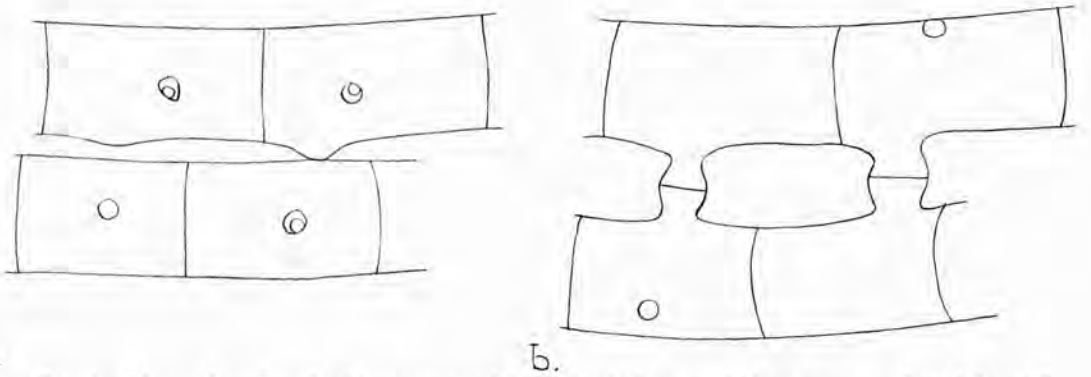
formed. These results have been collected from cultures kept under artificial light; no indication of the time taken for these processes under natural conditions can be given.

The Position of the Nucleus in Conjugating Cells.

Lloyd (8) states that during the growth of the conjugation tube in S.crassa, the nucleus moves from the centre of the cell towards the inner mouth of the tube, and thus passes through the tube sooner than it otherwise would. He attaches no importance to this fact, and does not regard it as giving support to Haberlandt in his conceptions that the nucleus lies in the place of the most intensive growth ( ). Before Lloyd's work Czurda (3) had stated that during the formation of papillae the nucleus leaves its original position in the centre of the cell, and becomes shifted to the side opposite to the papilla. On page 458 of the same paper, he states, that in spite of many observations, an exception to this rule was never found in any species, and further, that in spite of the most exhaustive and successive observations of the whole conjugation process, the nucleus is never met with in the neighbourhood of the conjugation papilla. He modifies these statements with regard to narrow species, such as S.weberi and S.varians, by adding, that although not demonstrated, it is quite conceivable that the nucleus may be drawn into the papilla entangled in the chloroplast turns if they sink into the papilla. Both Lloyd and Czurda criticise Haberlandt's statement that the nucleus lies in the place of the most intensive growth, and is therefore found near the growing tube.

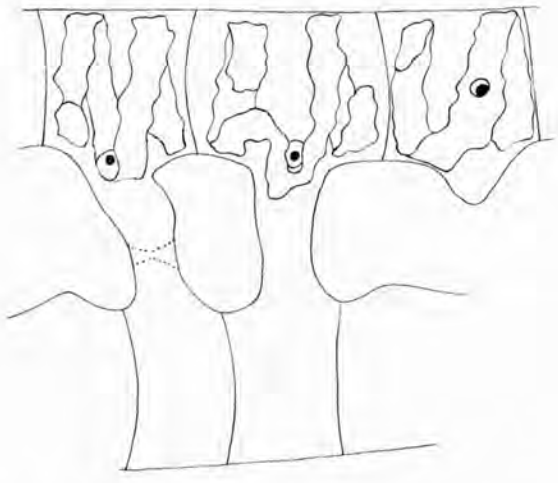
In the present investigation the position of the nucleus has only been obs erved in S.varians, since the other species available were so small, that the nucleus could not be seen in fresh material. In S.varians conjugating filament pairs were observed, and in all cases where the nucleus was seen in the living cell, it was in the middle of the cell when the papillae were small, and in the same place or on the side opposite to

the papillae when these had formed tubes. This indirect evidence has been supported by direct evidence obtained from a number of cultures which have been followed through the early stages of conjugation. Such cultures show that during the formation of thetube the nucleus passes from the middle of the cell to the side opposite the tube.(see text fig. 4).



Text fig. 4. Semi-diagrammatic sketches with camera lucida of Spirogyra varians (a) portion of filament pair showing position of nuclei in four cells (b) the same portion twenty-three hours later.

Exceptions to this statement have been noted in short celled filaments which have compact chloroplasts. In such cells it is impossible to observe the nucleus without staining since it is hidden by the chloroplast. Picronigrosin and dilute iodine have been found to be the best stains as they cause scarcely any shrinkage. Since they do not alter the position of the nucleus in cells where it can be observed in the living state, there is no reason to suppose that they alter its position in these short celled filaments. After staining such filaments it can be seen that when the tubes are fully grown, the most usual position of the nucleus is at the side of the cell which is opposite to the tube, but when the chloroplast has grown into the tube the nucleus is also frequently to be found there. It has never been observed in or near the region of the tube unless buried in the chloroplast, and thus as Czurda suggests it is probably carried there passively with the chloroplast (see text fig. 5).



Text fig. 5. Semi-diagrammatic sketch with camera lucida of a filament pair of Spirogyra varians stained with picronigrosin.

It might be contended that this unexpected position of the nucleus at the side farthest from the conjugation tube, is a sign of degeneration in the cell; however the condition has been observed in many cases and it has been attained while natural and vigorous growth of papillae is taking place. It also occurs in material which is observed immediately after collection from the native habitat. Moreover in those few cases where a nucleus is found near to the tube, the majority of the cells of that filament have the nucleus on the opposite side as though this were the natural position.

Size and Growth of Conjugating Cells.

S.weberi.Lloyd (8) states with regard to S.weberi that with very few exceptions the relative size of the male and female gametes is such that the male is smaller; he adds that "the exceptions show that the smaller size of the male is not essential to successful conjugation". S.weberi, as far as here investigated, does not conform to this rule; in the same filament pair the female cells may be longer or shorter than their partner male cells. The following are measurements, in the case of four pairs of conjugating filaments, of corresponding cells of the female filament (A), and the male filament (B). The lengths are given in microns.

S.weberi.

A	B	A	B	A	B	A	B
61.8	47.4	66	78	7611	47.4	59.5	40.5
56	52.3	52.3	76.1	64	76.1	40.5	64
64	56	52.3	71.3	59.5	76.1	50 odd	
61.8	52.3	59.5	66	56	69	35.6	73.6
						45.2	43
female cells longer.		female cells shorter.		female cells longer or shorter.		female cells longer or shorter.	

This variability in the relative size of the male and female cells is also shown by examination of zygote material. Unequal length of the gametes results in the production of odd, i.e. unpaired, cells in one or other filament or in both. Fifty-two filament pairs with zygotes were examined; fourteen had no odd cells, eleven had odd cells in both filaments, eighteen had odd cells only in the male filament, amd nine had odd cells only in the female filament. If the male cells were consistently shorter than the female cells, odd cells would be restricted to the male filament.

Both growth in length of cell, and cell division may occur after approximation of the filaments for conjugation. Only one of the filament pairs, whose cells were measured at the beginning of conjugation, was available for a further measurement when the filaments were separated by opposite papillae. Five pairs of cells were measured, in three the female showed the greatest growth, and in two the male. The measurements are given below and fourteen hours elapsed between measurement (1) and measurement (2)

S.weberi.

A(1)	A(2)	B(1)	B(2)
73.6	83	118	118
73.6	78	69	71.3
64	73.6	61.8	61.8
71.3	73.6	61.8	66
64	64	71.3	73.6

S.varians. In S.varians a similar variation in the relative size of male and female cells is found as the following tables will show. In each case A is the female filament and measurement (1) was made when the two filaments were in contact, and (2) when they were separated by conjugation tubes. Nine to twelve hours elapsed between the two measurements.

S.varians.

A(1)	A(2)	B(1)	B(2)
43	52.4	40.5	47.4
40.5	43	35.6	40.5
40.5	45.2	38	43
45.2	45.2	35.6	38
female cells longer.			

A(1)	A(2)	B(1)	B(2)
50	50	45.2	47.4
38	38	47.4	47.4
35.2	40.5	47.4	50
40.5	43	43	47.4
female cells longer or shorter.			

A(1)	A(2)	B(1)	B(2)
38	38	38	38
47.4	47.4	38	38
38	38	38	38
35.6	35.6	40.5	40.5
female cells longer or shorter.			

A(1)	A(2)	B(1)	B(2)
43	43	72	72
35.6	35.6	72	72
35.6	35.6	66	66
35.6	35.6	66	66
female cells shorter.			

A(1)	A(2)	B(1)	B(2)
45.2	50	56	56
45.2	47.4	80	80
47.4	47.4	78	78
47.4	50		
female cells shorter.			

A(1)	A(2)	B(1)	B(2)
43	43	71.3	71.3
35.6	35.6	71.3	71.3
35.6	35.6	66	66
35.6	35.6	66	66
female cells shorter.			

A(1)	A(2)	B(1)	B(2)
50	59.5	64	66
43	52.3	56	61.8
40.5	45.2	59.5	73.6
47.4	61.8	59.5	83
female cells shorter.			

A(1)	A(2)	B(1)	B(2)
50	50	40.5	40.5
40.5	40.5	40.5	40.5
45.2	45.2	40.5	40.5
50	50	40.5	40.5
female cells longer or equal.			

This variability in the relative size of male and female cells

is also shown by observations on zygote material. Thirty filament pairs with zygotes were examined, in eight there were no odd cells, in two there were odd cells in both filaments, in ten there were odd cells only in the female filament, and in ten there were odd cells only in the male.

S.cataeniformis.

Four or five cells and their partners were measured in sixty-five conjugating filaments, and in thirty-four of the filaments the longer cell of a pair occurred indifferently in either filament. Only eight filament pairs showed sexual differentiation, in two the female cells were shorter and in two they were longer, while in the other four sometimes the male, sometimes the female cell was the longer. These measurements are given below and in each case filament A is the female filament. This species was very difficult to keep under culture and no observations were made on growth during conjugation. Also, no observations were made on zygote material, since this was so scanty.

S.cataeniformis.

A	B	A	B	A	B	A	B
37.1	31.9	35.6	21	33.3	28.5	37.1	33.3
52.3	33.3		18.5 odd	35.6	35.6	35.6	37.1
43	30.9	35.6	21	35.6	35.6		21.4odd
43	30.9		18.5 odd	33.3	33.3	40.5	21.4
		35.6	16.6				21.4odd
		35.6	18.5			35.6	21.4
female cells longer.		female cells longer.		female cells equal or longer.		female cells longer and shorter.	
A	B	A	B	A	B	A	B
23.8	33.3	23.8	33.3	40.5	35.6	37.1	35.6
23.8	28.5	23.8	28.5	35.6	28.5	35.6	40.5
23.8 odd		23.8	30.9	33.3	33.3	40.5	35.6

23.8 30.9 21.4 28.5 female cells shorter.	23.8 odd 21.4 28.5 female cells shorter.	30.9 35.6 female cells longer and shorter.	43 33.3 female cells longer and shorter.

Thus in S.weberi, S.varians and S.cataeniformis, length of cell is not a sexual character. In the early stages of conjugation width is not a sexual character in S.weberi, where the filaments are of the same width. In S.varians, and S. cataeniformis, the filaments may be of a different width from the very beginning of conjugation. This is shown for S.varians in case 43 (see case 43 and Plate 1), but although the filaments were followed through conjugation, development ceased before the differentiation of female cells and the sex of the filaments was never known. Amongst vegetative material of S. cataeniformis, which was under observation from November 1927 to June 1929, a narrow type of filament frequently appeared. During observations on conjugation of this species, five filament pairs were noted in which one of the filaments was of the narrow type. In two cases this narrow filament was clearly the male as the other filament, of normal width at the cross walls, had developed the characteristic swollen female cells (see case 18). In neither of these cases were zygotes developed and it is not known whether these two types, the wide and the narrow, are varieties of the same species or whether they are different species, and their conjugation a hybridisation.

In S.varians, when growth of the cells occurred during conjugation, that of the female cells was not found to be regularly greater than that of the males. Czurda found that in S.setiformis the female filament became much twisted round the male during conjugation due to the fact that greater growth occurs in the female cells. No marked twisting of the filaments round each other has been seen in any of the three species here described; the conjugation tubes are usually orientated at right angles to the paired filaments and are not distorted.

These facts and the tables given above prove that greater growth in length of the cells is not confined to one filament of a pair.

Division during conjugation has been noted in all three species. In S.cataeniformis it has been observed in only two cases, in S.weberi in three cases (two of these showing division in both filaments), and in S.varians there was much more material available and division has been noted in twenty cases. In two of the twenty cases division was noted in both filaments but in eighteen it was only noted in filament A. There is the possibility that it may also have occurred in B and been unnoticed since at first only filament A was observed in detail. In three of the eighteen cases A later developed into the female filament and in three cases into the male, showing that this late division during conjugation is not concerned with sex.

Female Cells and Zygotes. In all three species a visible differentiation of sex occurred some time after the early stages of conjugation had been passed through. This differentiation involved a swelling of the female cells but in S.varians it was sometimes very slight and was merely a bulging out of the female cell towards the conjugation tube. In S.weberi and S. varians female cells were never differentiated until after tube formation, the shortest time being twenty-six hours in S,weberi. and twenty-two hours in S.varians, from the time the filaments of the pair were in contact. In S.cataeniformis female cells were sometimes differentiated when the filament pairs were still only united by papillae, but the shortest time was thirty-nine hours from the time the filaments were in contact. Zygotes were never formed in the cultures of S.cataeniformis; in cultures of S.weberi and S.varians they were occasionally formed but by the time the filament pairs had developed from an early stage of conjugation to the stage of protoplasmic fusion the cultures were stale.

CONCLUSION.

In the present account it has been shown that in three species of Spirogyra, S.weberi, S.varians and S.cataeniformis, the generally accepted view of the method of tube formation must be called in question. In two of these species, S.weberi and S.varians, this has already been reported by Czurda. It seems very probable that the new method ofdescribing tube formation may apply to all species of Spirogyra, especially since it has been found to take place in species which have been reported to become laced together in the "usual way". Hemleben (4) was the first to question the old story and he used S.crassa a species which de Bary evidently used, for he refers to it stating that it is difficult to culture for any length of time. In his experimental work on conjugation, Klebs used Spirogyra weberi, but he clearly did not observe early stages of conjugation similar to those reported by Czurda and in the present account. Lloyd, in a study on adhesions and geniculations, used, among other species, S.varians and he states that "during conjugation no adhesions were observed (except of course of the conjugation tube". Both in Czurda's paper and in the present account it has been shown that in S,varians adhesions play a prominent part in the early stages of conjugation.

Obviously if the new story of tube formation is accepted there must be some explanation why such a widely distributed and generally used plant as Spirogyra this method has so long remain undetected. Here are a few possible reasons why the early stages of conjugation have been so generally passed over.

1) Early stages may be quickly initiated and passed through in nature; thus in S.setiformis which Czurda used where conjugation is simultaneous in a wad, the early stages would be missed unless the wad was constantly under observation.

2) Early stages may be restricted to a set time of the day, Czurda has reported this to be the case in S.setiformis.

3) Early stages are not initiated after the material has

been removed from its native habitat. This is evident from the present work and had been previously observed by Czurda.

4) Indirect evidence as to the early stages of conjugation, drawn from observations of later stages, might give a mistaken impression. For instance in filament pairs laced together by conjugation tubes, the presence of opposite cells which have papillae which have apparently not yet met, would indicate the way in which the tubes have been formed in the rest of the filament pair, i.e. by opposite papillae which grow towards one another and meet. However in following through slide cultures of S.varians several of these opposite cells have been seen, and in each case the cells were formerly in contact, the separation of their papillae being brought about by the more rapid growth of the neighbouring papillae.

5) The examination of fresh material of conjugating Spirogyra is frequently most discouraging to the investigator, because the limits of the filament pairs are often almost completely hidden by a copious coating of detritus. During conjugation the filaments become sticky and if growing in water containing sediment they become coated with it. As may be imagined, it is difficult to see even mature conjugation tubes, and it is still more difficult to see the young papillae.

6) In culture, papillae may be put out irregularly with no relation to another filament. This has been seen in S.weberi and S.varians, and, in the absence of early stages, might be used as evidence for the generally accepted view of conjugation, the absence of the opposite papillae being explained as due to loss of the fellow filament in mounting.

7) It is true that occasionally cells which are not in contact may conjugate by papillae which grow towards each other and meet. This has been found in S.varians, but only rarely and in filament pairs which have elsewhere conjugated. This is possibly abnormal, but if early stages were being

deduced from later stages, one such case might be used to indicate the generally accepted view of conjugation.

8) Fixed material may always give evidence in support of the generally accepted view of conjugation. This has been shown to be the case when the three species here investigated were fixed in medium chrom-acetic acid. Whereas before fixation the material contained a number of filament pairs in contact, or slightly separated by small papillae, and no single filaments with papillae, after fixation the same material showed no paired filaments unless they were separated by conjugation tubes or larger papillae, but numerous single filaments with papillae of various sizes. However, part of the same material fixed in picronigrosin, still showed the early stages seen before fixation. This material, fixed in chrom-acetic acid, may then be interpreted as giving support to the generally accepted view of conjugation. Czurda has reported that he used medium chrom-acetic acid as a fixative and that every examination of living material was accompanied by fixation of some of this. He has not mentioned that the early stages of conjugation are destroyed by fixation, although amongst the species he used for his observations were two of the three species which have been used in this investigation, but his photograph of a portion of a conjugating wad indicates that even large papillae may become separated after fixation.

It is clear then that direct observations on the early stages of conjugation can only be made under special conditions. Czurda has stated that they can only be made on the spot, but it has been found possible to make such observations in the laboratory, immediately after collection if special methods of culture are used. In the papers of the older workers there is no indication that such methods have been used; de Bary's only mention of cultures is that wide species, such as S.crassa, are not suitable for observations on protoplasmic fusion, for they are not able to stand even a short culture on the slide. Had

the special manipulations been used that are necessary for the observation of such stages, they would surely have been mentioned. As Czurda has said the early descriptions of conjugation deal almost entirely with the later stages of this process: viz: protoplasmic fusion and zygote formation; the descriptions of the method by which the filaments become laced together are very short. There is no indication of the distance between the filaments when papillae are first formed, no drawings of this stage, and no records of the time taken for the production and growth of the papillae. In consideration of these facts, and of the great difficulty which would be encountered in mounting parallel filaments without displacement, it seems probable that the observations of these early workers on the beginnings of conjugation were not directly obtained, but were deduced from the later stages, or possibly from observations on fixed material.

SUMMARY.

Records of observations on early stages of conjugation in S.weberi, S.varians and S,cataeniformis are given.

Early stages of conjugation are only found in freshly collected material.

In all three species the filaments first lie in contact and are stuck together by a mucilage sheath.

Papillae are later put out from the cells of the filaments where they are in contact.

The first formed papilla may arise in either filament and is independent of sex. The second papilla arises at the place of contact of the first.

The two papillae are in contact from the first moment of their formation and by their growth in length the filaments are pushed apart.

The papillae become flattened on each other at their apices and conjugation tubes are formed, and the familiar appearance of the filaments laced together is attained.

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Key to the Symbols used in the following Tables.

Filaments closely in contact -(mucilage sheath very thin?)

Filaments in contact through mucilage (mucilage sheath wide?)

Filaments separated.

Filaments diverging.

Papilla from cell of filament A closely in contact with filament B.

Papilla from cell of filament B closely in contact with filament A.

Papilla in contact with opposite filament through mucilage.

Papilla separated from opposite filament.

Opposite papillae in contact.

Papillae flattened on each other forming a conjugation tube though the common wall is not necessarily dissolved.

N.B. with regard to symbols and tables.

(a) A may be the male or female filament; where sex is later differentiated it is noted in the tables.

(b) The numbers refer to the cells of filament A, not necessarily to those of B. Number 1 merely denotes the first cell observed.

(c) Each symbol refers to one cell in filament A. It may correspond to more or less than one cell of filament B.

(d) Odd cells are always noted in filament A and in S.weberi and S.cataeniformis they have also been noted in B. In S.varians they have been noted where drawings have been made and also in the follwoing cases: 70, 73, 75, 76, 79, 82, 84, 86, 87, 88, 89, 91, 92, 94, 96, 97,100, 101, 104.

(e) The size of the papilla varies and is not in anyway indicated by the symbol: a papilla indicated in the first column is often merely a slight swelling, in columns two amd three it may be a well developed protuberance.

(f) All drawings have been made with camera lucida; slight discrepencies in the drawings are due to the depth of the water that had to be focussed through.

(g) For drawings of S.cataeniformis and S.weberi Objectives $\frac{1}{3}$" and $\frac{1}{6}$" have been used with Ocular 4 giving approximate magnifications of 100 and 420 respectively.
For drawings of S.marians, a larger species, Objectives $\frac{1}{3}$" and $\frac{1}{6}$" have been used with Ocular 2 giving approximate magnifications of 60 and 250 respectively. In the case of drawings 1-37 the magnification has not been noted since various microscopes have been used the cultures being kept permanently on the stage.
With the following drawings a scale will be found giving the total magnification of the drawings made with camera lucida.
S.weberi drawings 1 and2.
S.varians drawing 60.
S.cataeniformis drawings 3 and 4.

(h) Division has been noted where it occurs.

(i) Filament C is another filament that may be found pairing with A.

(j) All the times given refer to Summer Time.

(k) The observations follwo on through the day and from day to day. The date is given where there may be doubt as to which day the time refers to

(l) Terms such as "dead", "free" indicate the condition of cells which have served as distinguishing marks in the filament pairs.

(m) Where there are no symbols (except in the last column) it may be assumed that observations have been rendered impossible by the overlapping of the filaments the condition of these cells may be deduced from that of neighbouring cells.

Cases 1-111.

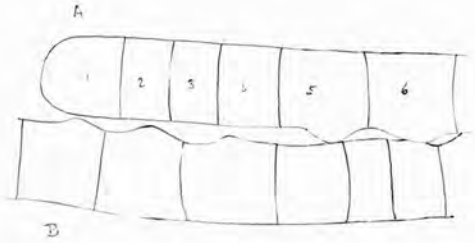
Tables of conjugation stages in

Spirogyra varians,

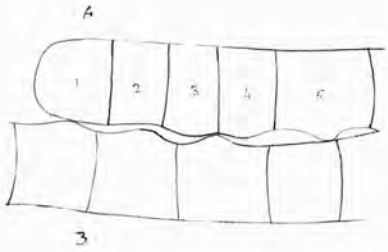
illustrated by drawings made

with the camera lucida.

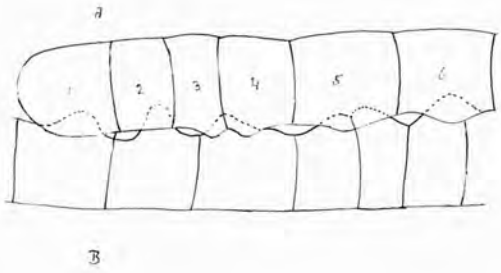
1.



9:20 A.M.



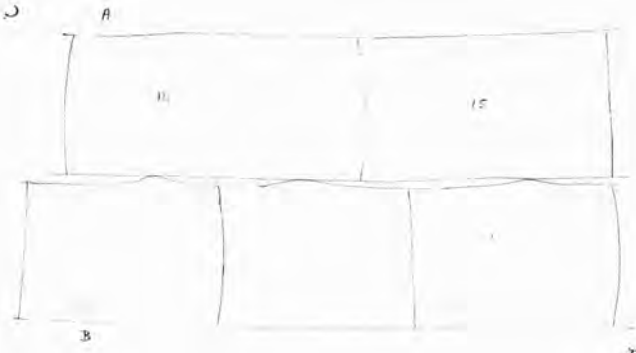
1:50 P.M.



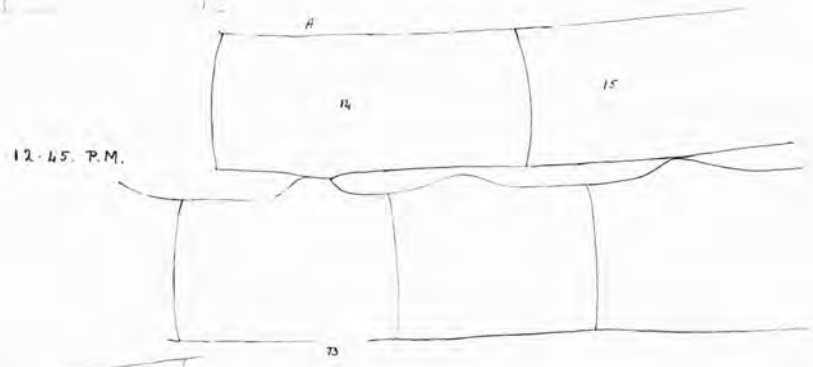
7:45 P.M.

No.		TIME OF OBSERVATIONS.			
		9.20.am.	7.45.pm.	9.15.am.	5.0.pm.
1. A	1.				A shews female cells
A♀	2.				
	3.	Odd.	Odd	Odd	
	4.				
	5.	Odd cell B.	Odd cell B.	Odd cell B.	
	6.				
		5.30.pm.	10.50.am.		
2. A	1-∞				
		6.20.pm.	10.0.am. 19.5.29.	11.0.am. 19.5.29.	
3. A	1.				
	2.				
	3.				
	4.				
	5.				
	6.				
	7.				
	8.				
	9.				
	10.				
		10.20.pm.	10.0.am.	11.30.am.	
4. A	1.	In division			
	2.				
	3.				
	4.				
	5.				
	6.				
	7.				
	8.				
	9.				
	10.		A conjugating with filament C of 2 cells.	A conjugating with filament C of 2 cells.	
	11.				
	12.				
	13.				
	14.				

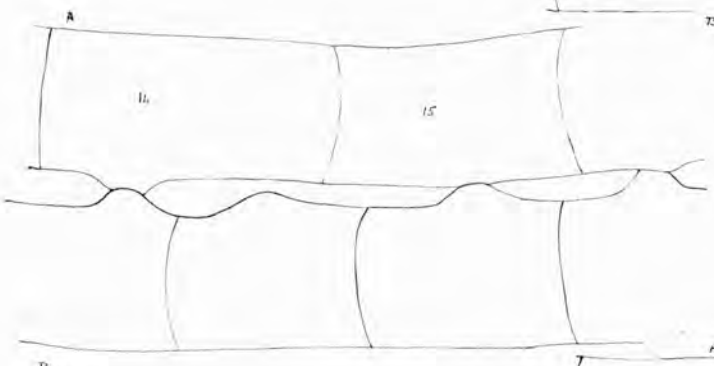
5



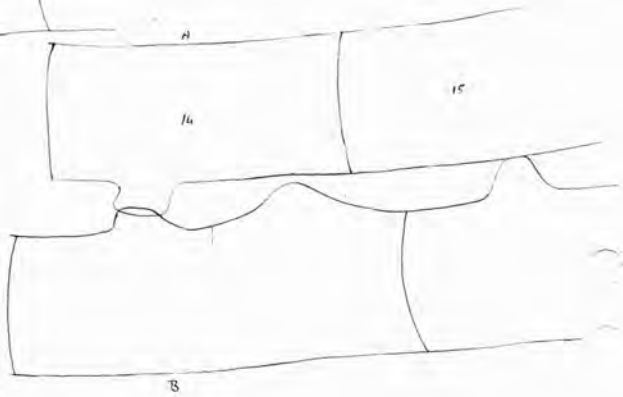
9.35. A.M 30. 5. 29.



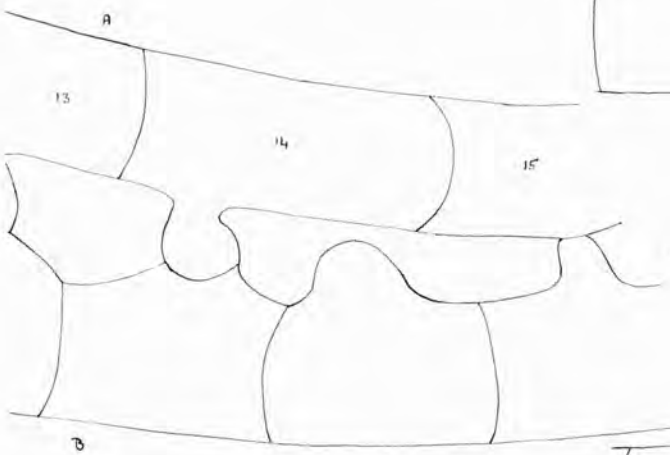
12.45. P.M.



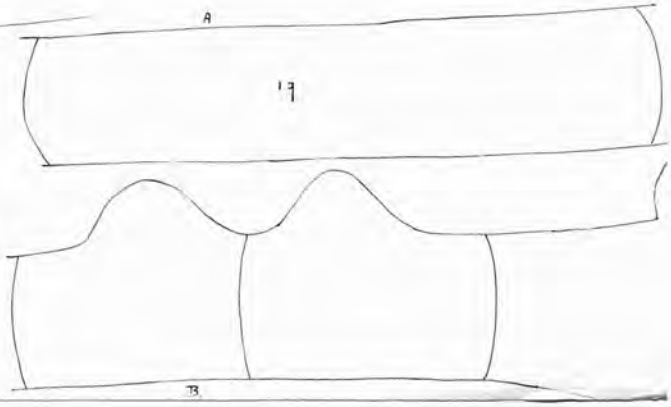
1.50 P.M.



5.10 P.M.

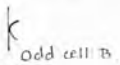
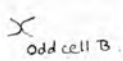
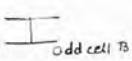
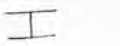
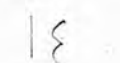
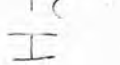


2. P.M 31. 5. 29.

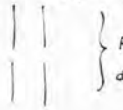
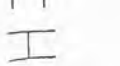
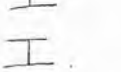


2 P.M. 31.5.29

No. TIME OF OBSERVATIONS.

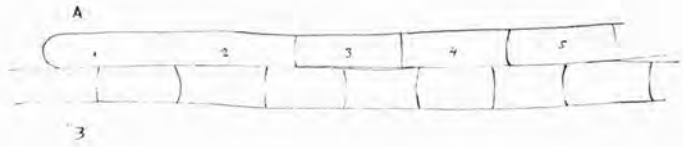
	9.35.am.	1.50.pm.	5.10.pm.	9.30.am.
5. A 14.	 Odd cell B	 Odd cell B.	 Odd cell B.	B shows female cells
B ♀ 15.				
16.				
17.				
18.				
19.				
20.				

	9.35.pm.	10.0.am.
6. A 1.		
2.		
3.		
4.		dying
5.		"
6-∞		"

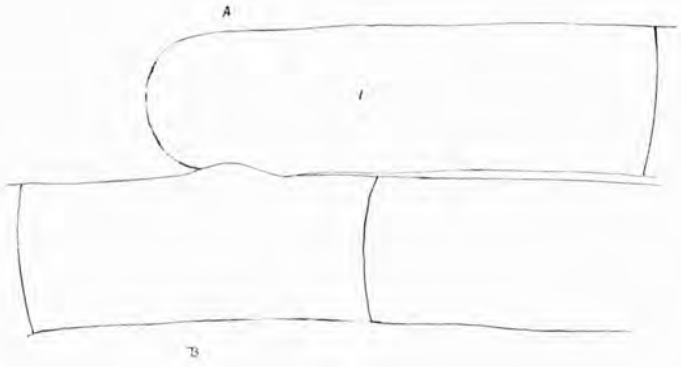
	11.0.pm.	9.0.am. [18.5.29.]	9.0.am. [19.5.29.]
7. A 1.			 A1 has divided.
2.			
3.			
4.			
5.			
6.			
7.			

	10.0.pm.	11.15.am.
8. A 1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

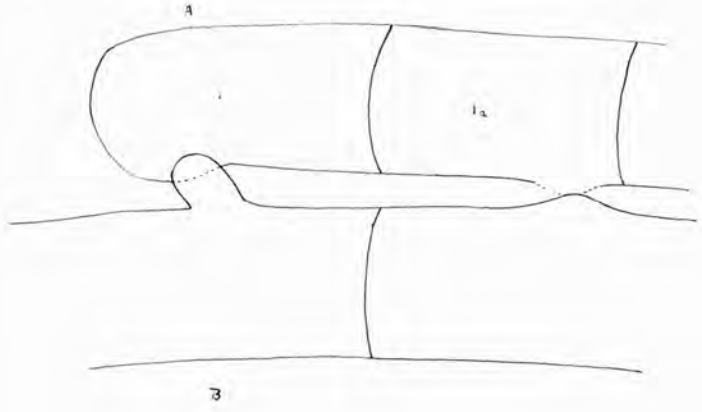
9.



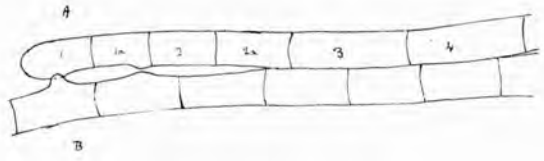
9.35. AM.



10 35. P.M

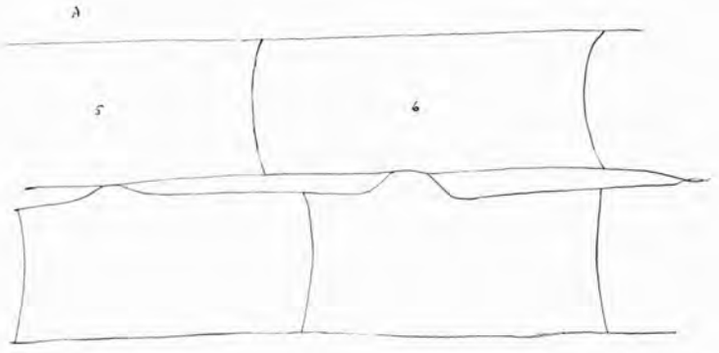


8. P.M.

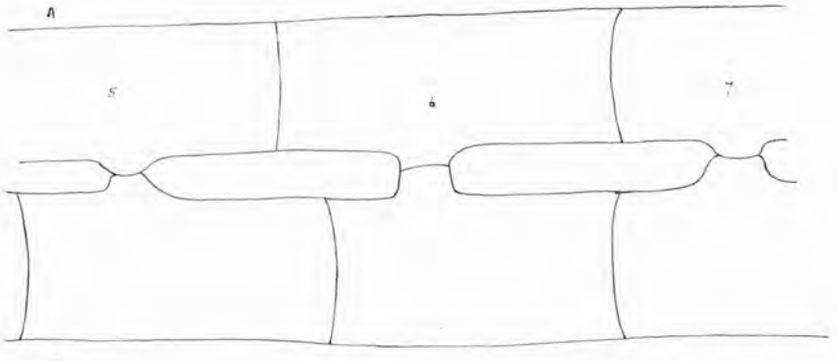


8 P.M.

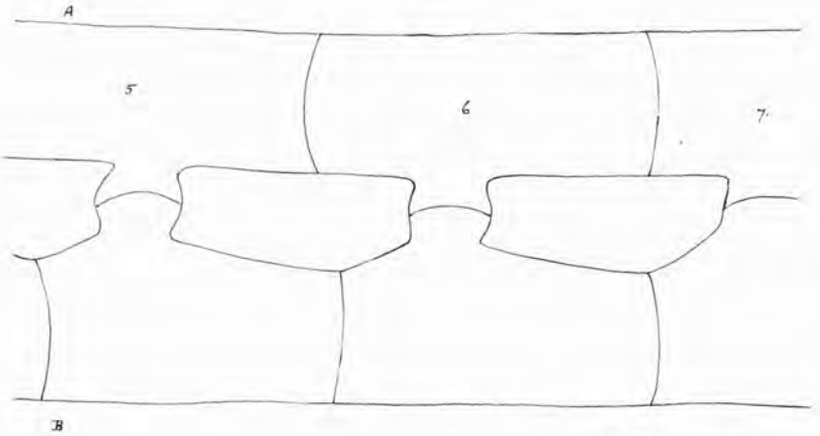
No.	TIME OF OBSERVATIONS,			
	9.35.am.	12.45.pm.	3.45.pm.	9.30.am.
			×	(1) 
9. A 1.		 A, dividing	division complete	(1.) 
2.		 A_2 dividing	division complete	
3.				
4.				
5.				
	10.15.pm. 19.6.29	11.50.am. 20.6.29	12.5.pm. 21.6.29	
10. A 1.			5 zygotes in A.	
A♀ 2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13-∞				
	10.20.pm. 19.6.29	11.20.am. 20.6.29.	12.10.pm. 21.6.29	
11. A 1.			5 zygotes in B	
B♀ 2.				
3-8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17.				
18.				



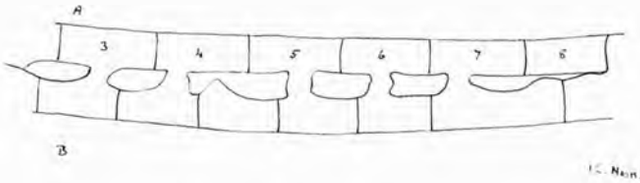
10. A.M. 30. 5. 29.



12.30 P.M. 30.5.29.

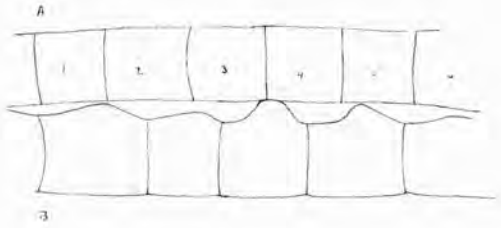


12. Noon. 31. 5. 29.

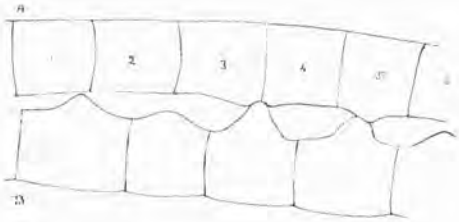


12. Noon. 31. 5. 29.

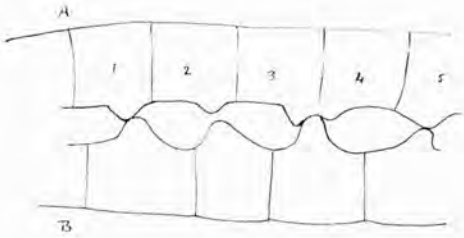
No.		TIME OF OBSERVATIONS.				
		10.0.am.	11.0.am.	2.0.pm.	5.0.pm.	q. 30. A.M
12. A	1.					B shows
B ♀	2.					female cells.
	3.					
	4.	odd cell B	odd cell B.	odd cell B.	odd cell B.	
	5.					
	6.					
	7.					
		10.30.pm.	12.5.pm.			
13. A	1.					
	2.					
	3.					
	4.					
	5.					
	6.					
	7.					
		10.45.pm.	12.10.pm.			
14. A	1.		dying			
	2.					
	3.					
	4.					
	5.					
	6.					
	7.					
	8.					
	9.					
	10.					
	11.					
	12.					
	13.					
	14.					



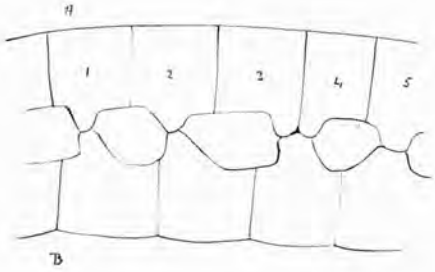
11. A.M.



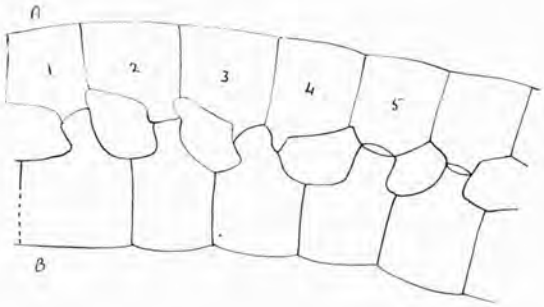
2 P.M.



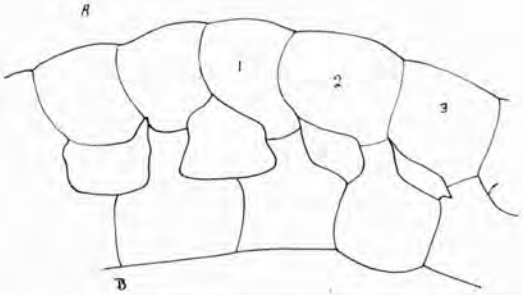
4. P.M.



6.30 P.M.
30.5.29.

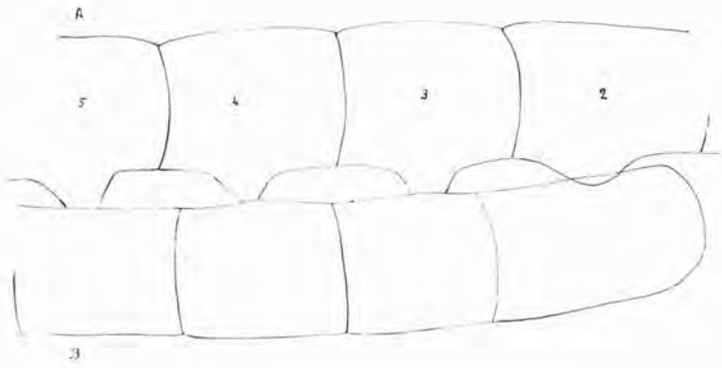


11. A.M.
31 5. 29.

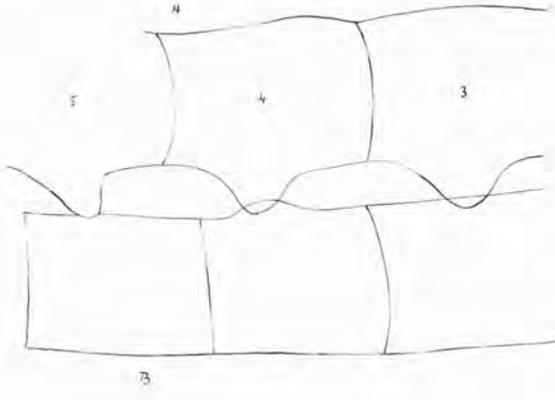


12 Noon
3.6.29.

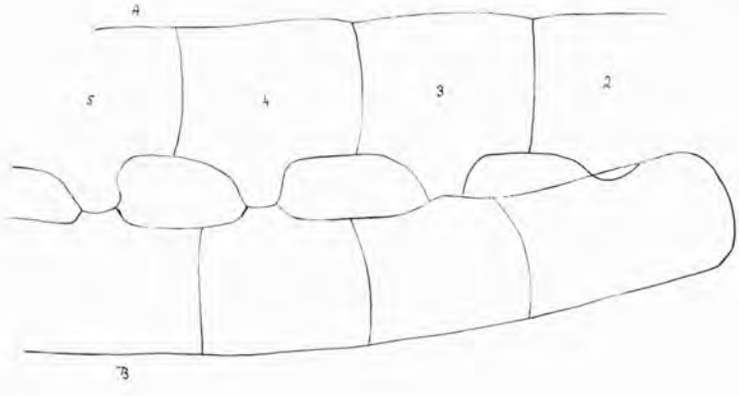
No.		TIME OF OBSERVATIONS.			
		11.0.am.	2.0.pm.	4.0.pm.	9.30.am.
15. A 1.					 A shows female cells.
A♀	2.				
	3.				
	4.				
	5.				
	6.				
		10.30.pm.	12 Noon.		
16. A 1.					
	2.				
	3.				
	4.				
	5.				
	6.				
	7.				
	8.				
	9.				
	10.				
	11.				
	12.				
		10.45.pm.	12.5.pm.		
17. A 1.					
	2.				
	3.				
	4.				
	5.				
			A6 has divided.		
	6.				
	7.				
	8.				
	9.				
	10.				
	11.				



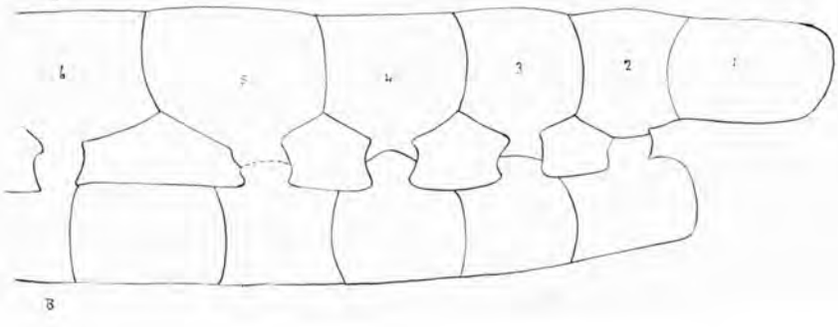
2.45 P.M. (30.5.29)



4.45 P.M

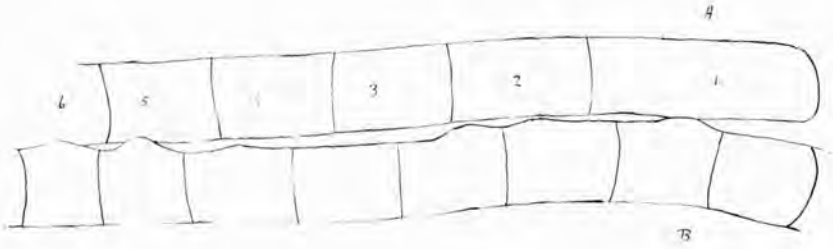


6.15 P.M.

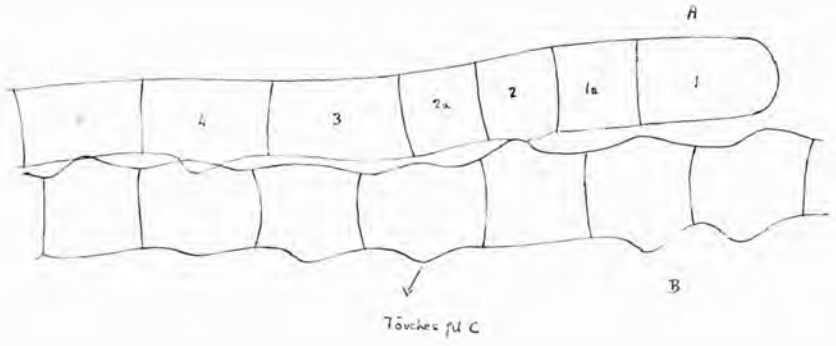


9.6.29.
12 Noon.

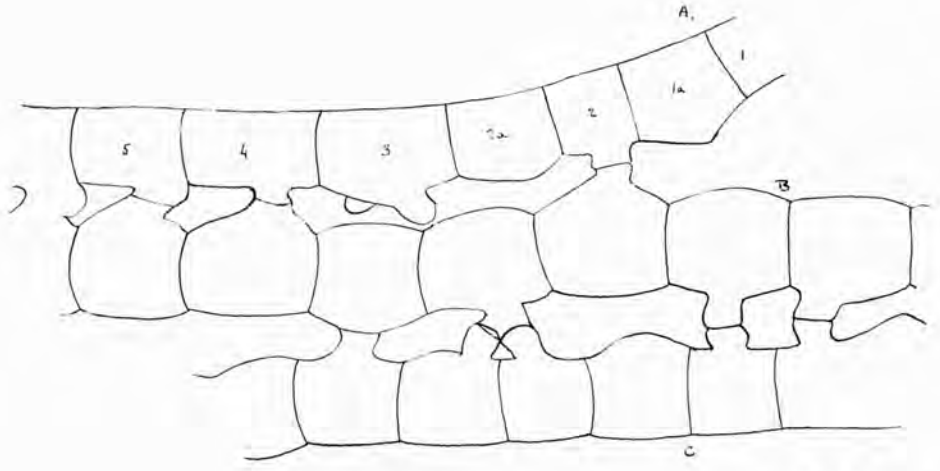
No.		TIME OF OBSERVATIONS.			
		2.45.pm.	4.45.pm.	6.15.pm.	9.15.am.
18. A	1.	Free	Free	Free	Free.
A♀	2.				 A shows
	3.				 female cells.
	4.				
	5.	 Odd cell 7.	 Odd cell 3	 Odd cell 13.	 Odd cell 13.
	6.				
	7.				
		11.0.pm.	2.0.pm.		
19. A	1.				
	2.				
	3.				
	4-8.				
	9.				
	10.				
	11.				
	12-24.				
		11.0.pm.	2.0.pm.		
20. A	7.				
	8.				
	9.				
	10.				
	11.				
	12.				
	13.				
	14.				
	15.				
	16.				
	17.				
	18.				
	19.				
	20.				



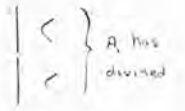
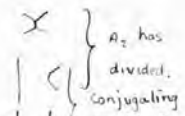
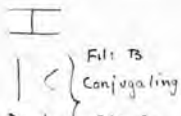
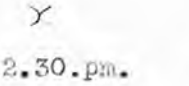
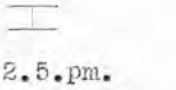
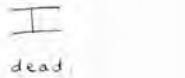
4 . P.M. 30. 5. 29.



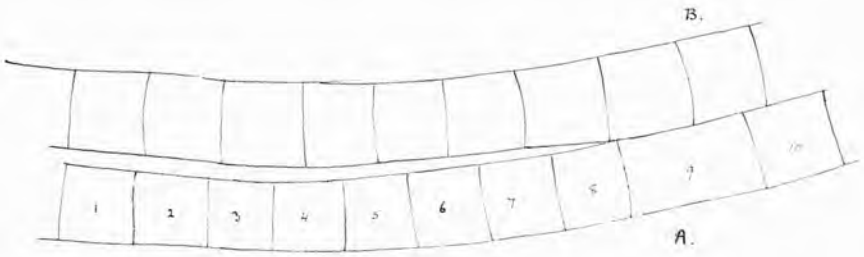
9 . P.M.



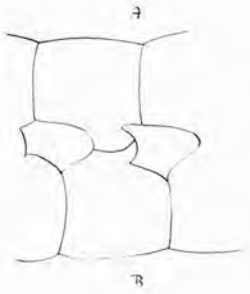
11 . 30 A.M. 2. 6. 29.

No.		TIME OF OBSERVATIONS.			
		4.0.pm.	9.0.pm.	9.30.am.	11.30.am.
21.			 A₁ has divided	 Fil: B conjugating with Fil: C	B shows female cells.
B♀	A 1.		 A₂ has divided. Conjugating w: fil: C	 Fil: B Conjugating w. fil: C.	
	2.				
	3.				
	4.				
	5.				
	6.				
		11.30.pm.	2.30.pm.	12.5.pm.	
22.	A 1.			6 Zygotes in B.	
B♀	2.				
	3.				
	4.				
	5.	dead	dead		
	6.				
	7.				
	8.				
	9.				
	10.				
	11.				
	12.				
	13.				
	14.				
		11.0.pm.	10.30.am.		
23.	A 1.				
	2.				
	3.				
	4.				
	5.				
	6.		Odd.		
	7.				
	8.				
	9.				
	10.				

24.

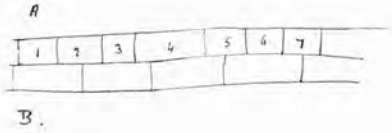


4. P.M 30. 5. 29



6. PM. 26. 29

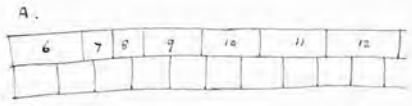
26.



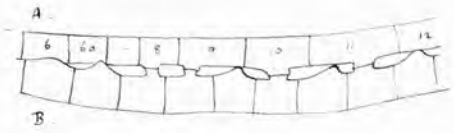
6. 10. P.M 30. 5. 29.

9:45. A.M. 31. 5. 29.

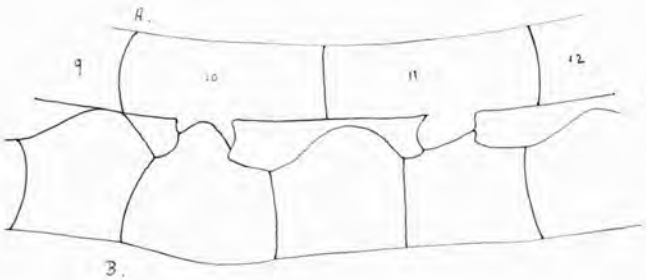
No.		TIME OF OBSERVATIONS.			
		4.0.pm.	5.30.pm.	2.0.pm.	9.0.am.
24. A	1.	 A still in division			B shows female cells.
B ♀	2.				
	3.				
	4.				
	5.				
	6.				
	7.				
	8.		Odd	Odd.	
	9.		 Aq has divided.		
	10.				
		11.45.pm.	2.30.pm.	10.0.am.	
25. A	1.			Zygotes in B.	
B ♀	2.				
	3.				
	4.				
	5-9.				
	9-16.				
	17.				
	18-24.				
	25-26.				
	27-∞	or			
		6.10.pm.	9.45.am.	9.50.pm.	
26. A	1.				
	2.				
	3.				
	4.				
	5.				
	6.				
	7.				



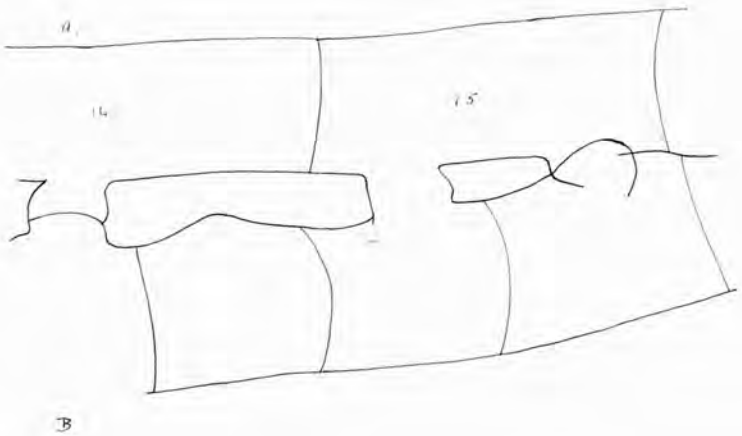
7. 20. 1911. 30. 5. 23



10. A.M. 31. 5. 29.

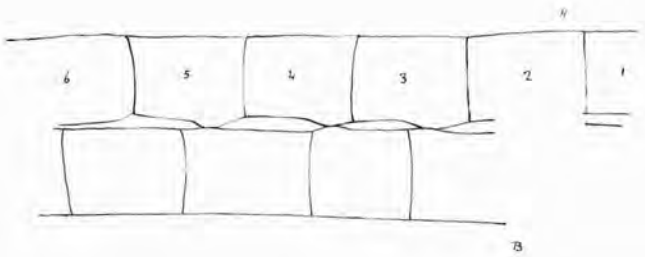


9. A.M. 1. 6. 29.

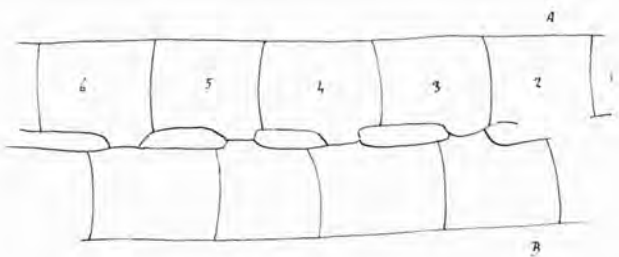


12 Noon.

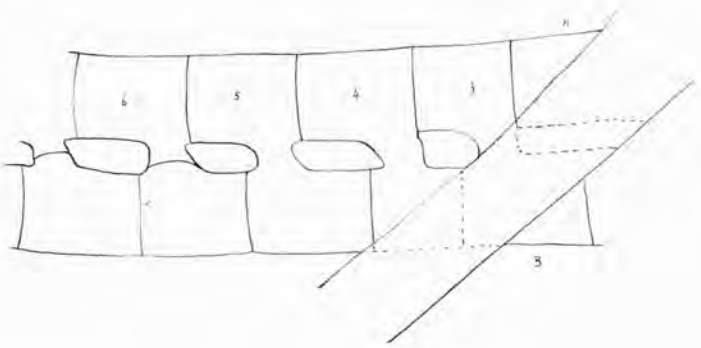
No.		TIME OF OBSERVATIONS.			
		7.30.pm.	8.40.pm.	9.30.pm.	10.0.am.
27.					
	A 6.		A6 has divided.		
	7.			Odd	Odd.
	8.				
	9.				Odd cell B
	10.		Odd cell B	Odd cell B	Odd cell B
	11.		Odd cell B	Odd cell B	Odd cell B.
	12.	Odd cell B	Odd cell B	Odd cell B	Odd cell B.
	13.	Odd cell B		Odd cell B	Odd cell B
	14.			Odd cell B	Odd cell B
	15.	Odd cell B			
		3.30.pm.	9.0.am.	9.0.am.	
28.	A 1.			2 Zygotes in A.	
A♀	2.				
	3.				
	4.				
	5.				
	6.				
	7.		dead.		
	8.				
	9.				
		11.30.pm.	11.0.am.		
29.	A 10.				
	11.				
	12.				
	13.				
	14.				
	15.				
	16-19.				
	20.				
	21.				
	22.				
	23.				



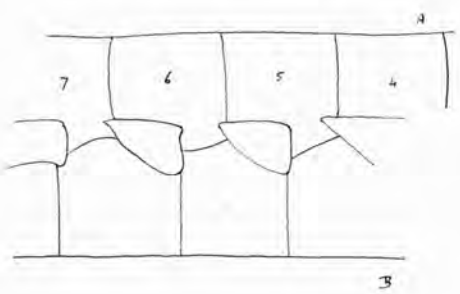
2. 30. P.M. 1. 6. 20



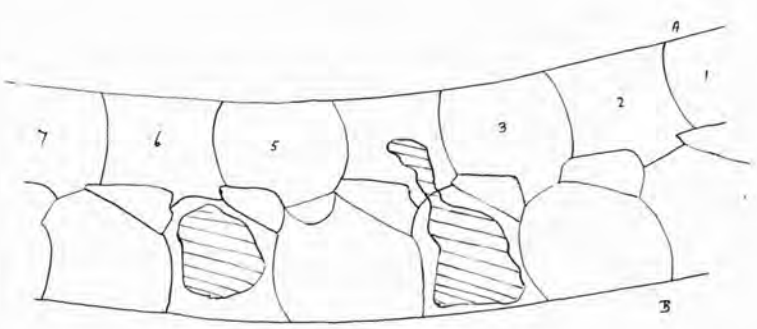
4. P.M.



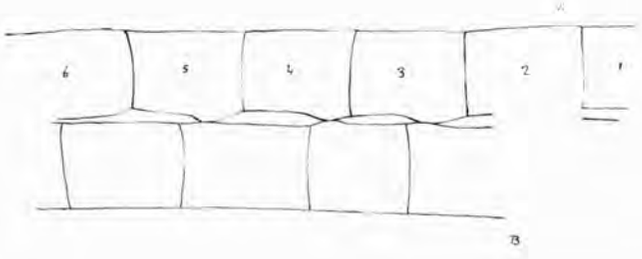
7. P.M.



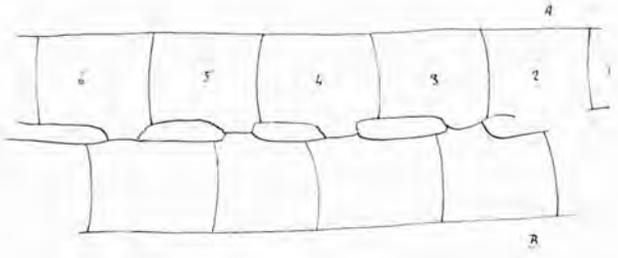
12. Nov. 2. 6. 20.



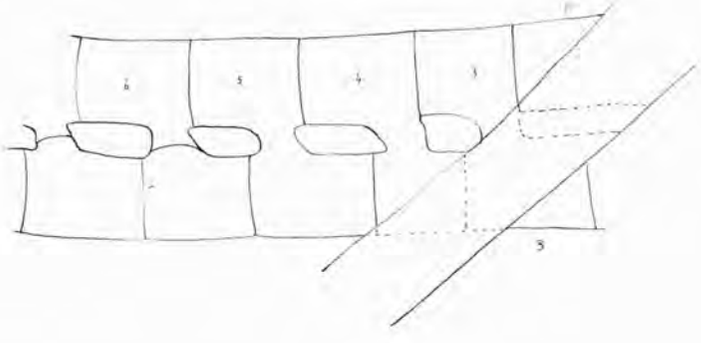
9. A.M. 3. 6. 29.



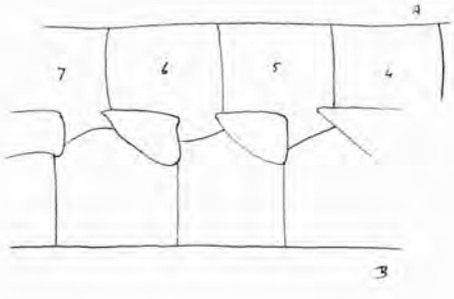
2.30. P.M. 1.6.29



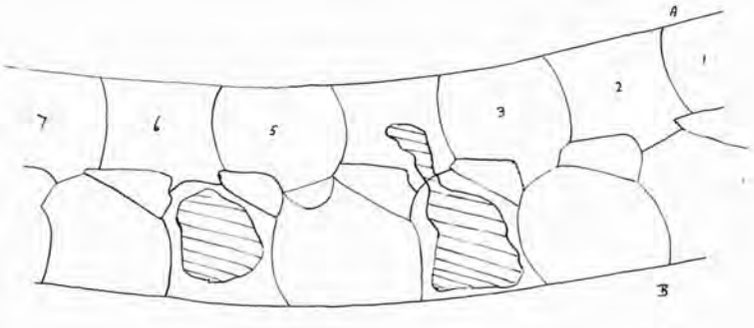
4. P.M.



7. P.M.

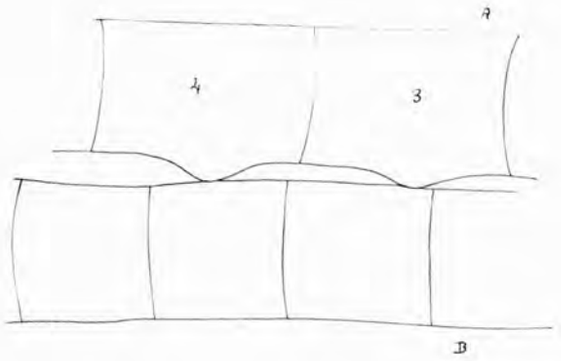


12. Noon. 2. 6. 29.

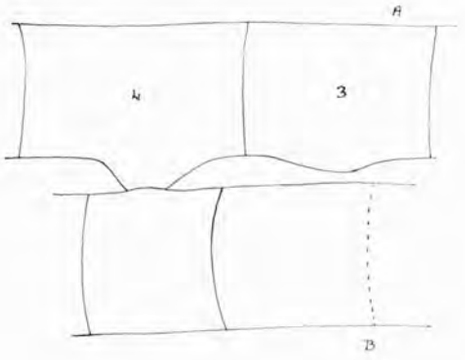


9. A.M. 3. 6. 29.

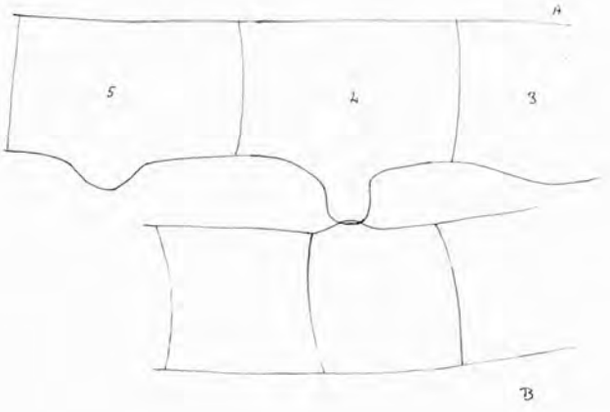
No.	TIME OF OBSERVATIONS.			
	2.30.pm.	4.0.pm.	7.0.pm.	12.0.Noon.
30. A 1.				B shows female cells
B ♀ 2.				
3.				
4.				
5.				
6.				
	10.0pm.	9.30.am.		
31. A 1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.		dead.		
11.	dividing			
12.				
13.		Odd.		
14.				
15.				
16.				
	9.15.pm.	9.30.am.	9.0.am.	
32. A 1-9.			Dead.	
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17.				
18.				
19.				



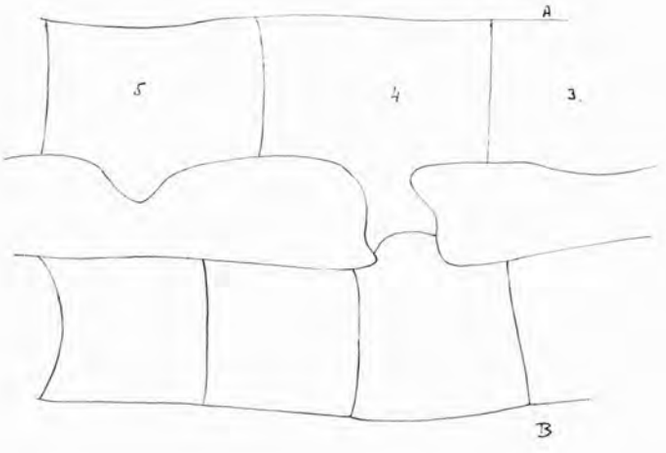
6. P.M. 1. 6. 29.



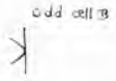
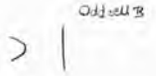
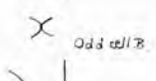
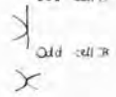
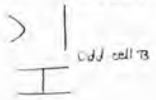
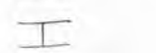
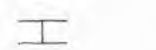
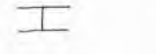
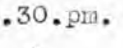
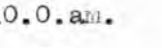
8. P.M.



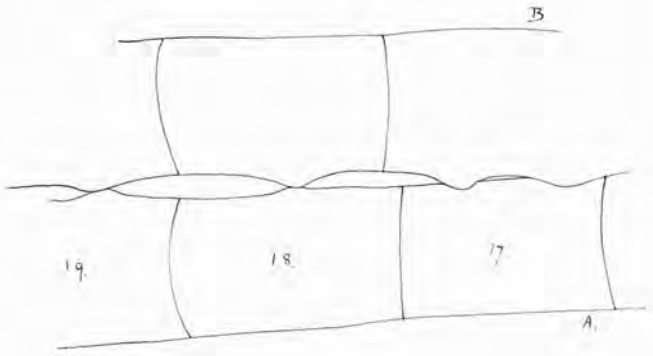
11. P.M.



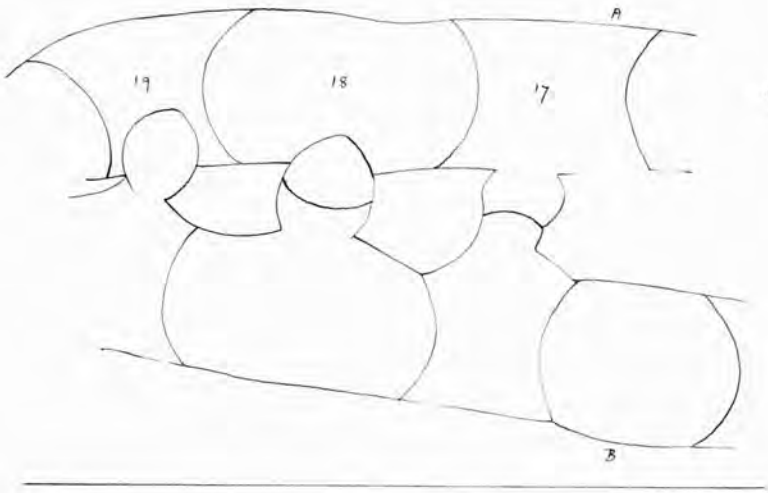
10-40. AM. 2. 6. 29

No.	TIME OF OBSERVATIONS.		
	6.0.pm.	11.0.pm.	10.40.am.
33. A 1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
	8.30.pm.	9.30.am.	9.30.am.
34. A 1.			B is the female
B ♀ 2.			filament for
3.			11 cells show
4.			partial fusion
5.			
6.			
7-			
	9.30.pm.	10.0.am.	
35. A 1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12-			
	11.30.pm.	11.15.am.	
36. A 1.			
2.			
3.			
4-			

37.

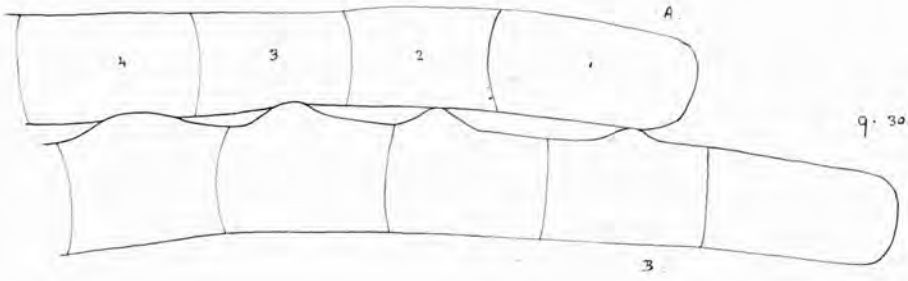


6.25 P.M. 1.6.29

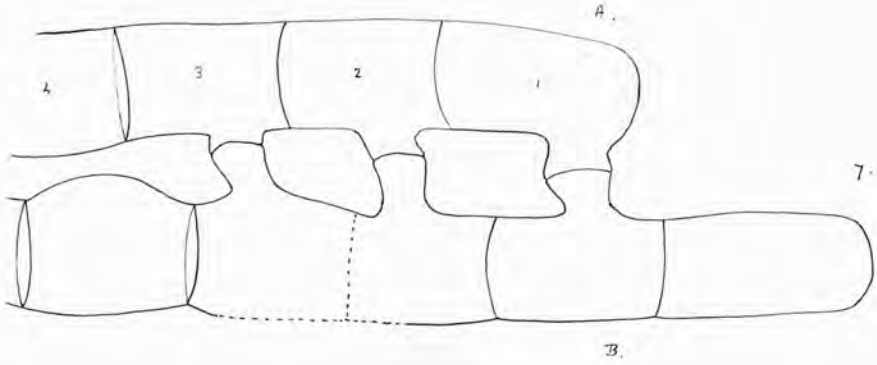


9.4 M. 3.6.29.

39.

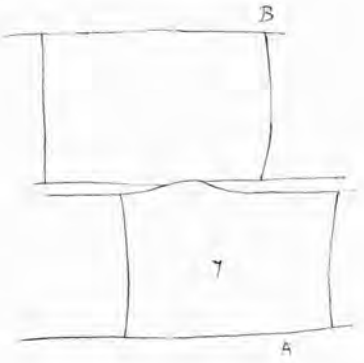


9.30 P.M.
16.5.29

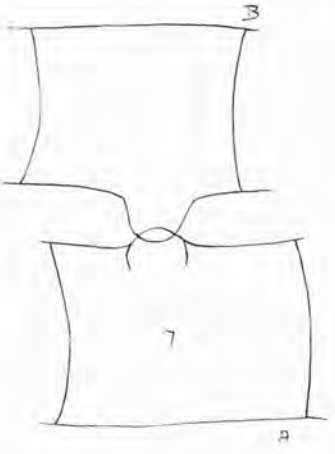


7.30. A.M.
17.5.29.

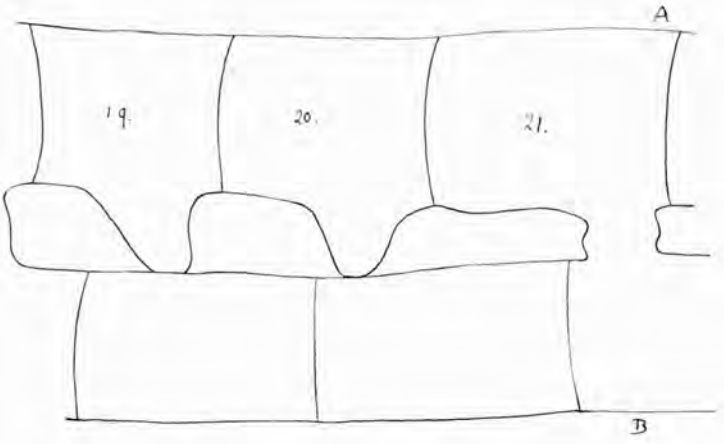
No.		TIME OF OBSERVATIONS.		
		6.25.pm.	11.0.am.	9.0.am.
37. A	11.			B shows female cells.
B♀	12.			- attempted fusion
	13.			
	14.			
	15.			
	16.			
	17.			
	18.			
	19.			
		9.45.pm.	11.0.am.	9.30.pm.
38. A	1.			B shows female cells.
B♀	2.			Zygotes in B.
	3.			
	4.			
	5-10.			
	11.			
	12.			
	13.			
	14.			
	15.			
	16.			
	17-			
		9.30.pm. 16 5.24	7.30.am. 17.5.24	2.25.pm. 18 5.24
39. A	1.			B shews female cells
B♀	2.			
	3.			
	4.			
	5.			
	6.			
	7.			
	8.			



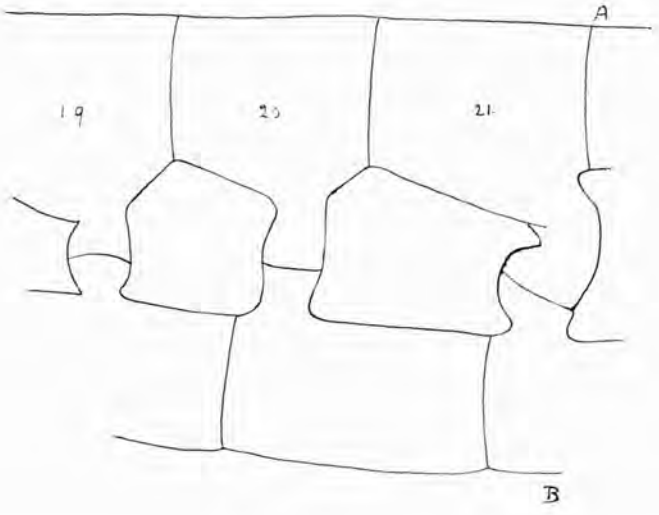
9.25 P.M. 30.5.29



9.9 P.M. 31.5.29



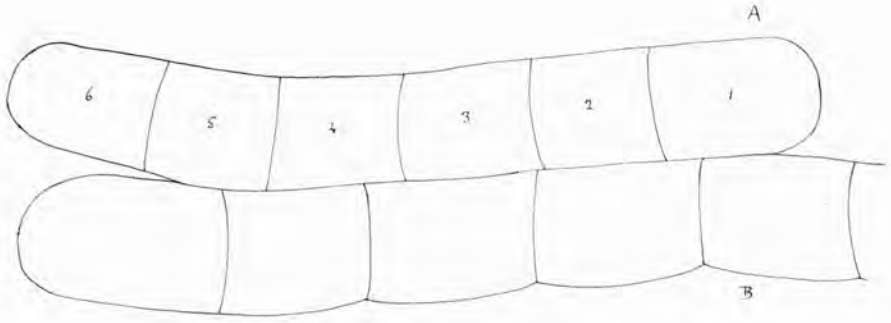
12.30 P.M. 3[illegible].29.



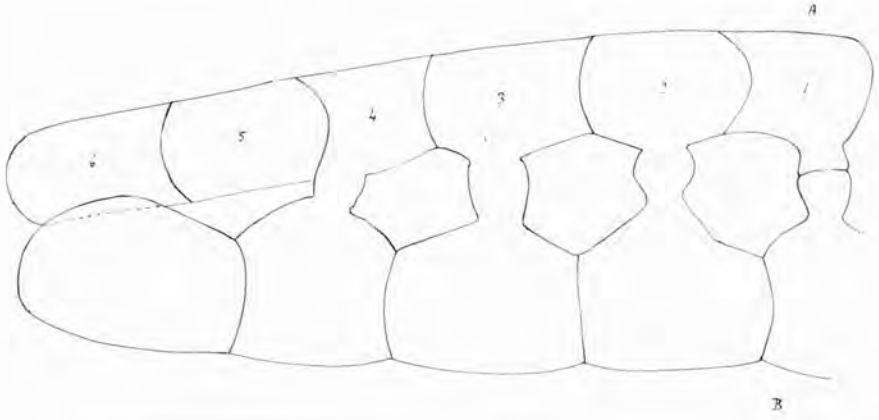
6. P.M. 2.6.29

No.		TIME OF OBSERVATIONS.			
		5.20.pm.	8.30.pm.	10.15.pm.	8.30.pm.
40.	A.1.				B shows female cells.
B ♀	2.	A and B A In division			
	3.				
	4.	A and B A In division			dead
	5.				dead
	6.	A In division			
	7.				
	8.	A In division			
	9.				
	10.	A In division			
	11.				
	12.				
	13.				
	14.				
	15.				Odd
	16-18.				
	19.				
	20.				
	21.				
	22.				
	23.				
	24.				
		10.30.pm.	10.0.am.		
41.	A 1-8.				
	9.				
	10.				
	11.				
	12.				
	13.				
	14.				
	15.				
	16.				
	17.				
	18.				
	19-29.				

42

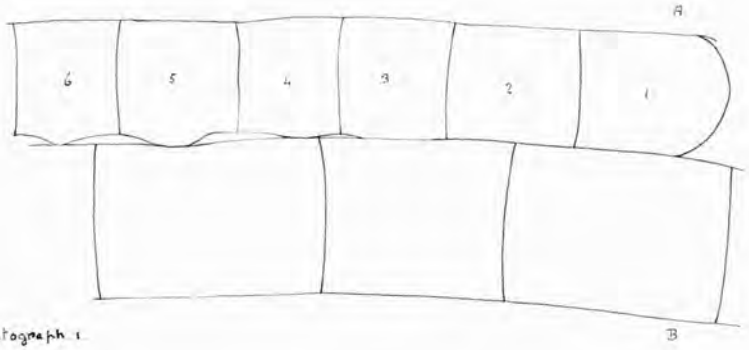


9.45. P.M.
18.5.29.



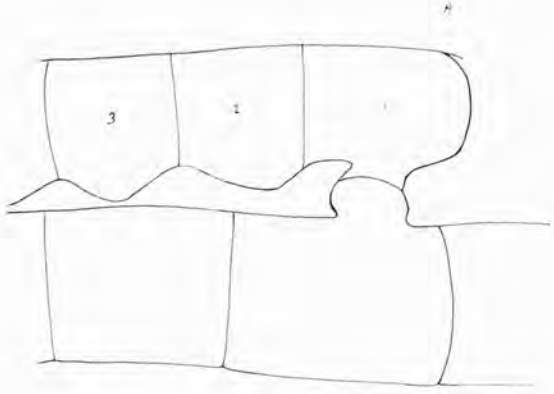
2 P.M.
18.5.29

43



8.20.P.M.
17.5.29.

Cf Photograph 1

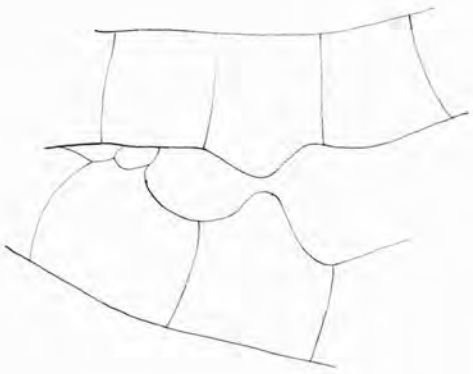


8.A.M.
18.5.29.

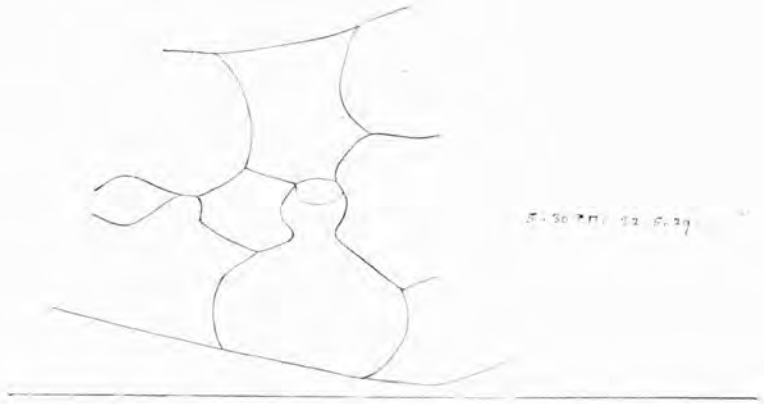
Cf Photograph 3

No.	TIME OF OBSERVATIONS.		
	9.45.pm.	9.0.am.	2.0.pm.
42. A 1.			
2.			
3.			
4.			
5.			Odd.
6.			
	8.30.pm.	7.30.am.	
43. A 1.			
2.		odd)	
3.			
4.		odd.)	
5.			
6.			
7.			
8.			
9-			
	11.30.pm.	3.30.pm.	9.30.am.
44. A 1.			4 Zygotes in B.
B♀ 2.			
3.			
4-9.			
10.			
11.			
12-13.			
14.			
15.			
16.			
17-21.			
22.			
23.		Odd	
24.			
25.			
26.			

25.

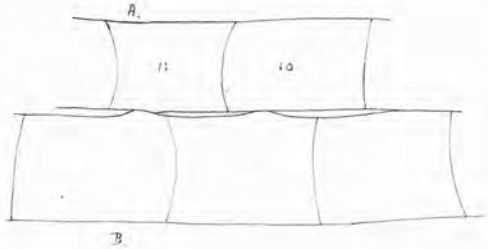


11:30 A.M. 24 Sq.

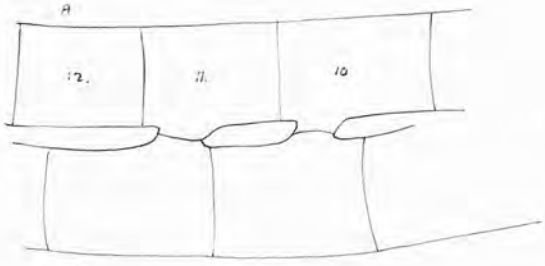


5:30 P.M. 12 Sq.

26



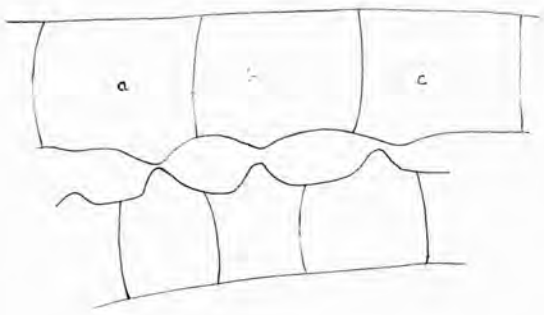
8 P.M.



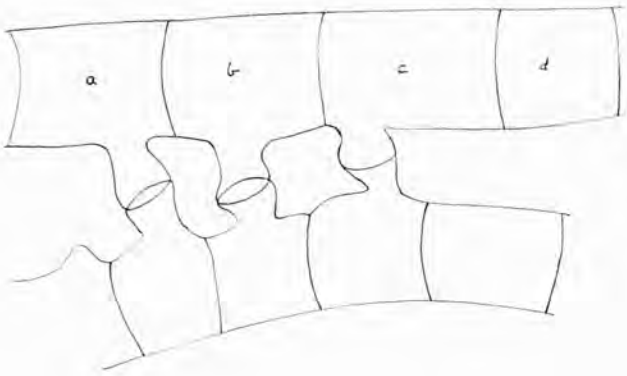
10.45 P.M.

No.	TIME OF OBSERVATIONS.	
	11.30.am.	5.30.pm.
45. A		
1.		
2.		
3.		
4 etc.		
	8.0.pm.	10.0.am.
46. A 1-9.		
10.		
11.		
12.		odd.
13.		
14-17.		
18.		
19.		
20.		
	11.45.pm.	11.15.am.
47. A 1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
	10.0.pm.	11.15.am.
48. A 1.		
2.		
3.		
4- ∞		

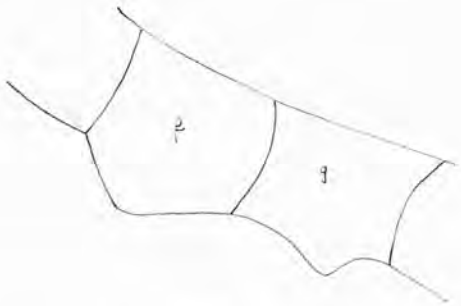
49.

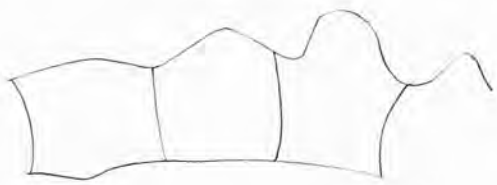


8. P.M. 17.5.29

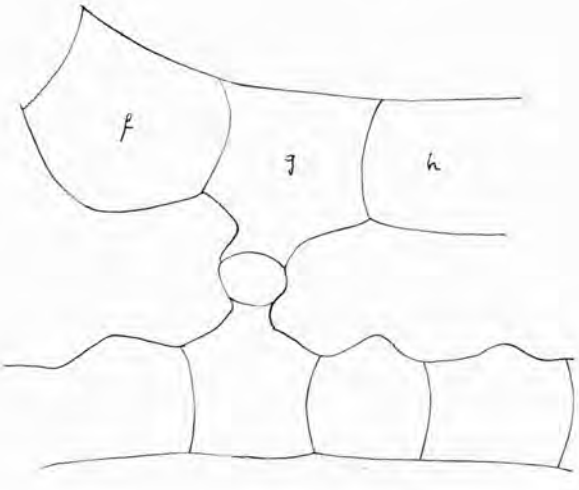


10.30 A.M. 18.5.29.





10.30. 18.5.29.



11.A.M. 19.5.29.

No.	TIME OF OBSERVATIONS.		
	8.0.pm.	10.30.am.	11.0.am.
49. A 1-∞			
a			
b			
c			
d			
e			
f			
g			
h-			
i-∞			
	11.20.pm.	3.15.pm.	9.30.am.
50. A 1.			Zygolë in B
B ♀ 2.			Zygolë in B
3.			Zygolë in B
4.			
5.			Zygolës in B.
6.			Zygolë in B
7.			Zygolë in B.
8.			Zygolë in B
9.			
10.			
11.			Zygolë in B
12.			
13.			Zygolë in B.
14.			
15.			
16.			
	10.0.pm.	9.30.am.	
51. A 1.			
2.			
3.			
4-∞			

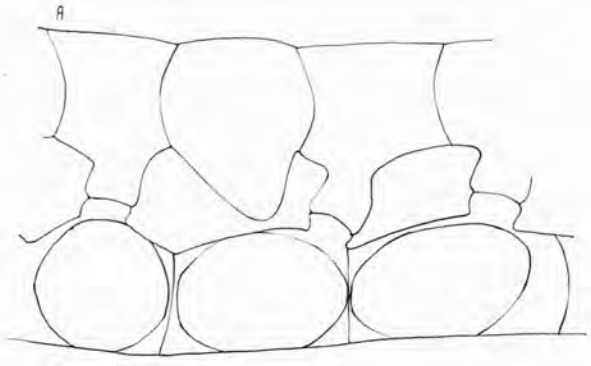
B shows female cells

52.

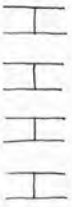
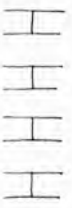
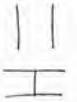
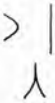
9.12 M

9.20 A.M

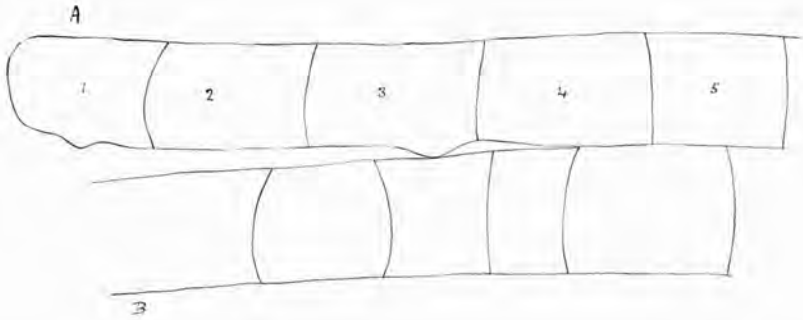
53.



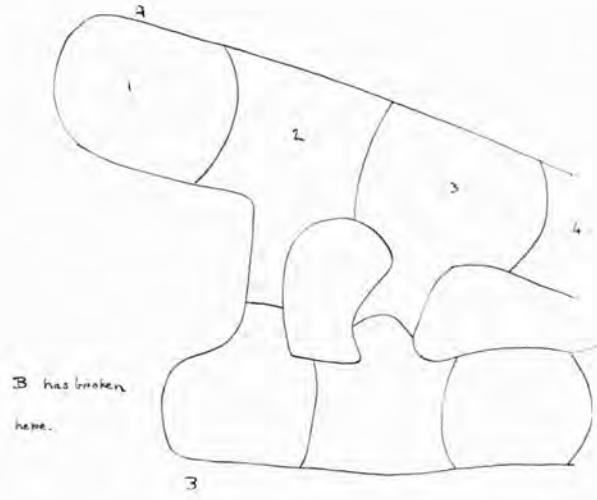
3.

No.	TIME OF OBSERVATIONS.		
	9.0.pm.	9.30.am.	
52. A 1.			
2.			
3.			
4-∞			
	11.15.pm. 20.5.29.	10.40.am.	5.30.pm. 22.5.29.
53. A 1.			Zygotes in B- no odd cells
B♀. 2.			Odd cells in A wheel Bulge.
3.			B shows female cells.
4-∞			
	9.10.pm.	12.5.pm.	9.0.am.
54. A 1-9.			Dying.
10.			
11-13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			
21.		dead.	
22.			
23.			
24.			
	10.15.pm.	11.30.am.	
55. A 1.			
2.			
3.			
4-∞			
	10.15.pm.	11.30.am.	
56. A 1.			
2.			
3.			
4-∞	or.		

57



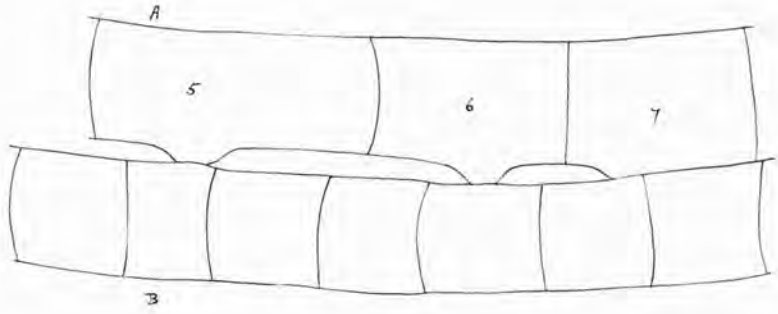
10 PM 17.5.20

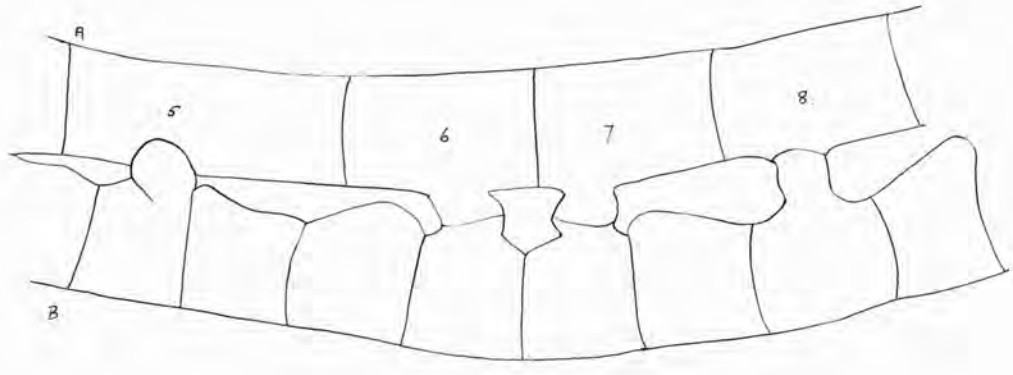


B has broken here.

10.30 PM 17.5.20

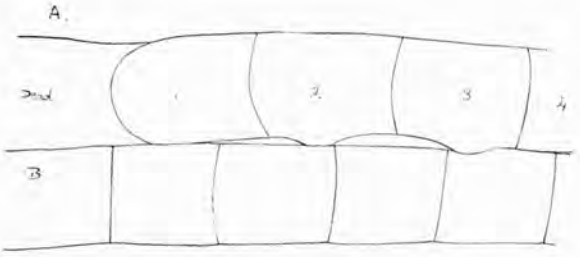
58



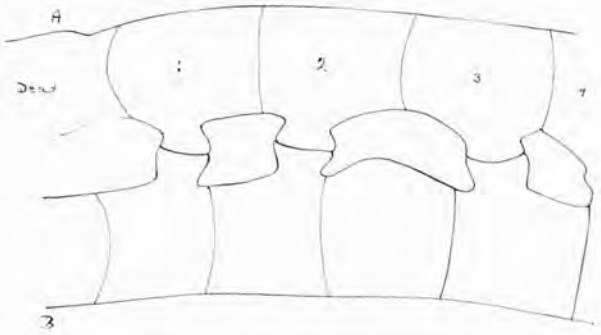


No.	TIME OF OBSERVATIONS.		
	10.0.pm.	9.30.am.	10.30.am.
57. A 1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
	10.0.pm.	9.30.am.	
58. A 1.			
2.			
3.			
4.			
5.			
6.			
7.			
8,			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
	8.0.pm.	9.0.am.	
59. A 1.			
2.			
3.			
4- ∞			

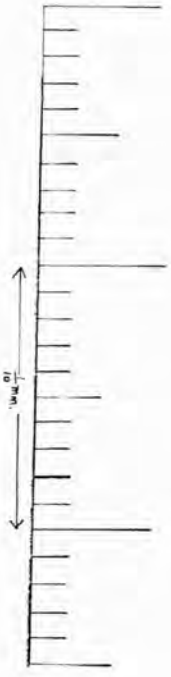
60.

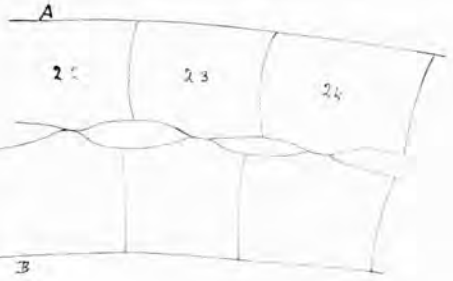


10-30 PM 18.5.29

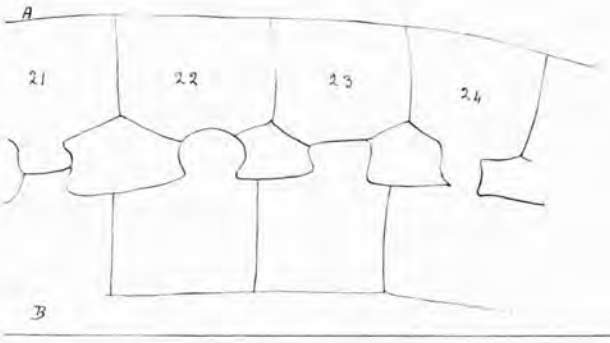


12 Noon 19.5.29

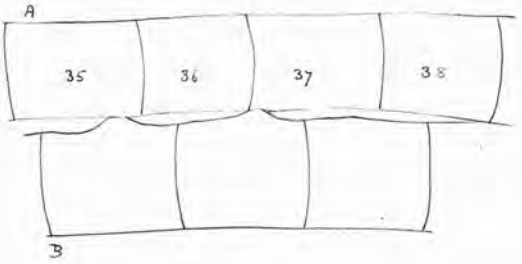




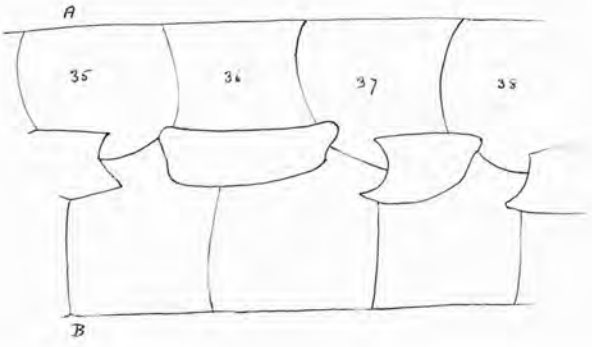
10-30 PM 18.5.29



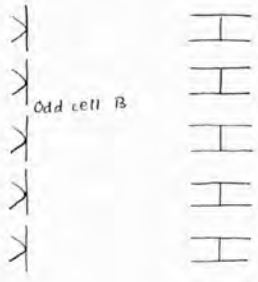
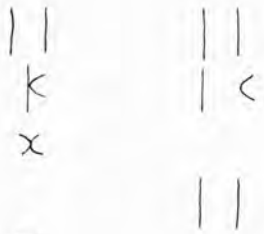
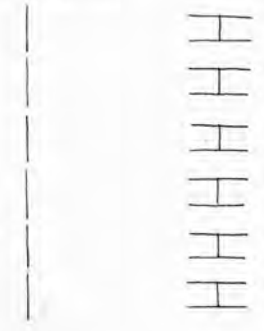
12 Noon 19.5.29



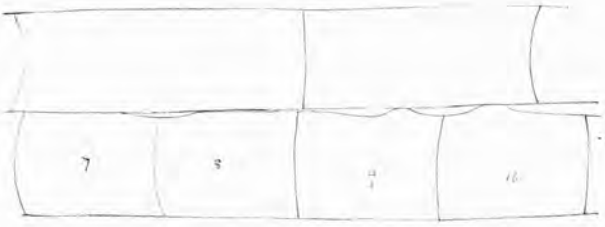
10-30 PM 18.5.29.

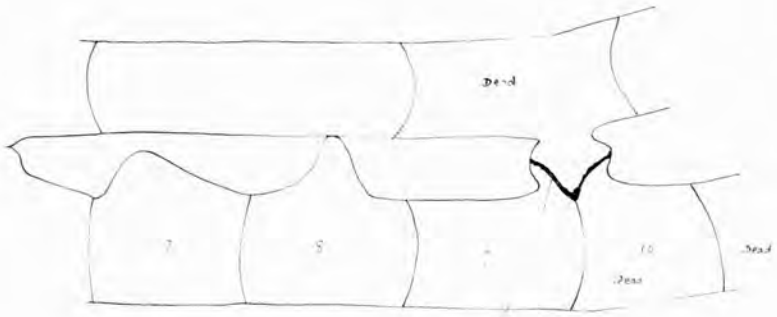


12 Noon 19.5.29.

No.	TIME OF OBSERVATIONS.	
	10.30.pm.	12.5.pm.
60. A 1.		
2.	Odd cell B	Odd cell B.
3.		
4.		
5.		
6-19.		
20.		
21.		
22.		
23.		
24,		
25.		
26.		
27-34.		
35.		
36.		
37.		
38.		
	10.30.pm.	1.0.pm.
61. A 1-8.		
9-10.		
11.		
12.		
13.		
14.		
15.		
	11.15.pm.	2.0.pm.
62. A 1.		
2.		
3.		
4.		
5.		
6-∞		

63

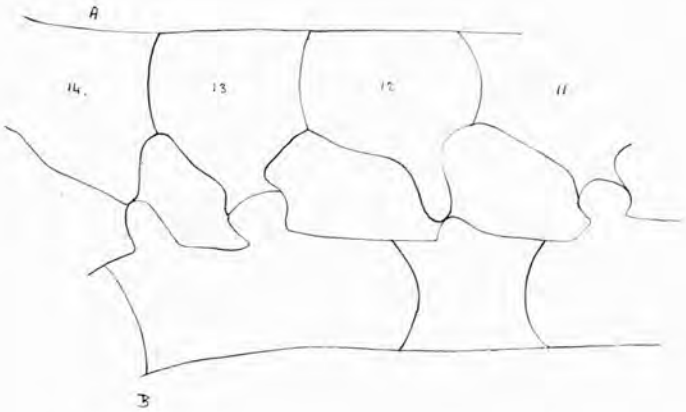




cf Photograph 10.

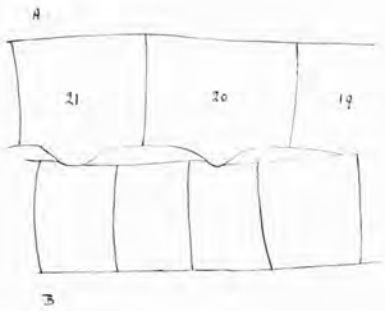
Both walls dissolved

64

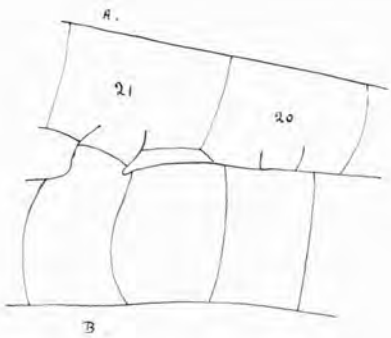


No.		TIME OF OBSERVATIONS.		
		8.0.pm.	9.30.am.	
63.	A 1.			
	2.			
	3.			
	4.			
	5.			
	6.			
	7.			
	8.			
	9.			dead.
	10.			
		10.30.pm.	1.0.pm.	2.45.pm.
64.	A 1.			
A ♀.	2.			
	3.			Zygolē in A.
	4.			Zygolē in A
	5.		Odd	Odd
	6.			Phm ie Fusion stärling
	7.			Partial fusion.
	8.			
	9.			
	10.			Zygolē in A.
	11.			
	12.			
	13.			
	14.			
	15.			
		8.15.pm.	9.15.am.	
65.		Not yet divided	A_1 divided	
	A 1.			
	2.			
	3.			
	4.			
	5.			
	6.			
	7.			

66

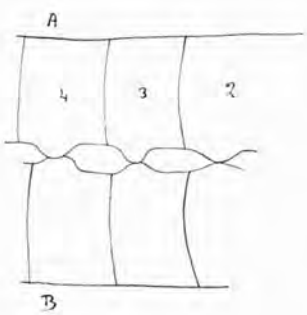


9.15. P.M

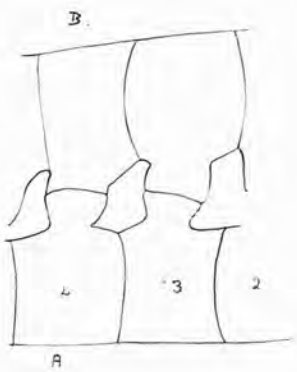


10. A.M.

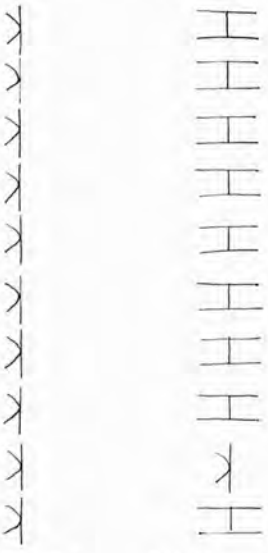
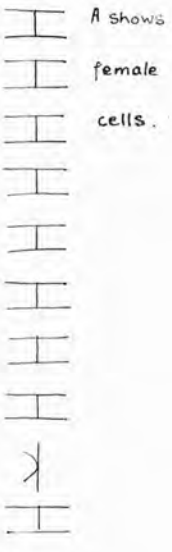
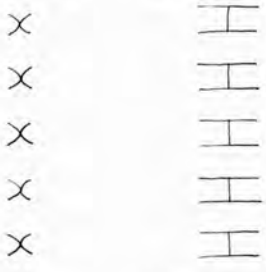
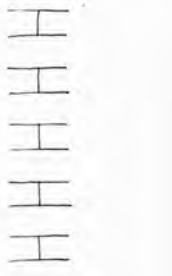
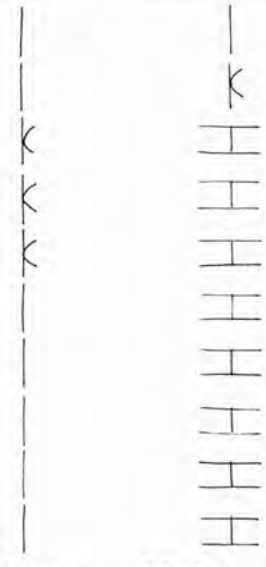
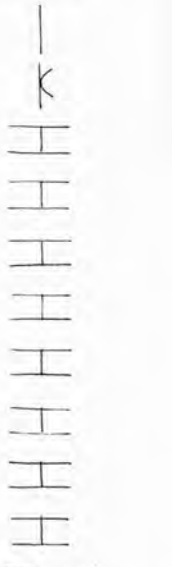
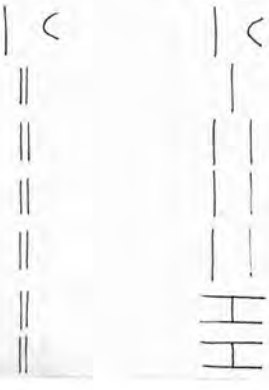
67



9.15. P.M.

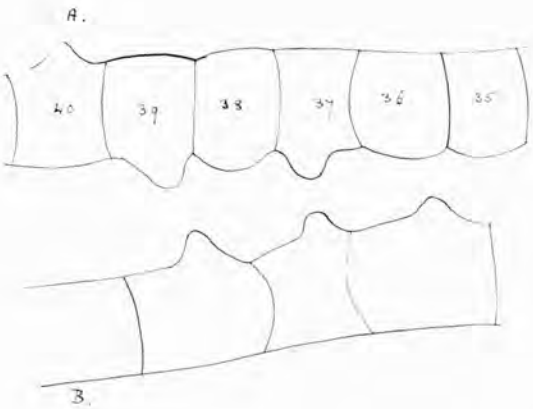


10. A.M.

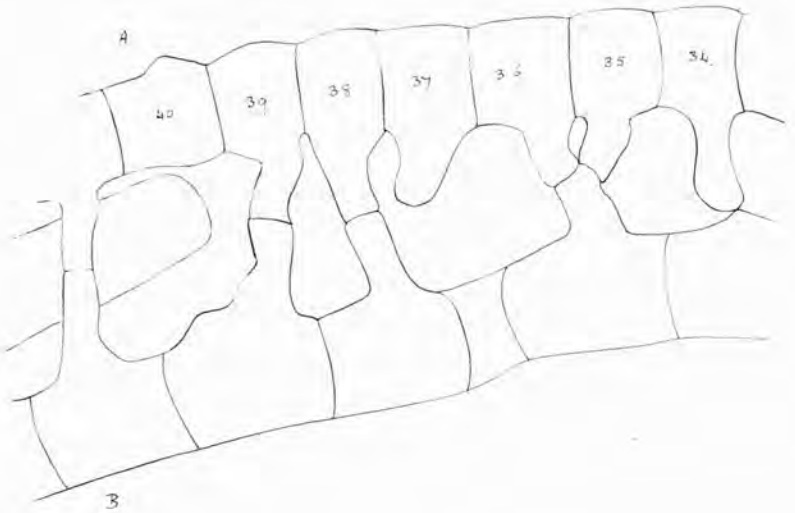
No.	TIME OF OBSERVATIONS.	
	9.15.pm.	10.0.am.
66. A 1.		
A♀ 2.		
3.		
4.		
5-19.		
20.		
21.		
22.		
23.		
24- ∞		
	9.15.pm.	10.0.am.
67. A 1.		
2.		
3.		
4.		
5- ∞		
	9.15.pm.	10.0.am.
68. A 1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
	10.0.pm.	12.30.pm.
69. A 1-4.		
5.		
6.		
7.		
8.		
9.		
10.		

A shows female cells.

70.

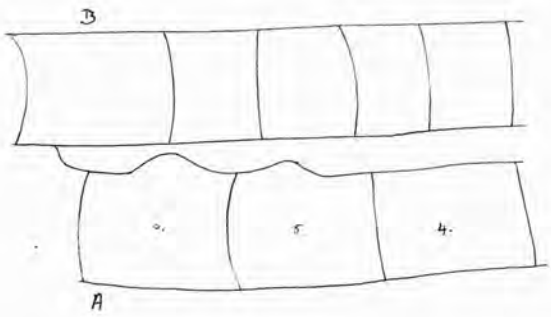


9.30 P.M.

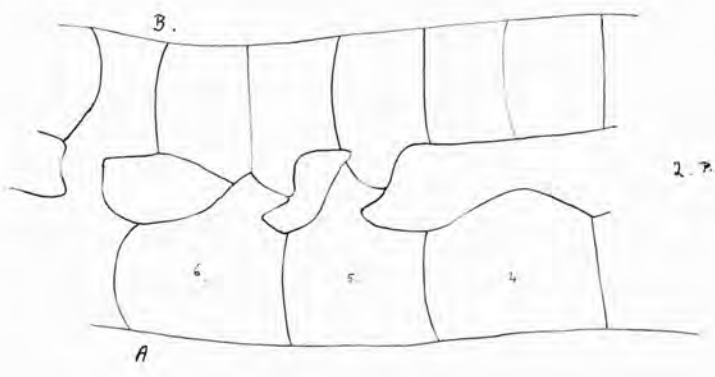


10.3[illegible] P.M.

71.



10.15 P.M.



2 P.M.

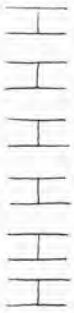
No.		TIME OF OBSERVATIONS.		
		8.15.pm.	11.25.am.	9.0.am.
70. A ♀	10-13.	‖	H	A shows female cells
	14-16.		H	
	17-20.		H	
	21.		H	
	22.		Odd	
	23-24.		H	
	25-26.	> |	H	
	27.	> |	H	
	28.	> |	H	
	29.	> |	H H — A_{29} has divided	
	30.	>	Odd	
	31.	> <	H	
	32.	> <	H	
	33.	Odd	Odd	
	34.	> <	H H — A_{34} has divided	
	35.	> |	⫫	
	36.	>		
	37.	> <	Odd >	
	38.	>	H	
	39.	> <	H	
	40.	| >	| >	
	41.	> <	H	
	42.	> <	H	
	43.	> <	H	
	44.	> <	H	

A & B united by tubes many cells further on in filament.

		10.15.pm.	2.0.pm.
71. A	1.	|	| |
	2.	| |	H
	3.	| |	> |
	4.	| |	> |
	5.	> |	H
	6.	> |	H
	7.	>	H

No.		TIME OF OBSERVATIONS.		
		10.15.pm.	1.0.pm.	
72.	A 8.			
	9.			
	10.			
	11.	Odd 	Odd 	
	12.			
		10.50.pm.	9.0.am.	9.0.am.
73.	A 11.			B shaws female cells.
B ♀	12.			
	13.			
	14.			
	15.			
	16.	 Odd cell B	 Odd cell B.	
	17.			
	18.			
	19.			
	20.	 Odd cell B	 Odd cell B	
	21.			
	22.		Odd 	
	23.			
	24.			
		11.30.pm.	3.15.pm.	9.0.am.
74.	A 1.			
	2-4.			
	5.			
	6.			
	7.			Odd.
	8.			
	9.			
	10.			
	11.			
	12.			
	13.			

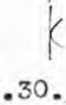
No.	TIME OF OBSERVATIONS.			
	5.45.pm.	6.45.pm.	8.50.pm.	10.30.pm.
75. A 1.				
2.				
		A3 has divided		
3.				
4.				
	6.40.pm.	9.0.pm.	10.30.pm.	11.0.pm.
76. A 1.	 still in division			A shows female cells.
A ♀. 2.		Odd	Odd	
3.				
4.				
5.		Odd	Odd	
6.	 dead	 dead	 dead	
7.				
	7.0.pm.	11.20.pm.	7.30.am.	10.0.am.
77. A 1.				
2.				
3.				
4.				
5.				
6.				
	11.0.pm.	1.45.pm.		
78. A 1.				
		A2 has divided		
2.				
		A3 has divided		
3.				
4.				
5.				
6.	B is dead	B is dead		
7.		dead		
8.				
9.				
10-				

No.	TIME OF OBSERVATIONS.			
	10.30.am.	2.0.pm.	3.30.pm.	5.0.pm.
79. A 1.				
2.				
3.				
4.				
5.				
6.				
	2.0.pm.	3.30.pm.	8.45.pm.	
80. A 1.				
2.				
3.				
4.			Odd.	
5.				
	11.15.pm.	3.0.pm.	9.0.am.	
81. A 1-7.			6 Zygotes in A.	
A♀. 8.				
9.		Odd	A 9 has divided.	
10.				
11.				
12.		Odd		
13.				
14.				
15.				
16.				
17.				
18.				
19.				
20.				

No. TIME OF OBSERVATIONS.

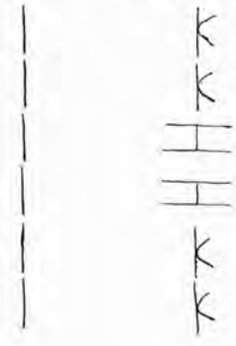
	6.0.pm.	10.30.pm.	10.0.am.	3.45.pm.
82. A 1.	All cells of B in division	X		
2.		X		
3.		X		
4.				
5.			A_5 has divided	A_5 has divided.
6.				
7.			Odd	odd
8.	A In division.			
9.				
10.				
11.		X	X	
12.	A In division.		X	
13.			X	
14.				

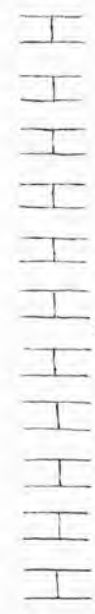
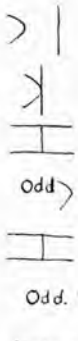
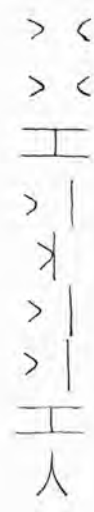
	9.20.pm.	12.5.pm.	12.5.pm.
83. A 1.			Phm^{ls} fusion
2.			Phm^{ls} fusion
3.			Phm^{ls} fusion
4.			
5.	X		
6.			
7.			
8.			
9.			
10.			
11.			

No.		TIME OF OBSERVATIONS.		
		6.15.pm.	10.0.am.	4.0.pm.
84.				B shows female cells.
B♀	A 1.			
	2.			
	3.			
	4.			
	5.			
	6.			
	7.			
	8.			
	9.			
	10.	Odd	Odd)	
	11.			
	12.			
	13.			
	14.			
	15.	Odd	Odd)	
	16.			
	17.			
	18.			
	19.			
	20.			
		9.30.pm.	12.0.Noon.	
85.	A 1-2.			
	3.			
	4.			
	5.			
	6.			
	7-14.			
	15.			
	16-20.			
	21.			

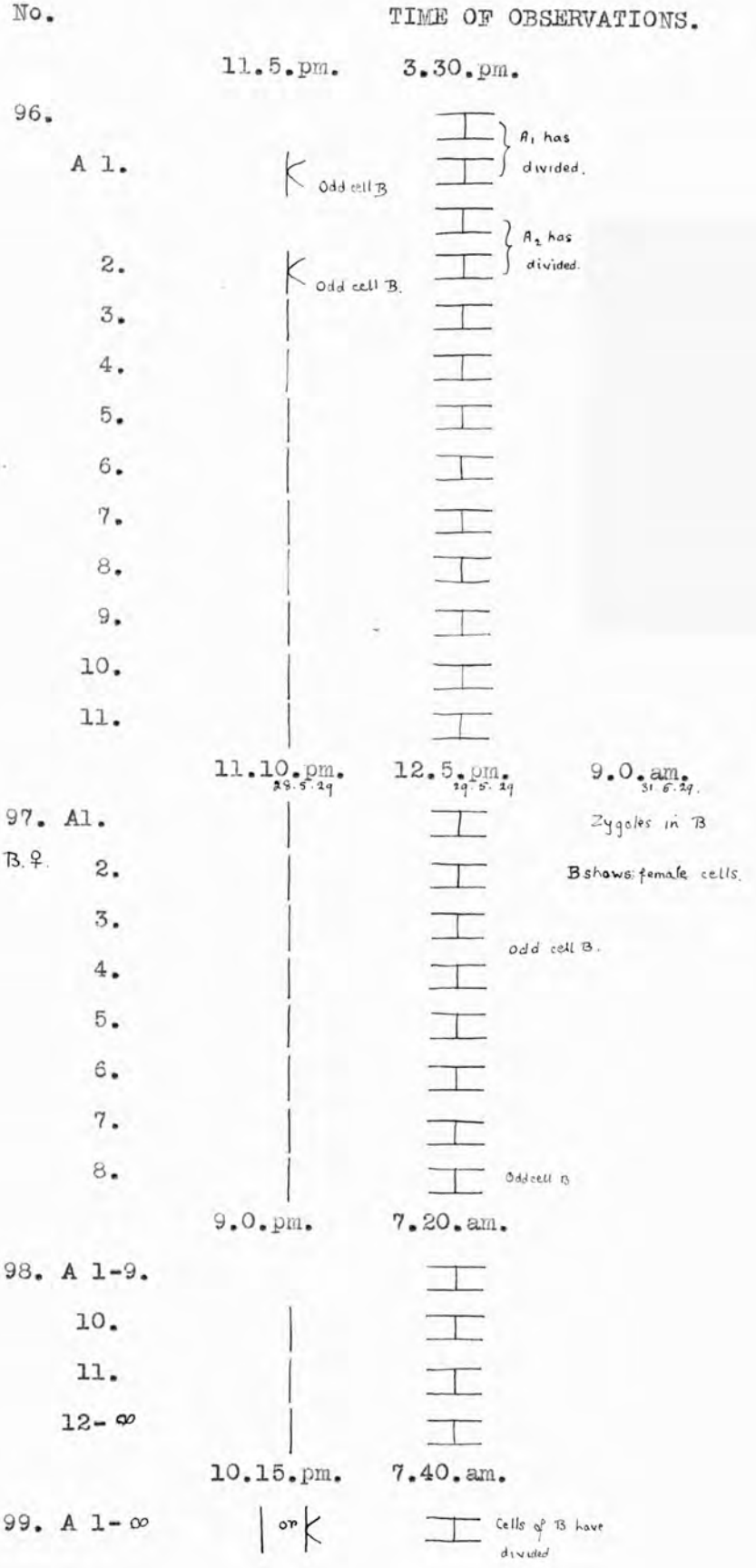
No.	TIME OF OBSERVATIONS.	
	8.45.pm.	11.0.am.
86. A 1.		dead.
2.		"
3.		
4.		
5.		
6.		
7.		
8.		dead
9.		
10.		
	9.15.pm.	11.30.am.
87.		A1 just divided
A 1.		
2.		
3.		
4.		
5.		
6.		
7-9.	B11 Odd	B16 Odd B17. odd.
10.		B18 Odd B 19 "
11.		
12.		B 17 Odd B 18 "
13.		B 20 Odd B 21 "
14.		
15.		B 23 Odd.
	10.0.pm.	2.0.pm.
88. A 1-3.		
4.		Odd cell B.
5.		Odd cell B
6.		Odd cell B
7.		
8.		

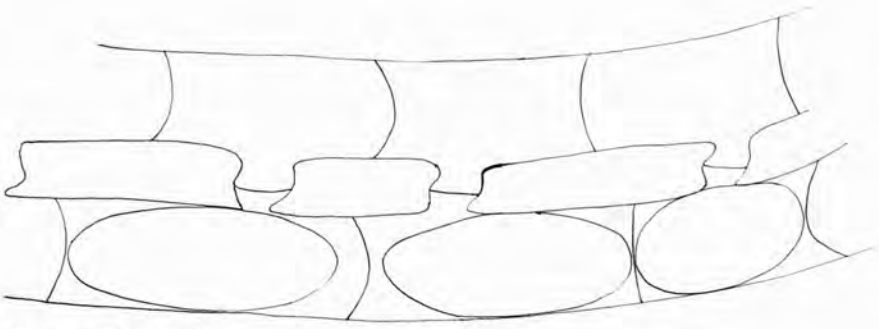
No.	TIME OF OBSERVATIONS.			
	10.50.am. 30.5.29.	5.0.pm. 30.5.29.	9.0.am. 31.5.29	1.6.29.
89. A 1.	Free	Free	A shows female cells.	B has 3 empty cells.
A8 2.			-	
3.		Odd		
4.				
5.				
6.		Odd		
7.				
8.				
9.		Odd		
10.				
11.		Odd		
12.				
13.		Odd		
14.				
15.				
	10.0.pm.	9.0.am.	11.10.pm.	2.0.pm.
90. A 1.			distance apart 1.9μ	distance apart = 3.3μ
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				

No.		TIME OF OBSERVATIONS.		
		9.30.pm.	12.5.pm.	9.0.am.
91. A	1.			A. is female filament
A♀	2.			A shows slightly swollen cells
	3.			2 empty cells in B.
	4.		Odd cell B	
	5.			
	6.			
	7.			
	8.			
	9.			
	10.			
	11.			
	12.	Odd cell B	Odd cell B.	
	13.			
	14.			
	15.			
		10.30.pm.	9.0.am.	
92. A	1-3.			
	4.			
	5.			
	6.		dead.	
	7.			
	8.	Odd cell B	Odd cell B.	
	9.			
	10.			
	11.			
	12-			
		9.45.pm.	9.0.am.	
93. A	1.			
	2.			
	3.			
	4.			
	5.			
	6.			

No.	TIME OF OBSERVATIONS.		
	11.0.pm.	9.0.am.	
94. A 1.			
2.			
3.			
4.			
5.			
6.		Odd cell B (B_4 divided).	
7.			
8.			
9.			
10.			
11.- ∞			
a.			
b.			
c.			
d.		Odd	
e.			
f.		Odd.	
g.			
	6.20.pm.	10.0.am.	11.0.am.
95. A 1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			

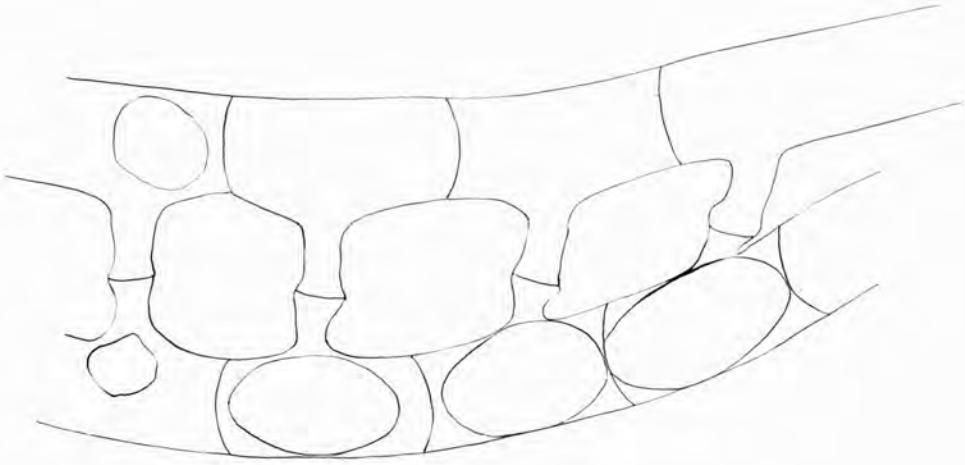
No. TIME OF OBSERVATIONS.





Filaments originally in contact

Cells 1–∞.



Filaments originally separated.

Cells a–f.

No.		TIME OF OBSERVATIONS.		
		11.50.pm.	12.5.pm.	9.0.am.
100. A 1.			B is dead	dead Cells of A not swollen.
A ♀	2.			
	3.		Odd	Odd
	4.			
	5.			
	6.			Zygote A
	7.			Zygote A
	8- ∞			Zygote A
	a.			
	b.			
	c.			
	d.			
	e.			
	f.			
		12.5.am. 29.5.29.	12.10.pm. 29.5.29.	9.0.am. 31.5.29.
101. A 1.				Zygotes in B.
B ♀	2.			
	3.			
	4.			
	5.			
	6.			
	7.			
	8.		Odd	
	9.			
	10.			
	11.			
	12.			
	13.		Odd	
	14.			
		12.15.am. 28.5.29.	11.50.am. 29.5.29.	9.0.am. 31.5.29.
102. A 2-18.				Zygotes in A.
A ♀	19.			
	20.			
	21.			
	22- ∞		or	

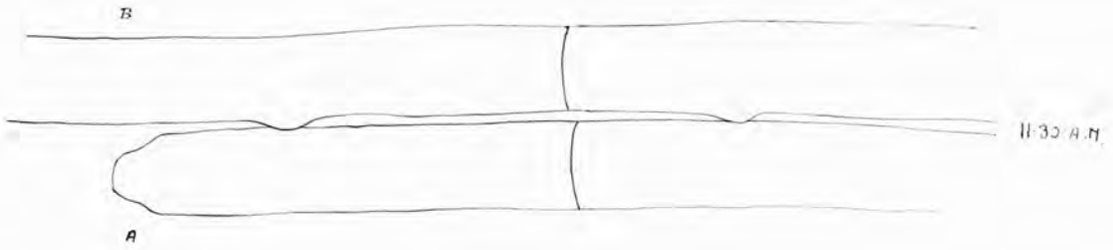
No.		TIME OF OBSERVATIONS.			
		12.15.am.	11.50.am.		
103. A	1.			Zygotes in A	
A ♀	2.			A shows female cells.	
	3.				
	4.				
	5.				
	6.				
	7.				
	8.		 Odd cell B.		
	9.				
	10.				
	11.				
	12.				
	13.		Odd		
	14.				
		11.0.am 29.5.29.	5.0.pm.	10.15.pm.	9.0.am. 31.5.29
104. A	1.			A shows female	Empty cells in B
A ♀	2.			cells	Zygotes in A.
	3.		Odd		
	4.				
	5.				
	6.		Odd.		
	7.				
	8.				
	9.		Odd.		
	10.				
	11.		Odd		
	12.				
	13.				
	14.				
		9.0.pm.	10.0.am.		
105. A	2-24.				
	25.				
	26.				
	27.				

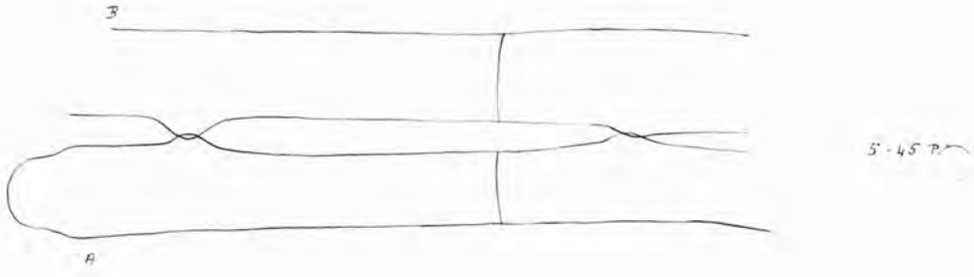
No.	TIME OF OBSERVATIONS.		
	9.15.pm.	10.30.am.	
106. A 1.			
2.			
3.			
4.			
5-8.			
9.			
	9.30.pm.	10.0.am.	10.0.am.
107. A 1-∞			
	9.45.pm.	10.0.am.	10.0.am.
108. A 1-∞			Female cells.
	9.50.pm.	10.0.am.	
109. A 1-∞			
	10.0.pm.	10.0.am.	10.0.am
110. A 1-∞			Female cells.
	10.15.pm.	10.0.am.	
111. A 1-∞			

Cases 1-29.

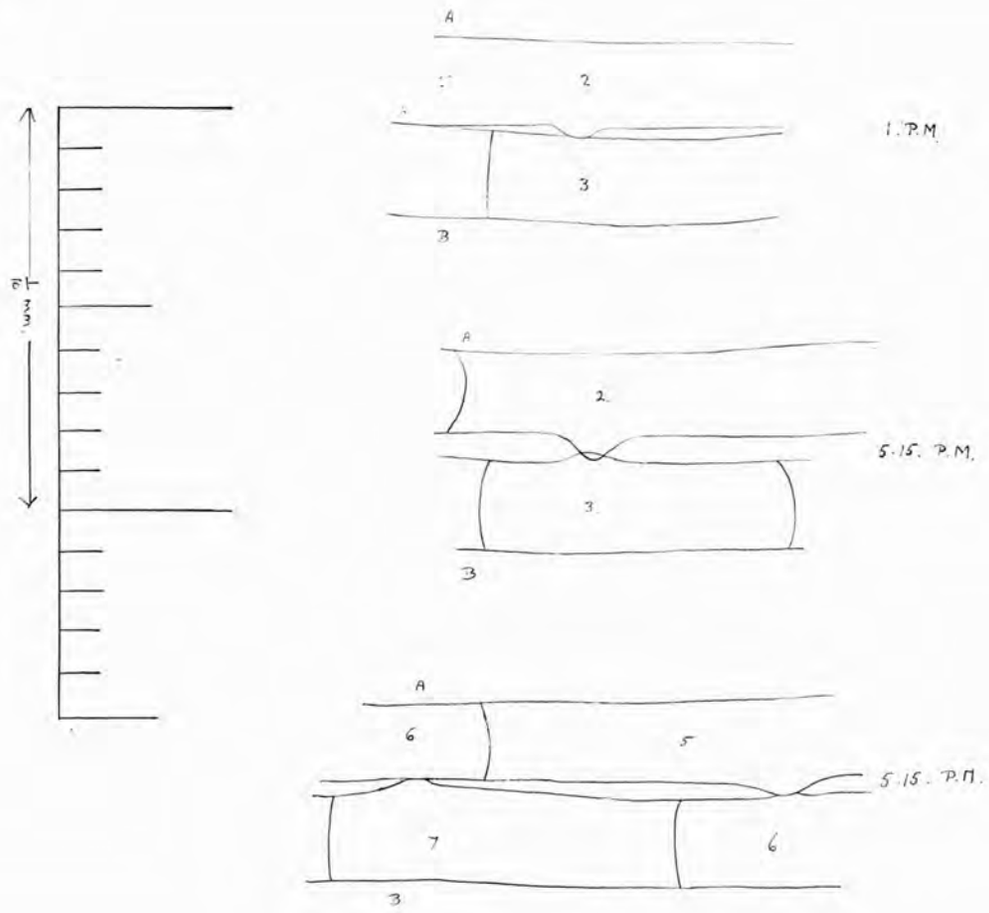
Tables of conjugation stages in
Spirogyra weberi,
illustrated by drawings made
with the camera lucida.

1



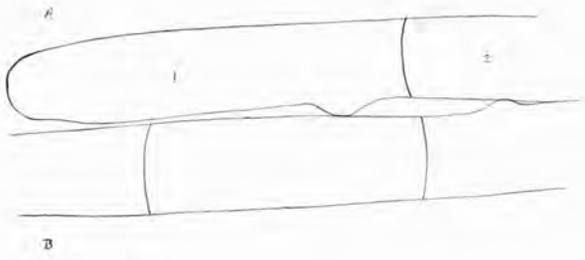


2.

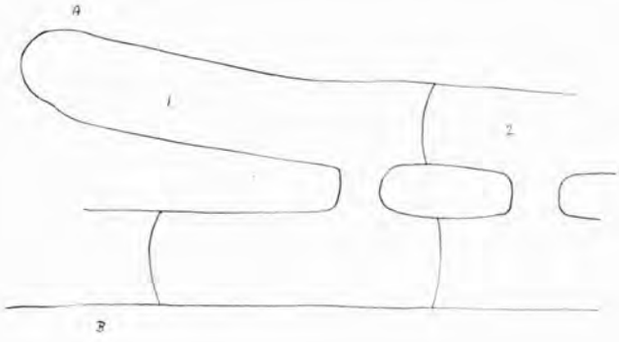


No.		TIME OF OBSERVATIONS.			
		11.30.am.	5.45.pm.	9.35.pm.	
1.	A 1.				
	2.				
		1.0.pm.	4.0.pm.	12.15.pm.	7.25.pm.
2.	A 1.	dead	dead	dead	dead.
	2.				No change.
	3.				in any cell
	4.				
	5.				
	6.				
	7.				
	8.				
	9.				
	10.				
	11.				
	12.				
		10.15.pm.	7.40.am.	6.40.pm.	
3.	A 1.				
	2.				
	3.				
	4.				
	5.				
	6.				

4.

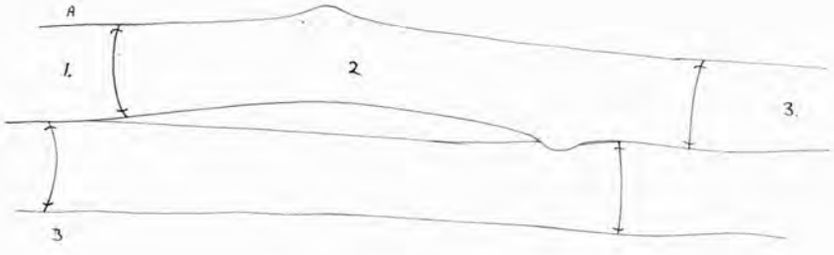


3. P.M.

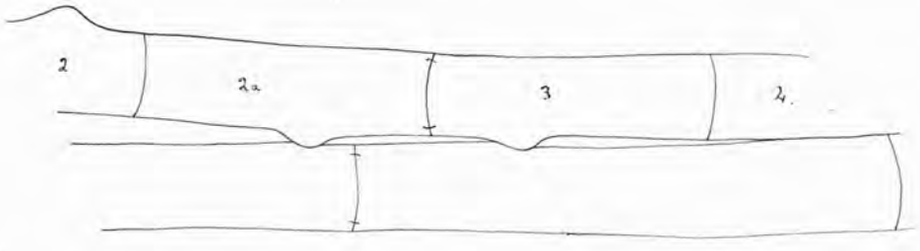


7.35. A.M

5.



12.45 P.M.



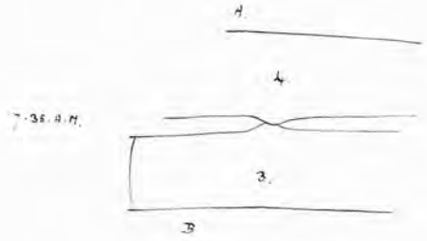
2.50 PM

Division has occurred

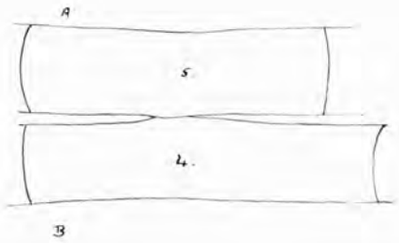
No.		TIME OF OBSERVATIONS.			
		12.50.pm.	3.0.pm.	9.50.pm.	9.40.pm.
4.	A 1.				
	2.				
	3.				
	4.				
	5.				
	6.				Female cells shown.
	7.				
	8.				
	9.				
	10.				
	11.				
	12.				
	13.				
	14.				

		12.45.pm.	5.40.pm.	10.0.pm.	7.20.am.
5.	A 1.		B_1 divided. A_2 divided.		
	2.				
	3.				
	4.				
	5.		A_5 divided.	A_5 divided.	
	6.			A_6 dividing	
	7.				

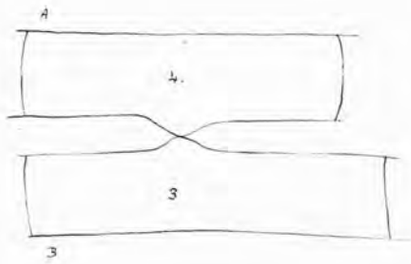
6



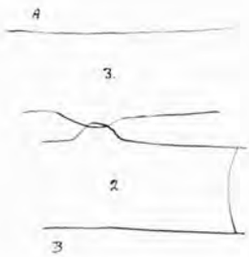
7·35. A.M.



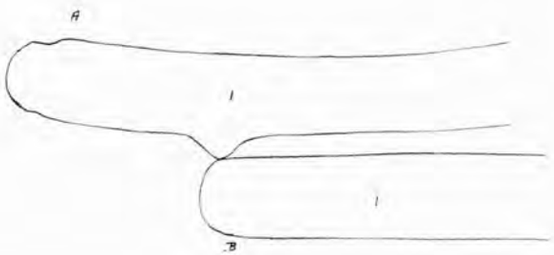
7 35. A.M.
5. 5. 29



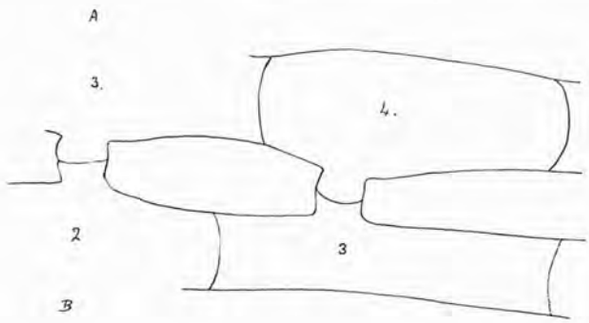
3. P.M.



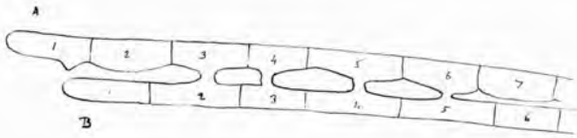
3 P.M.



3 P.M.



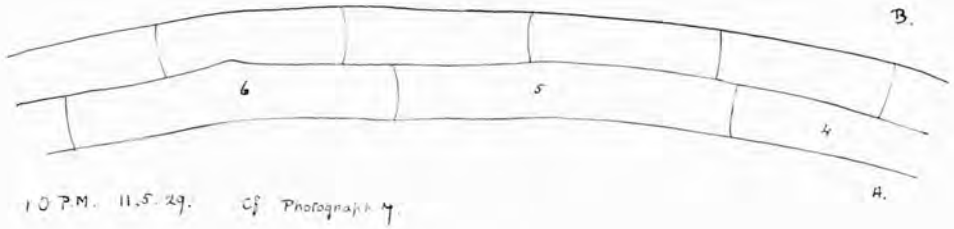
2·45. 8.5.29.



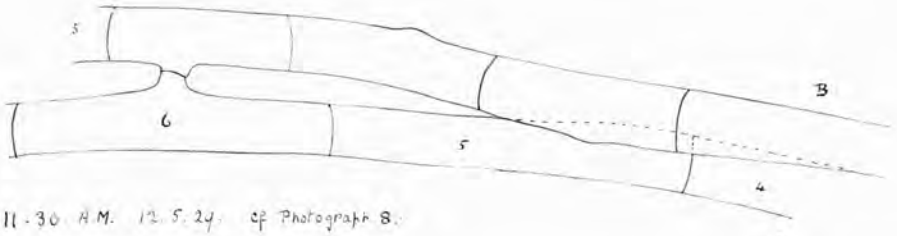
8 5 29.

No.		TIME OF OBSERVATIONS.			
		3.45.pm.	7.35.am.	3.0.pm.	12.50. m.
6. A♀	1.				A shows female cells
	2.			Odd	Odd
	3.				
	4.				
	5.				
	6.				
	7.			Odd	Odd
		4.0.pm.	9.10.pm.	11.55.pm.	7.15.am.
7. A	1.				
	2.				
	3.				
	4.				
	5.				
	6.				
		5.45.pm. 6.5.29.	9.55.pm. 6.5.29.	7.40.am. 7.5.29	10.0.am. 9.5.29
8. A	1.				
	2.				
	3.				
	4.				
	5.				
	6.				
	7-				

9.

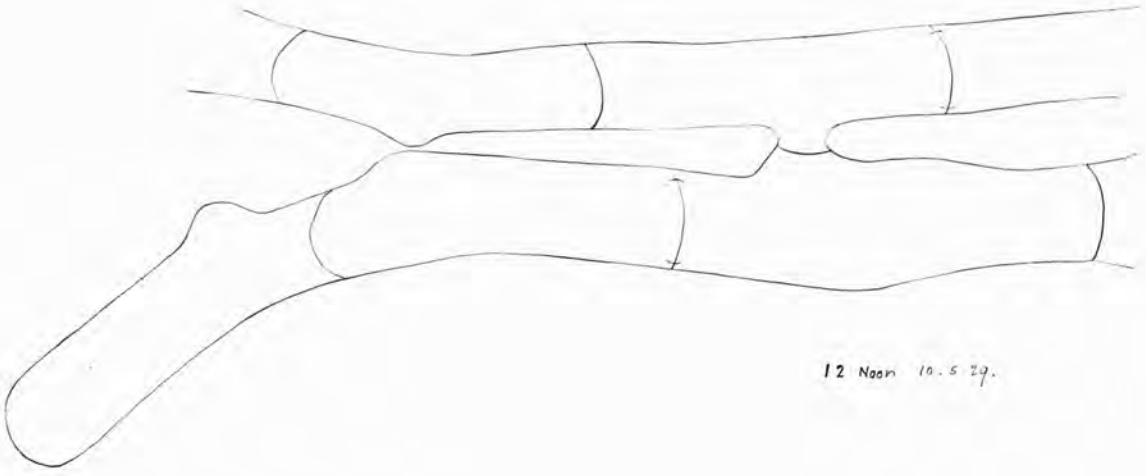


1 0 P.M. 11.5.29. cf Photograph 7.



11.30 A.M. 12.5.29. cf Photograph 8.

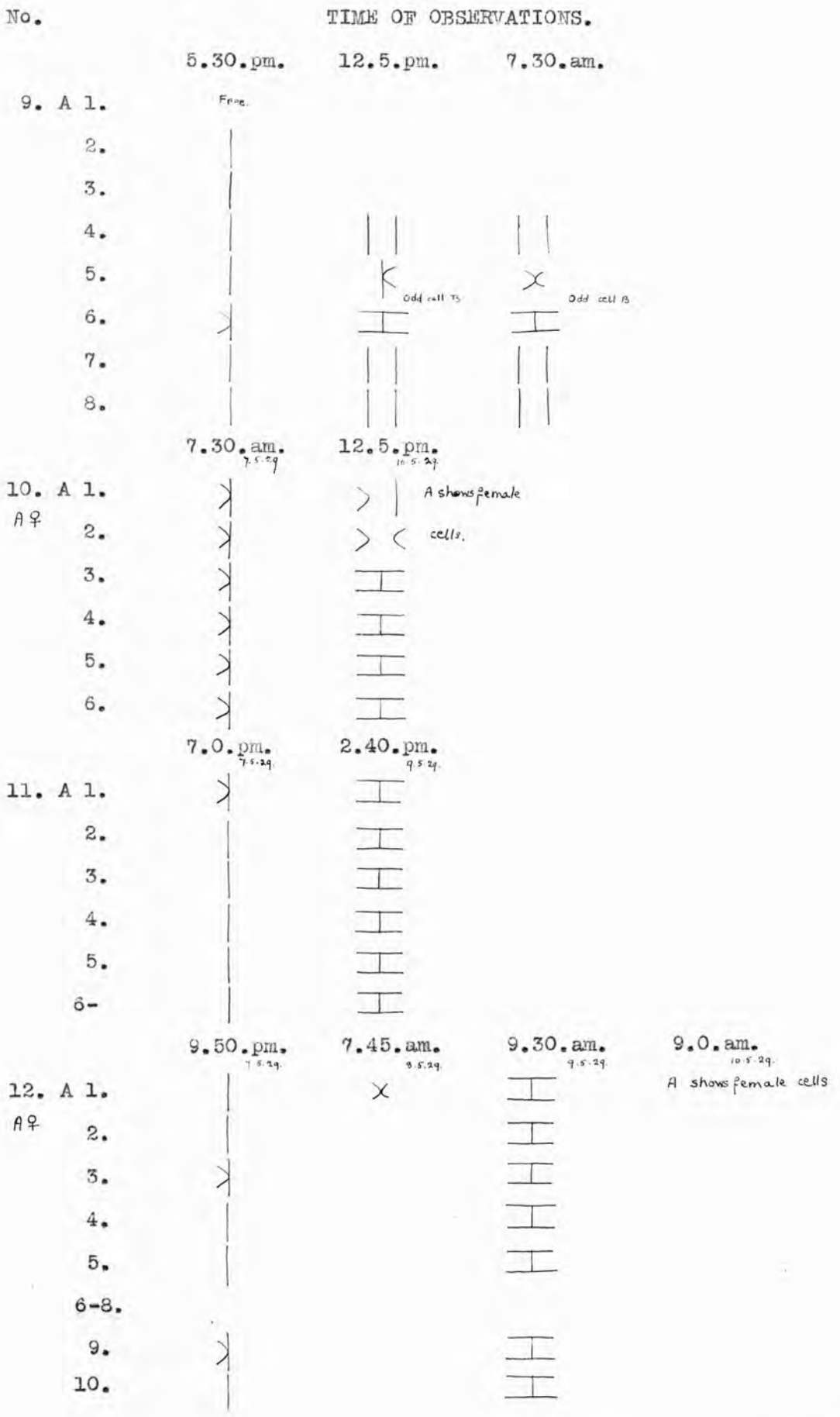
10.



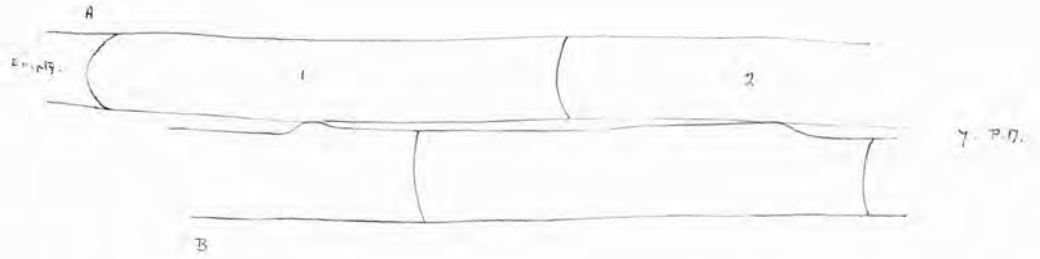
12 Noon 10.5.29.

Earlier Stage shown in Photograph 11.

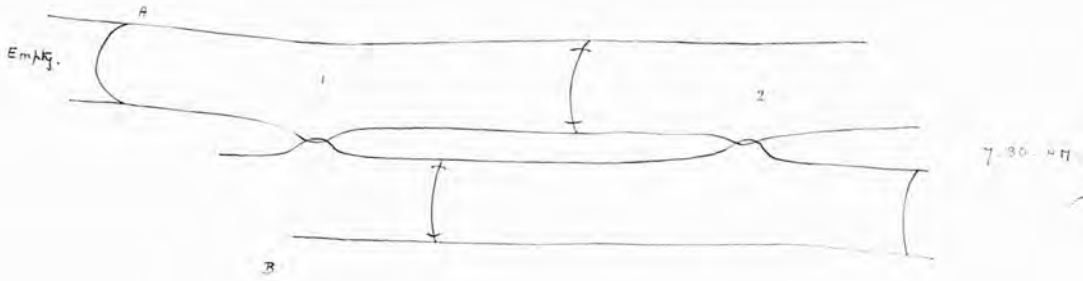
No. TIME OF OBSERVATIONS.



13.

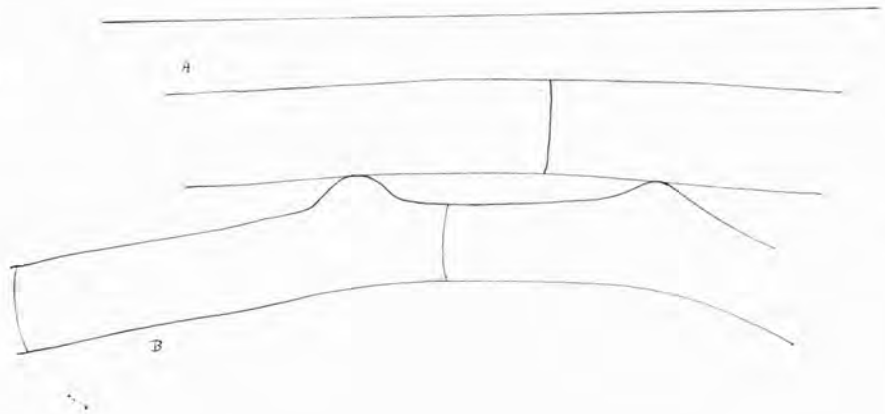


7. P.M.

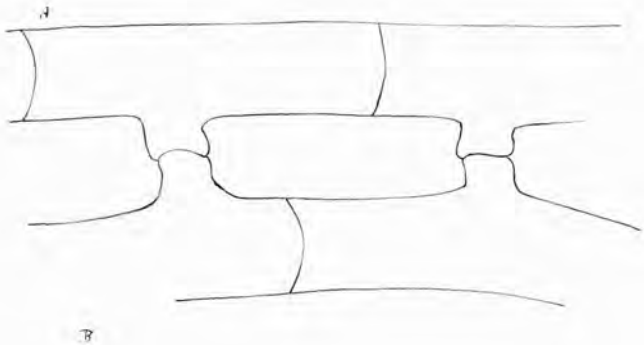


7.30 P.M.

14.

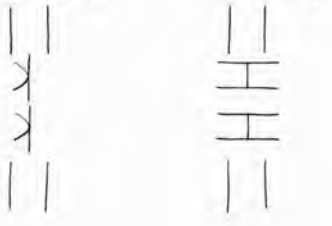


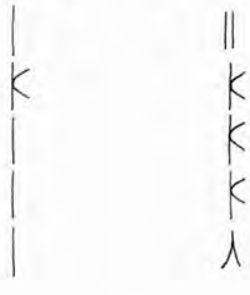
10. P.M.

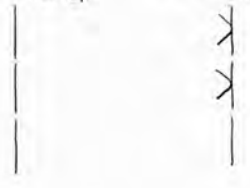


11. A.M.

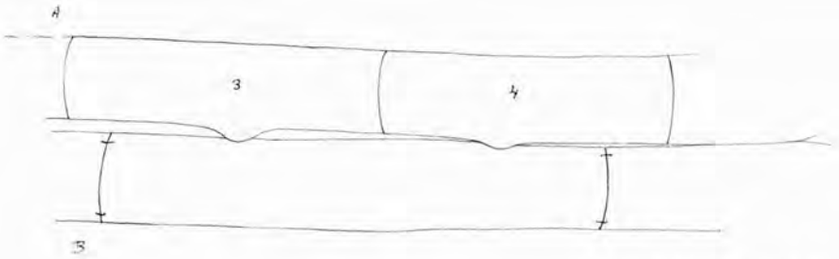
No.	TIME OF OBSERVATIONS.	
	7.0.pm.	7.30.am.
13. A 1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		

	10.0.pm. 9.5.29.	11.0.am. 10.5.29.
14. 1 - ∞		
a.		
b.		
2 - ∞		

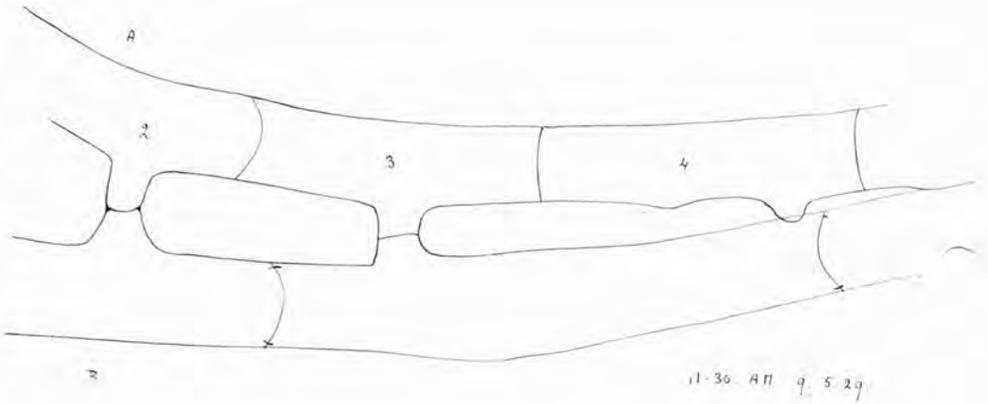
	12.25. m.	6.50.pm.
15. A 6.		
7.		
8.		
9.		
10.		

	10.0.pm. 12.5.29.	7.0.am. 13.5.29.	10.30.am. 14.5.29.
16. A 1.			Slight growth
2.			
3.			

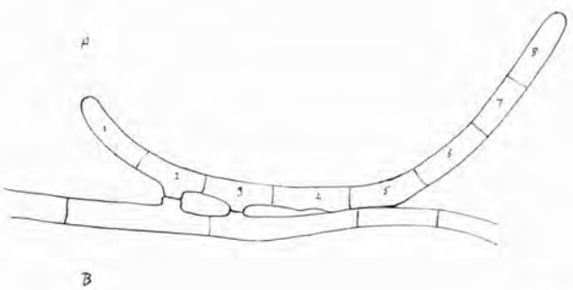
17.



12.25 P.M. 6.5.20



11.30 AM 9.5.29



11.30 A.M. 9.5.29.

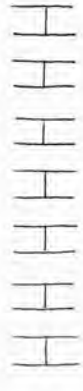
No.	TIME OF OBSERVATIONS.				
	12.25.pm. 6.5.29.	6.45.pm. 6.5.29.	11.30.am. 9.5.29.		
17. A 1.					
2.					
3.					
4.		Odd cell A_4	Odd cell A_4.		
5.					
6.					
7.					
8.					
	5.30.pm. 6.5.29.	9.50.pm. 6.5.29	7.30.am. 7.5.29.	1.50.pm. 7.5.29.	9.5.29.
18. A 1.					A. shows
A♀ 2.		Odd.	Odd	Odd.	female
3.					cells.
4.					
5.					
6.					
7.					
8.					
9.					
10.		dead			
	12.20.pm. 7.5.29	11.30.am. 9.5.29.			
19. A 1.					
2.					
3.					
4.					
5.					
6.					
7.					

DIAGRAM OF PHOTOGRAPH 4. 5.P.M. 7.5.29.

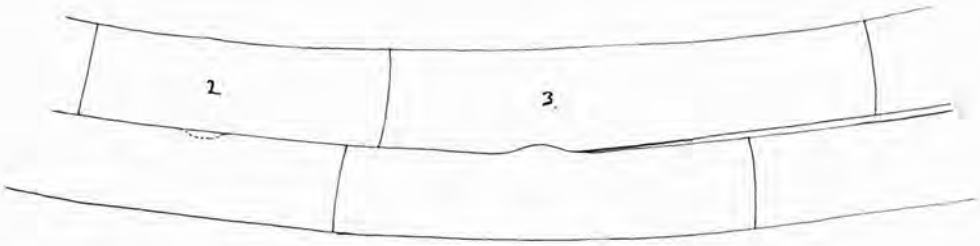
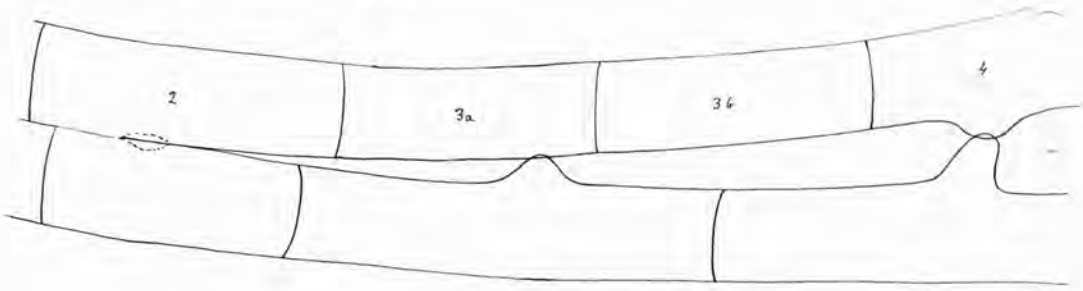


DIAGRAM OF PHOTOGRAPH. 5. 1.30P.M. 8.5.29.



SEMI-DIAGRAMATIC DRAWING OF CASE 20. 1.30 P.M. 8.5.29

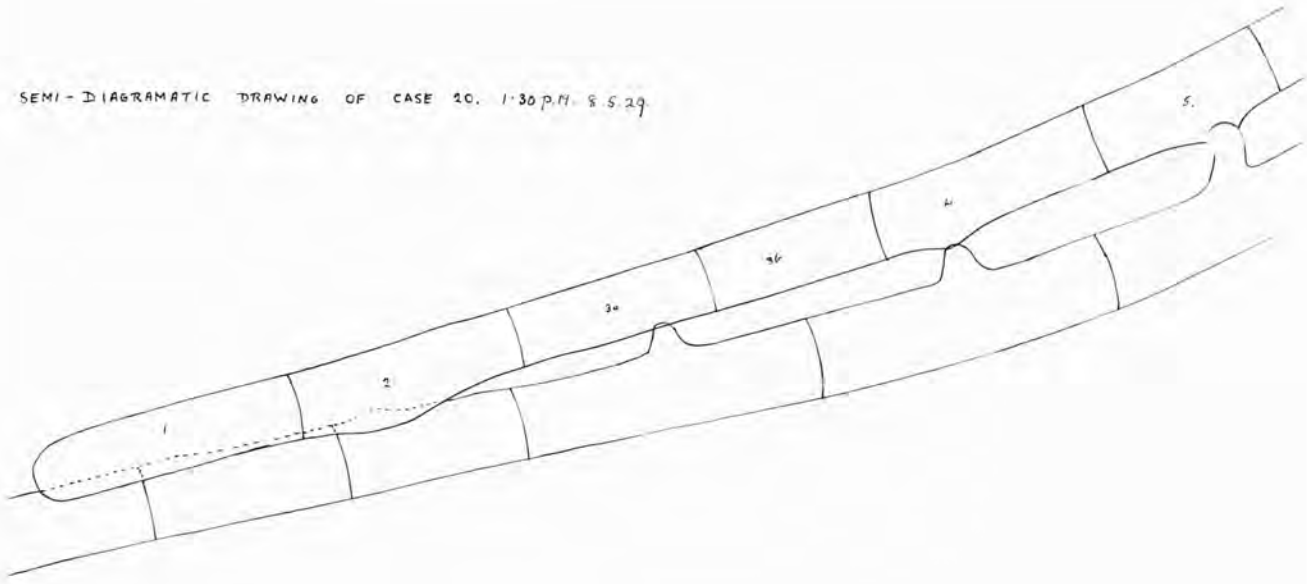


DIAGRAM OF PORTION PHOTOGRAPHED 5 P.M. 7.5.24.

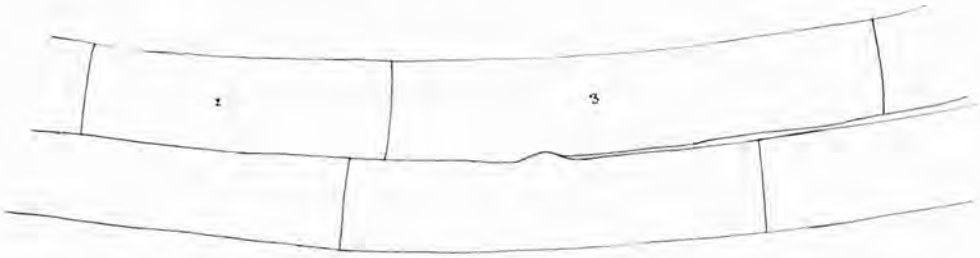
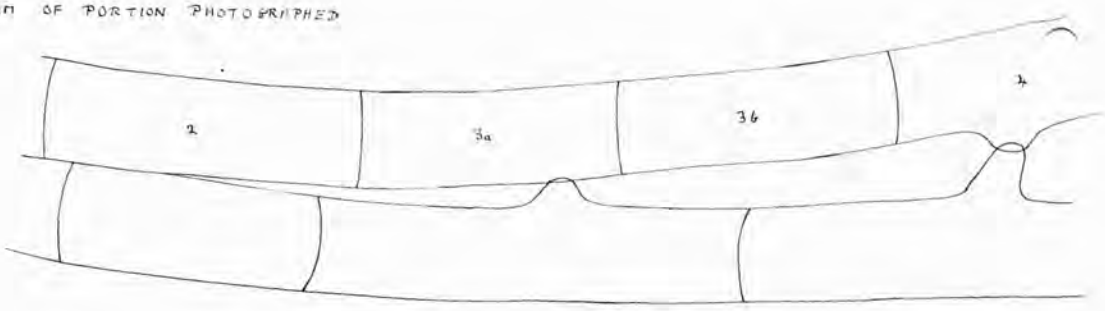
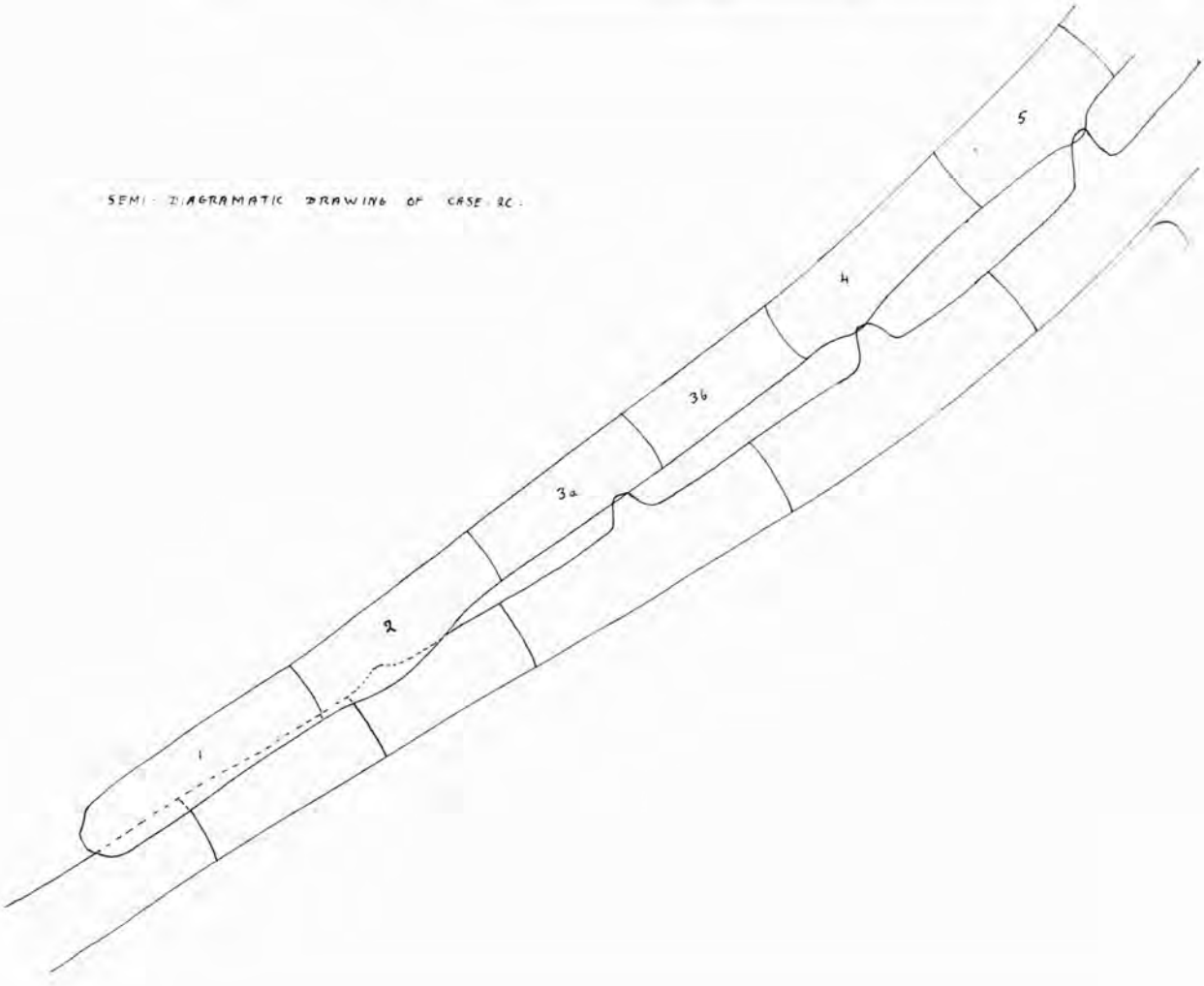


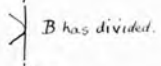
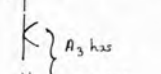
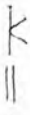
DIAGRAM OF PORTION PHOTOGRAPHED



SEMI-DIAGRAMATIC DRAWING OF CASE 2C.

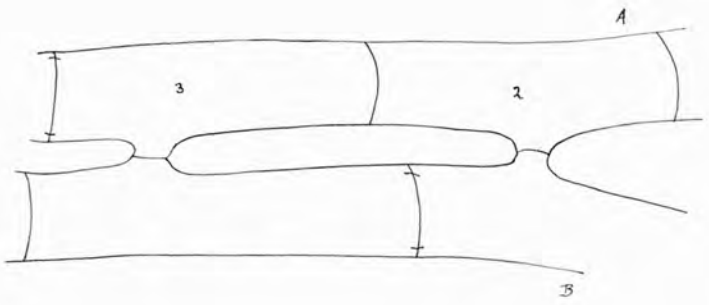


No. TIME OF OBSERVATIONS.

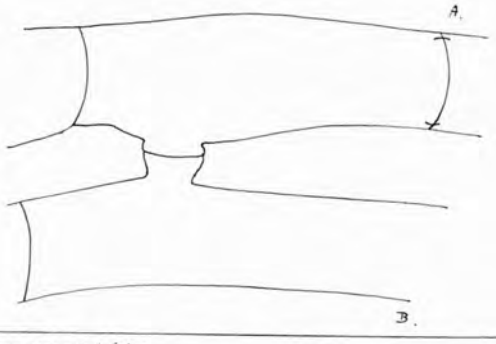
	12.25.pm.	3.35.pm.	9.30.pm.	7.50.am.
20. A 1.				
2.				
				
3.				Odd.
4.				
5.				
6.				
7-10.				
11.				
12.				
13.				

	12.35.pm. 7.5.29	7.45.am. 8.5.29.	9.30.am. 9.5.29.
21. A 1.			Female cells in B further on in filament.
B♀ 2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

	9.30.pm.	7.45.am.	3.0.pm.	2.0.pm.
22. A 1.				
2.				
3.				
4-				



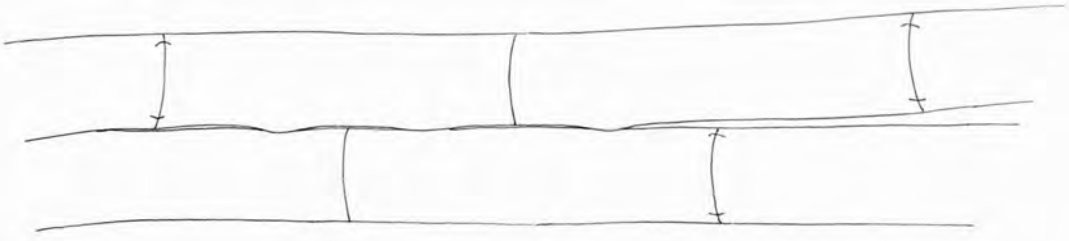
7.40. A.M. 5.5.29.



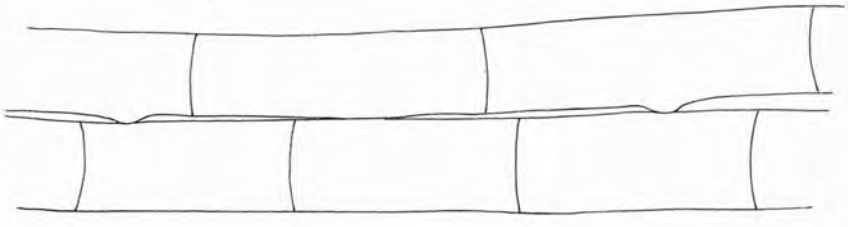
1. P.M. 6.6.29

Various Early stages. figured but not labeled.

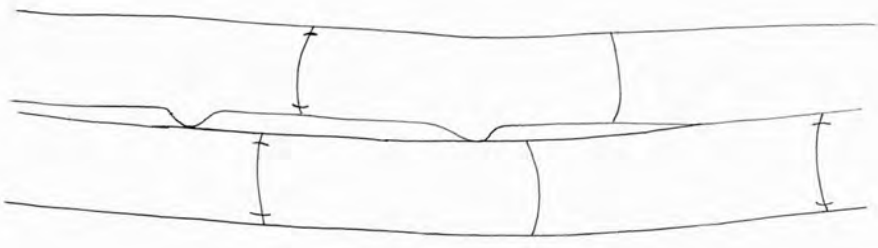
27



28



29



No. TIME OF OBSERVATIONS.

		10.20.pm. 4.5.29.	7.40.am. 5.5.29	1.0.pm. 5.5.29.
23. A	1.			d = .65μ A shows female cells.
A♀	2.			
	3.			d = 1·1μ.
	4.			
	5.			
	6.			

		10.0.pm.	9.0.am.	5.0.pm.
24. A	1.	Odd	Odd	Odd
	2.			
	3.			
	4.			

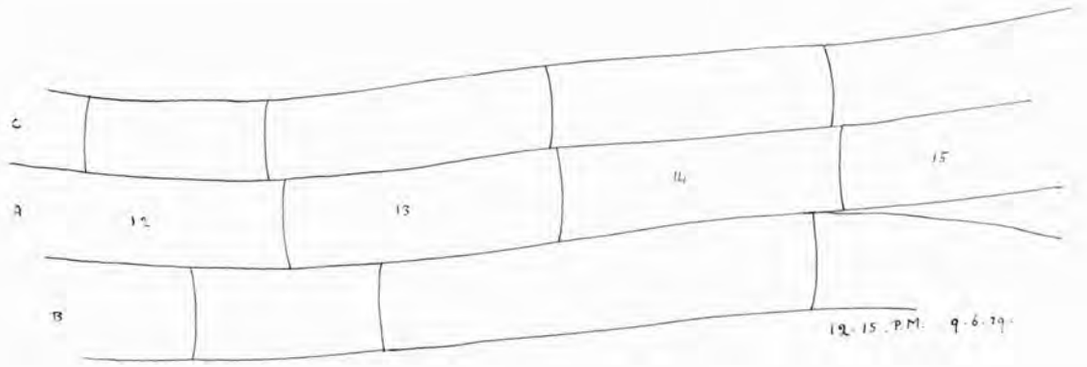
		5.30.pm.	11.30.am.	8.15.pm.
25. A	1.			
	2.			
	3.			
	4.			
	5.			Division has occurred in 2 cells of A.
	6.			
	7.			
	8.			
	9.			

		5.50.pm.	10.0.am	8.30.pm.
26. A	1.	free	free	free. Female cells shown.
	2.			
	3.			
	4.			
	5.			
	6.			

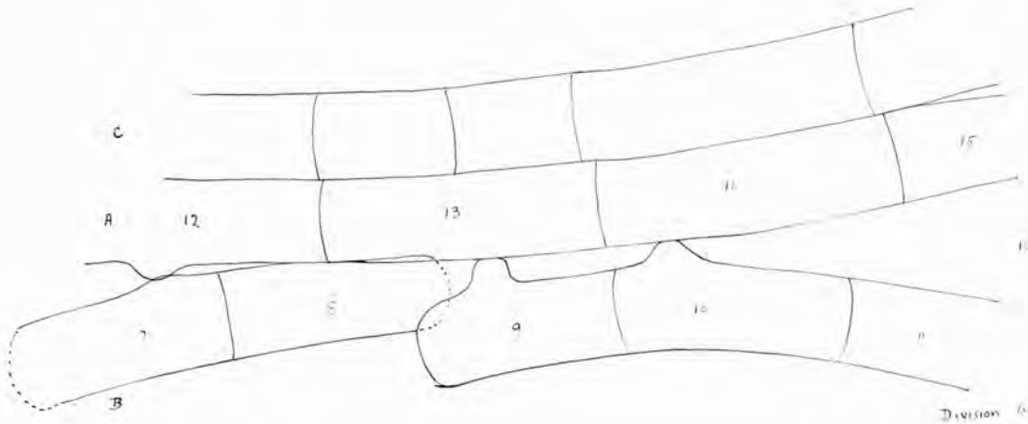
Cases 1-19.

Tables of conjugation stages in
Spirogyra cataeniformis,
illustrated by drawings made
with the camera lucida.

1

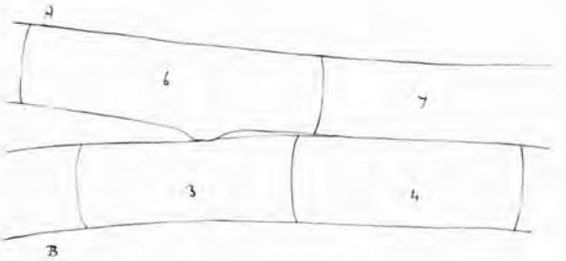


12.15 P.M. 9.6.79.

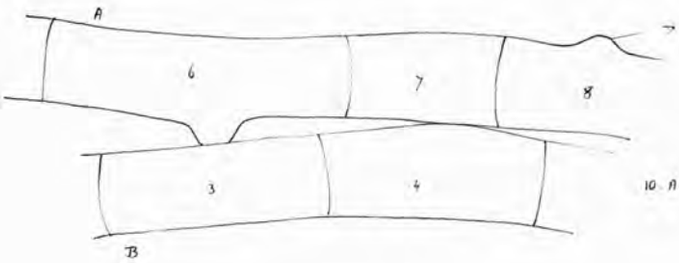


11 A.M. 10.6.79

Division has occurred.

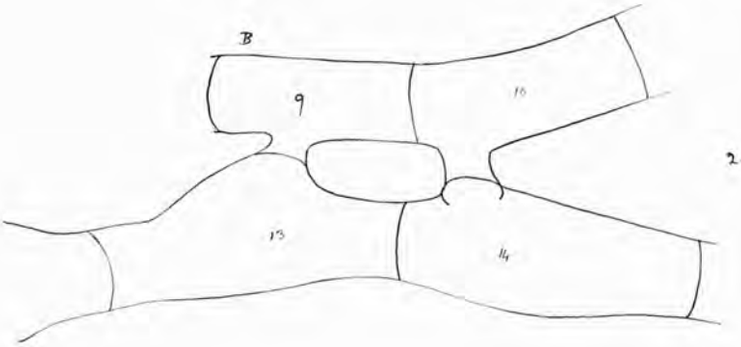


3 P.M. 3.6.20



→ Unites to fd C

10 A.M. 10.6.79.



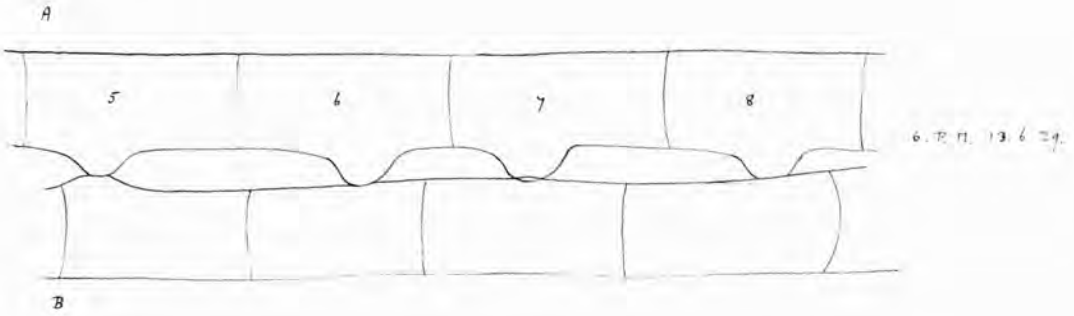
2 P.M. 12.6.79.

No. TIME OF OBSERVATIONS.

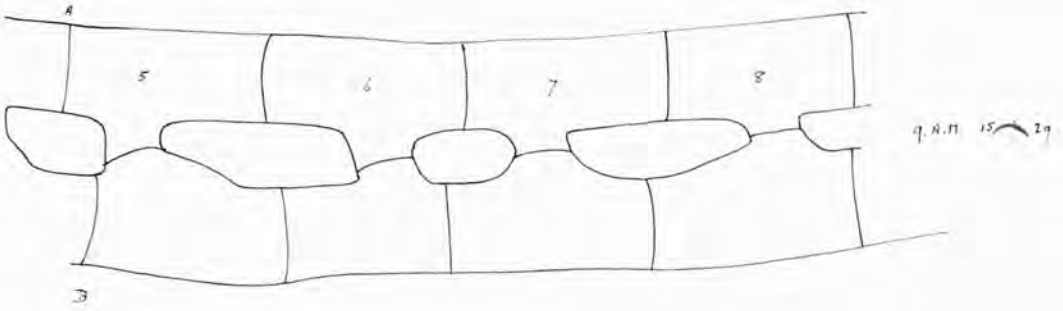
	12.15.pm. 9.6.29.	9.0.pm.	10.0.am. 10.6.29.	2.0.pm. 12.6.29.
1. A 1.	A, B & C in			Female cells in A.
A ♀ 2.	contact all			Protoplasmic
3.	4-way			fusion on 11.6.29.
4.		B broken.	B broken	B broken
5.				
6.		B broken	B broken	B broken
7.				
8.		A1: C B broken.	B broken	B broken.
9.				
10.				
11.				
12.			Odd cell in B	Odd cell in B.
13.			B has	
14.			divided	
15.				

	5.45.pm.	10.30.am.	9.0.am.
2. A 1.		No change.	Female
2.			cells shown.
3.			
4.			

3.

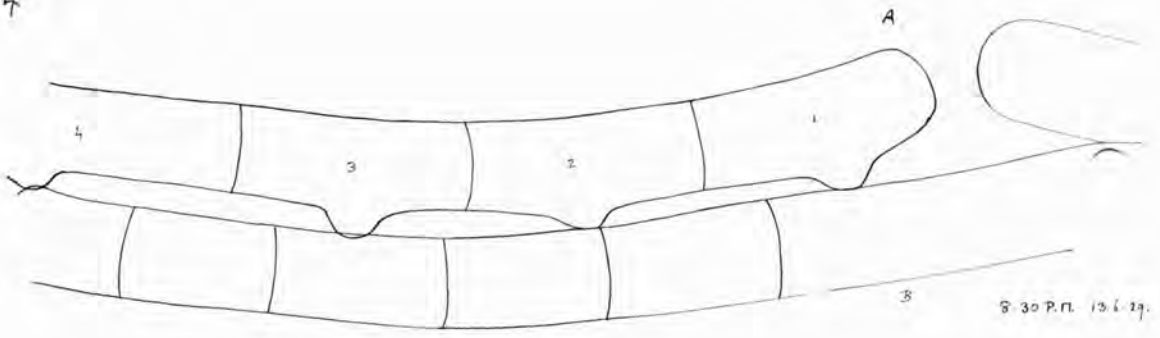


6. P.M. 13. 6. 29.

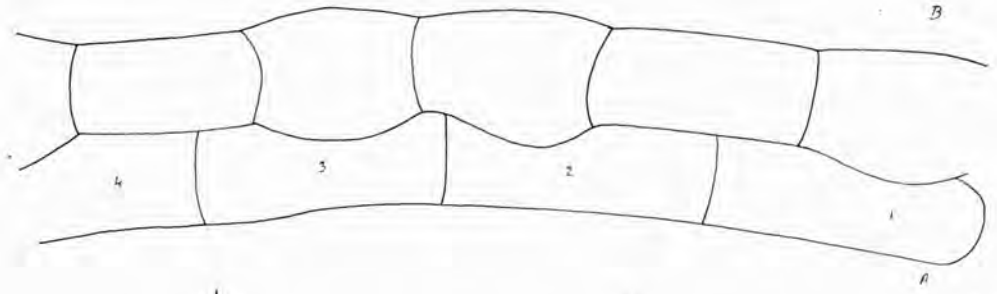


9. A.M. 15 6 29

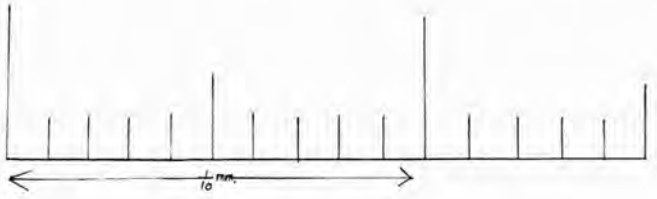
4



8. 30 P.M. 13. 6. 29.



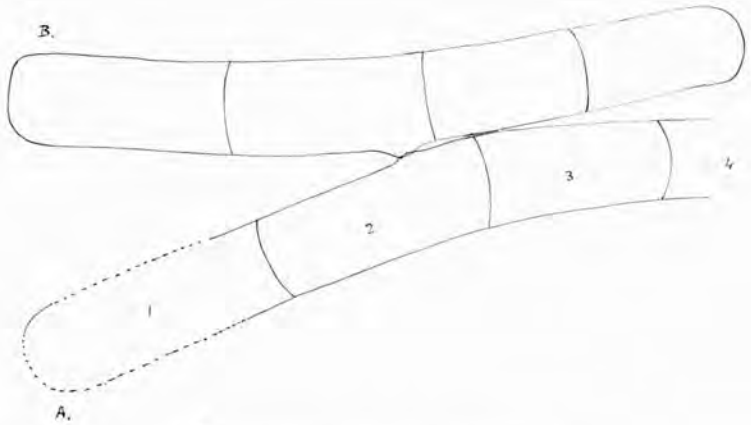
8. 30. A.M. 16. 6. 29.



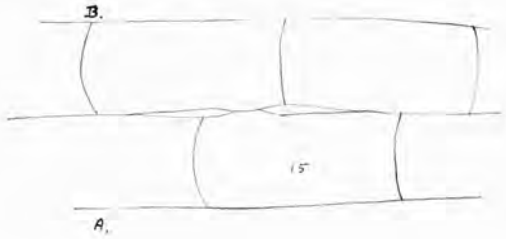
No. TIME OF OBSERVATIONS.

		6.0.pm. 13.6.29	9.0.am. 15.6.29.
3. A	1.		 B shows
B ♀	2.		female cells.
	3.		
	4.		
	5.		
	6.		
	7.		
	8.		
	9.		
	10.	free	free

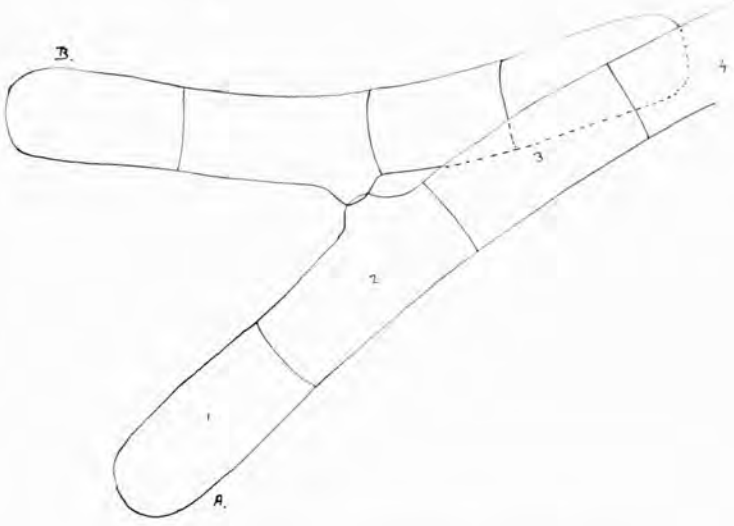
		8.30.pm.	7.0.am.	9.0.am.	8.30.am.
4. A	1.			 Odd cell B	B shows female cells.
B ♀	2.				
	3.			 Odd cell B	
	4.				
	5.				
	6.				
	7.		Odd.	Odd	
	8.				
	9.				



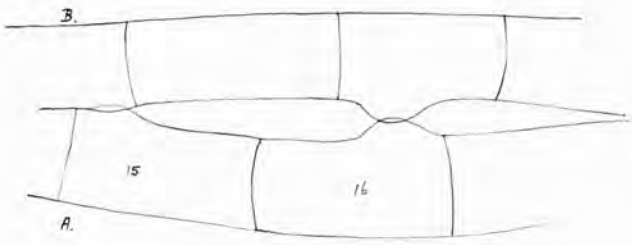
8. P.M 9. 6. 29.



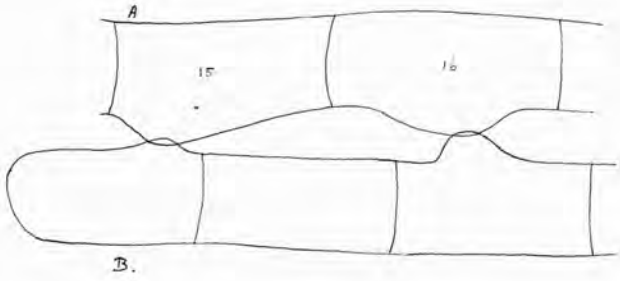
8. P.M 9. 6. 29



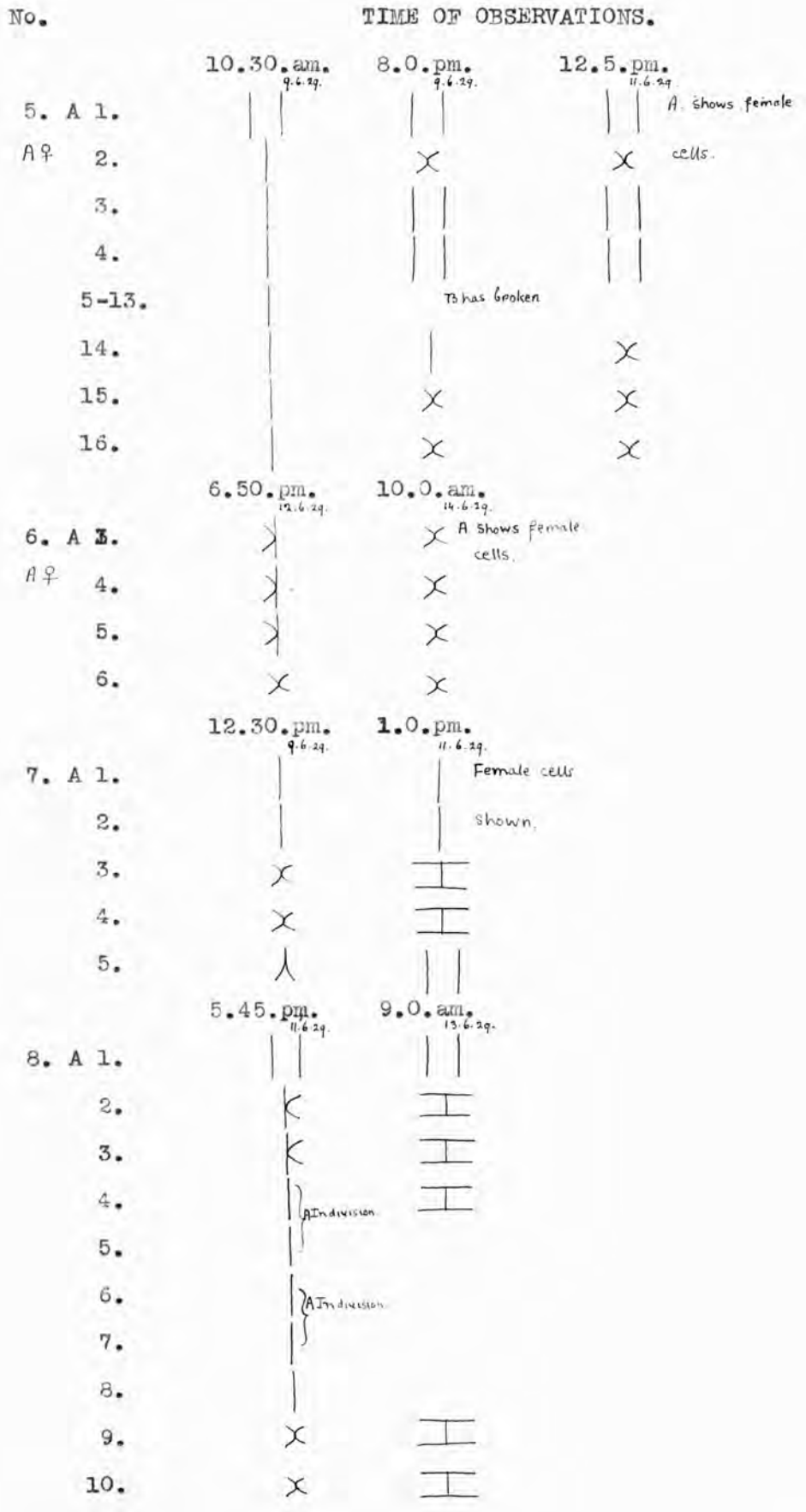
10. 30. A. M 10. 6. 29



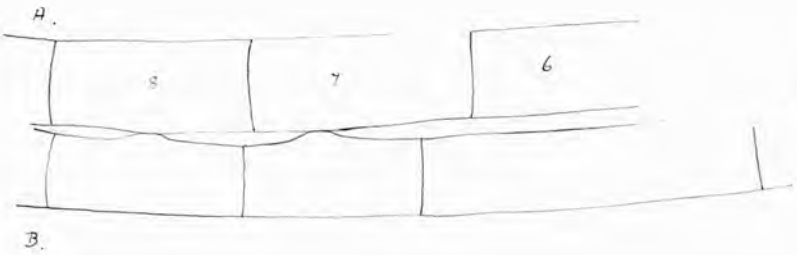
10. 30. A. M.



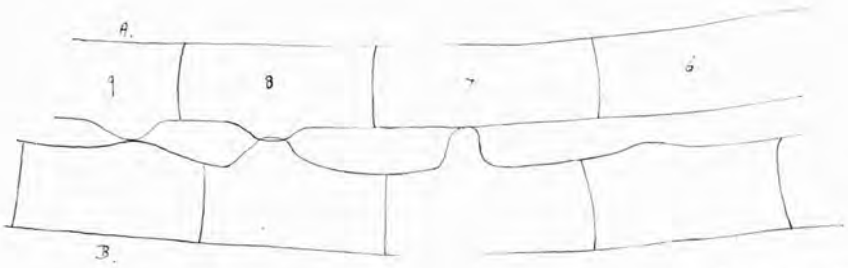
12. Noon 11. 6. 29.


No.
TIME OF OBSERVATIONS.
10.30.am. 9.6.29.
8.0.pm. 9.6.29.
12.5.pm. 11.6.29.
5. A 1.
A♀ 2.
3.
4.
5-13.
14.
15.
16.
A shows female cells.
T5 has broken
6.50.pm. 12.6.29.
10.0.am. 14.6.29.
6. A 1.
A♀ 4.
5.
6.
A shows female cells.
12.30.pm. 9.6.29.
1.0.pm. 11.6.29.
7. A 1.
2.
3.
4.
5.
Female cells shown.
5.45.pm. 11.6.29.
9.0.am. 13.6.29.
8. A 1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
A in division
A in division

9



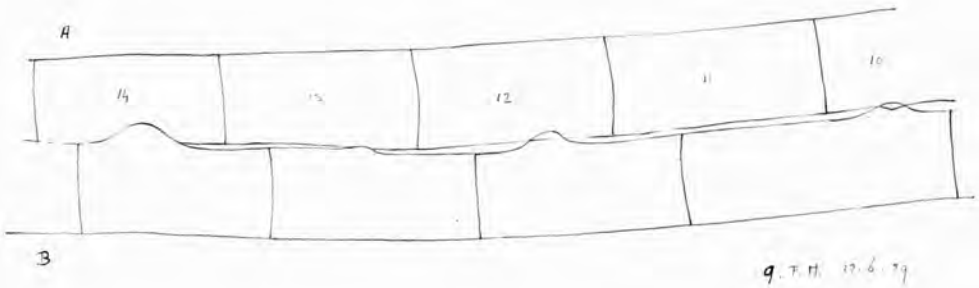
11. 30. A.M. 9. 6. 29.



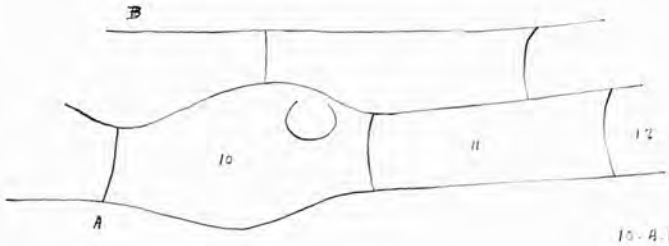
12. Noon 10. 6. 29.

Division has occurred.

10



9. P.M. 12. 6. 29

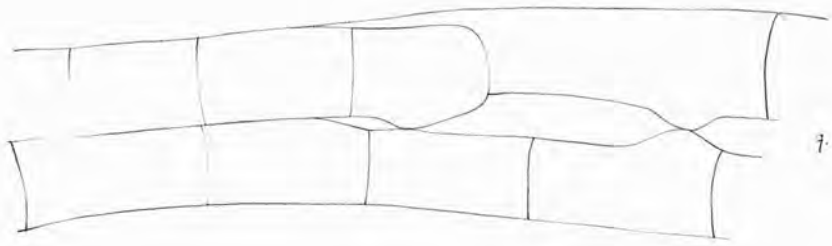


10. A.M. 15. 6. 29

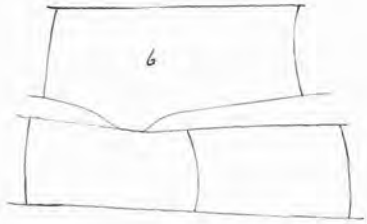
No.	TIME OF OBSERVATIONS.		
	11.30.am. 9.6.29	12.0.Noon. 10.6.29.	
9. A 1.	free	free	
2.	"	"	
3.	X	X	
4.	X	X	
5.	X	X	
6.	T3 has divided	T3. has divided.	
7.	K	K	
8.	K	X	
9.	|	X	
10.	X	X	

	9.0.pm. 12.6.29	10.0.am. 13.6.29.	9.0.am. 15.6.29.	
10. A 1. ♀	K	No change. in any cell.	H	A shows female cells.
2.	K		H	
3.	X		H	
4.			H	
5.			H	
6.	X		H	
7.	X		H	
8.	K		H	
9.	K		H	
10.	X		H	
11.	Odd		Odd	
12.	K		H	
13.	K		H	
14.	K		H	
15.	|		H	

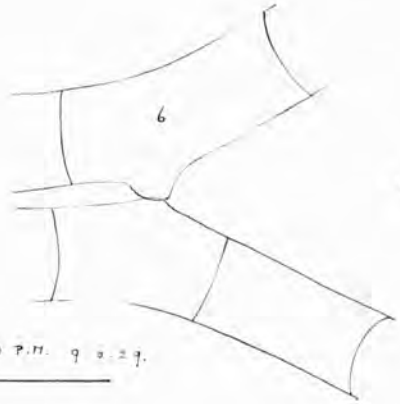
12



9. A.M. 8.6.29.

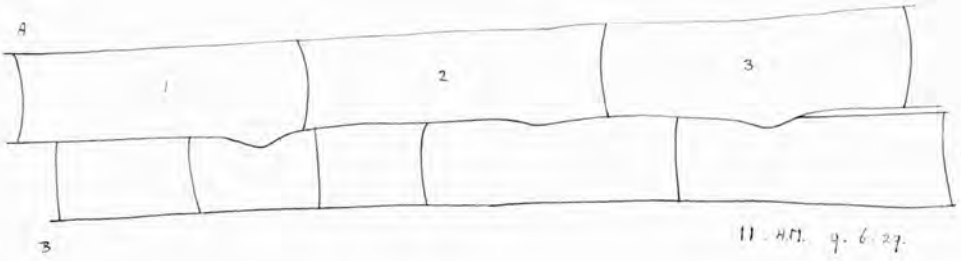


9. A.M. 8.6.29

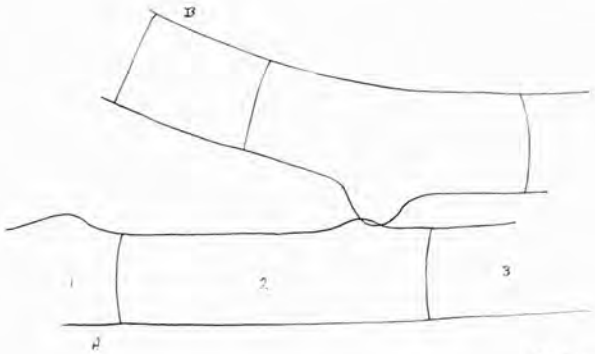


1.10 P.M. 9.6.29.

11.

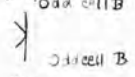
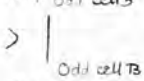


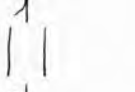
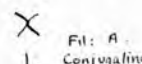
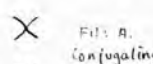
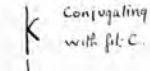
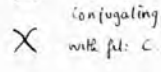
11. A.M. 9.6.29.



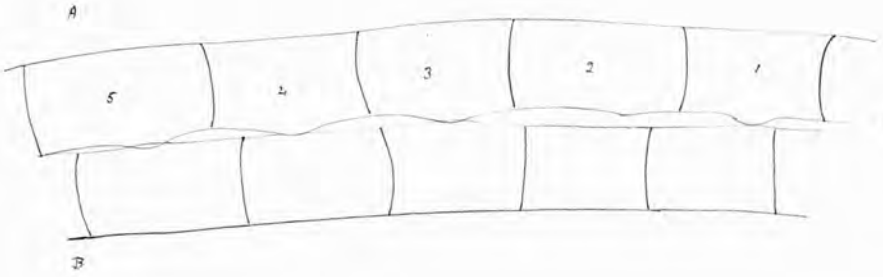
12. Noon. 11.6.29

No. TIME OF OBSERVATIONS.

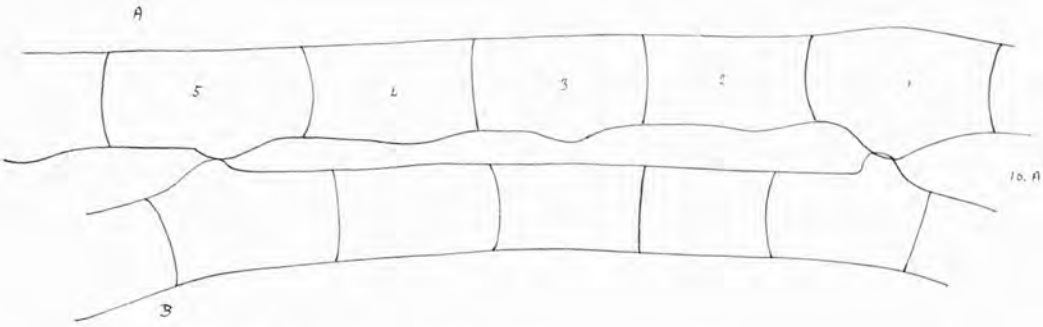
	11.0. am. 9.6.29	11.50. am. 11.6.29
11. A 1.	 Odd cell B	 Odd cell B
2.		
3.		

	10.30. pm. 7.6.29	1.10. pm. 9.6.29
12. A 1.		Filaments very much
2.		broken up.
3.		
4.		
5.		
6.		
7.		
8.		
9.	 Fil: A. Conjugating with fil: C.	 Fil: A. Conjugating with fil: C.
10.		
11.		
12.		

13



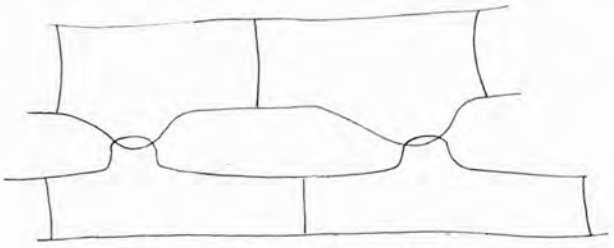
6.15. P.M. 11.6.29



10. A.M. 11.6.29

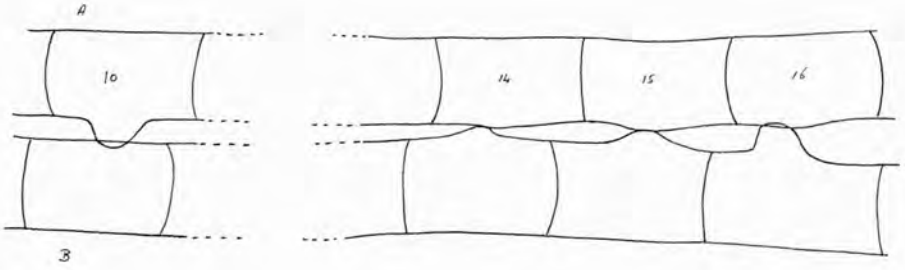
CASES FIGURED BUT NOT TABLED.

18



Wide & narrow filaments conjugating.

19



1st Papilla from A.

1st Papillae from B.

No.	TIME OF OBSERVATIONS.			
	6.15.pm. 11.6.29	10.0.am. 14.6.29		
13. A 1. A ♀		A shows female		
2.		swollen cells		
3.				
4.				
5.				
	11.50.am. 7.6.29	8.0.am. 8.6.29	9.0.pm. 9.6.29	
14. A 1- ∞	A, B & C Touching	from 2 cells	from 4 cells	
		from 2 cells		
	10.30.pm.	10.0.pm. Filaments much broken up.		
15. A 1- ∞	A, B & C touching	from a few cells		
	12.5.pm. 9.6.29.	10.0.am. 10.6.29 Filaments much broken up.	11.0.am. 11.6.29.	
16. A 1- ∞	Tangle of threads	few cells	few cells	
	12.35.pm. 9.6.29.	8.0.pm. 9.6.29.	11.50.am. 11.6.29.	
17. A 1-3.			B shows female cells	
3 ♀ 4.			further along the filament	
5.		Odd	Odd	
6.				
7.		Odd	Odd.	
8.				

DESCRIPTION OF PLATES.

PLATE I.

Photographs of living filaments of S.varians x 250.

Photo. 1. An early stage of conjugation in S.varians. Case 43 photographed at 8.30 p.m. x 250.

Photo. 2. The same cells photographed at 12 midnight 3½ hours after the first photograph. x 250.

Photo. 3. The same cells photographed at 7.30 a.m. 11 hours after the first photograph. x 250.

PLATE II.

Photographs of living filaments of S.weberi x 420.

Photo. 4. An early stage of conjugation in S.weberi. Case 20 photographed at 5. p.m. x 420.

Photo. 5. The same cells photographed at 1.30 p.m. 20½ hours after the first photograph x 420. Note that division has occurred in both filaments. Diagrams of these photographs accompanying Case 20.

Photo. 6. An early stage of conjugation in S.weberi. Case 10 photographed at 7.30 a.m. x 420. A drawing (made with camera lucida) of the later stage of conjugation accompanies Case 10.

PLATE III.

Photographs of living filaments of S.weberi x 250.

Photo. 7. An early stage of conjugation in S.weberi. Case 9 photographed at 9.30 p.m. x 250.

Photo. 8. The same cells photographed at 11.30 a.m. 14 hours after the first photograph. x 250.

Photo. 9. A typical early stage of conjugation in S.weberi x 250.

PLATE IV.

Photographs of living filaments x 250.

Photo. 10. A conjugating filament pair of S.varians. Case 63 photographed 13½ hours after the first signs of

conjugation were noted x 250.

Photo. 11. A typical early stage of conjugation in S.cataeniformis x 250. Mature conjugation tubes were formed between these filaments but a further photograph could not be obtained.

PLATE V.

Copies of paintings of portions of filaments of S.cataeniformis stained with dilute nile blue sulphate or 2% osmic acid.

Fig. 1. A filament collected on March 18th 1929 and treated with nile blue sulphate. There was a large amount of blue precipitation which was unaltered by dilute glycerine. The walls were at first stained purple but this rapidly faded.

Fig. 2. A filament collected at the same time and treated with osmic acid. The copious black precipitation was unaltered by dilute glycerine.

Fig. 3(a). A filament extracted from the culture under artificial light on March 20th 1929 and treated with nile blue sulphate. The protoplasm was coloured blue but very little blue precipitation could be seen. The purple colour of the walls faded while the filament was being painted but the blue colour remained.

(b). The same filament after the addition of dilute glycerine. The blue colour appeared to be confined to the outer protoplasmic layer.

Fig. 4. A filament extracted from the culture under artificial light on March 16th 1929 and treated with osmic acid. The precipitation was very slight (Cf. fig. 2).

Fig. 5. A filament extracted from the pond on March 20th 1929 and treated with nile blue sulphate. The walls were uncoloured but the blue precipitation was produced immediately. The filament was kept in a damp

chamber and painted on March 22nd its appearance being quite unaltered. There was no change in the blue precipitation after the addition of dilute glycerine.

PLATE I.

Photo 1.

Photo 2.

Photo 3.

PLATE 2.

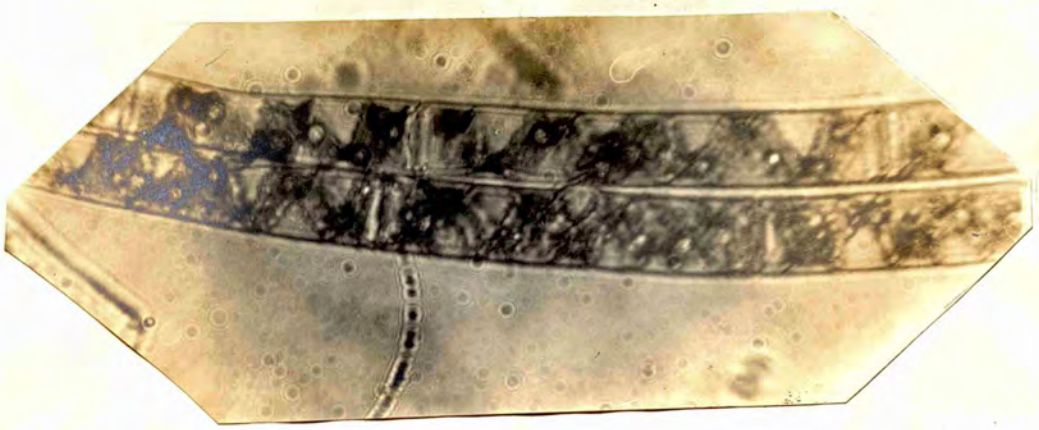


Photo 4

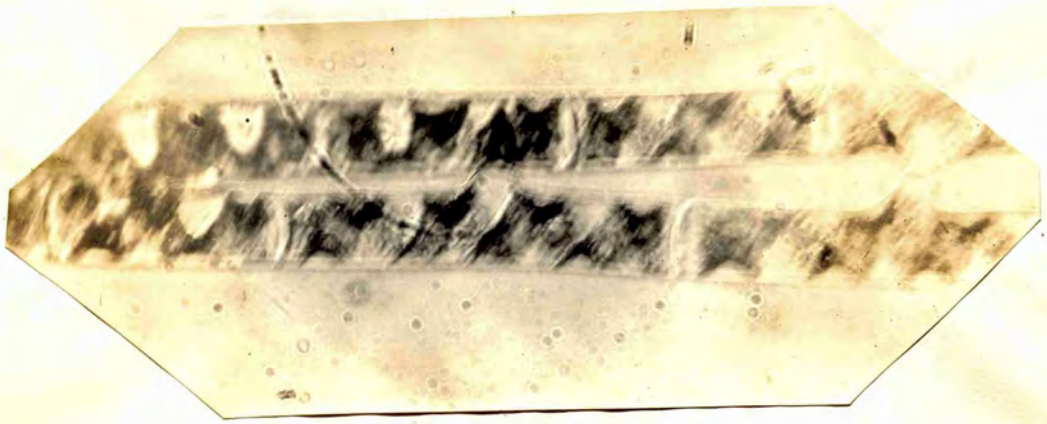


Photo 5.

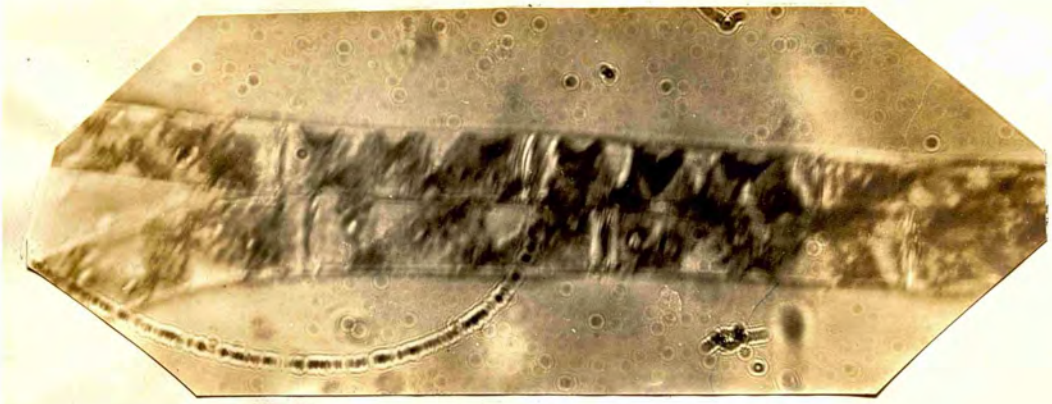


Photo 6

PLATE 3.

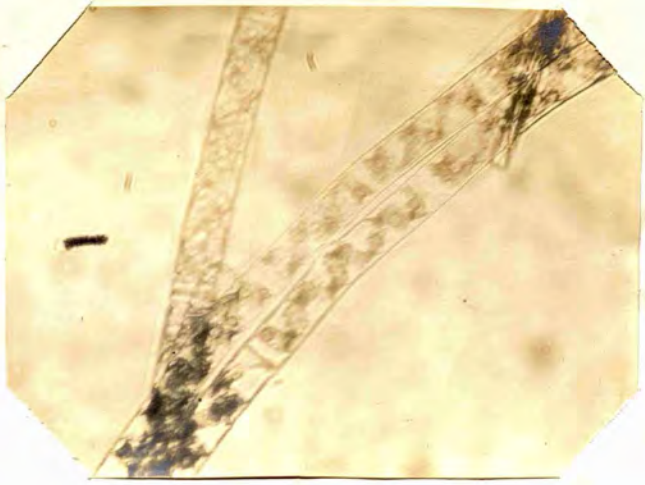


Photo 7.

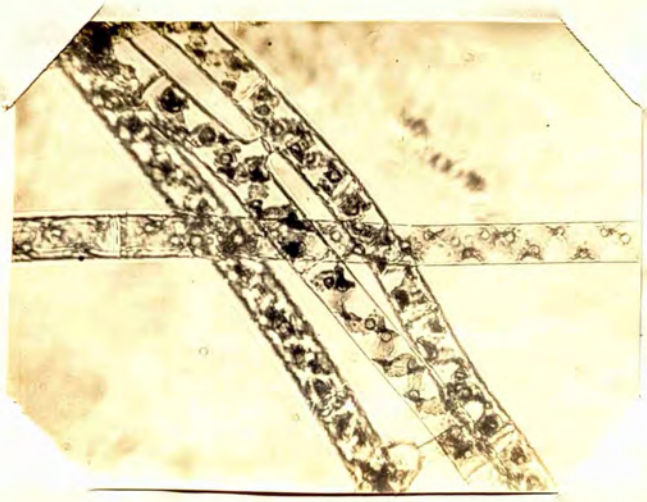


Photo 8.

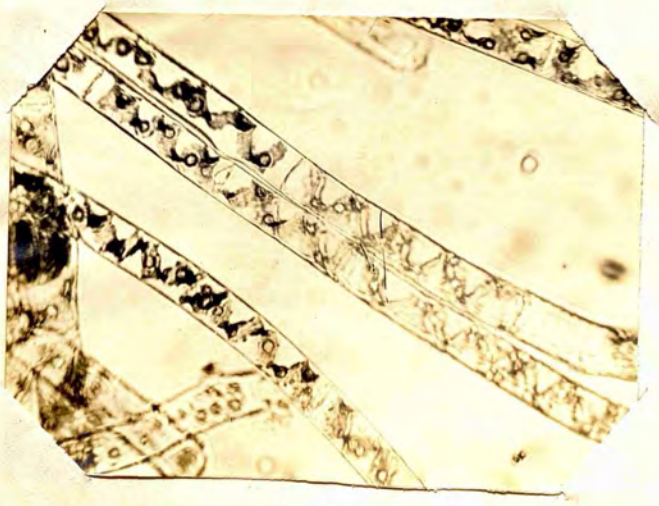


Photo 9.

PLATE 4.

Photo 10.

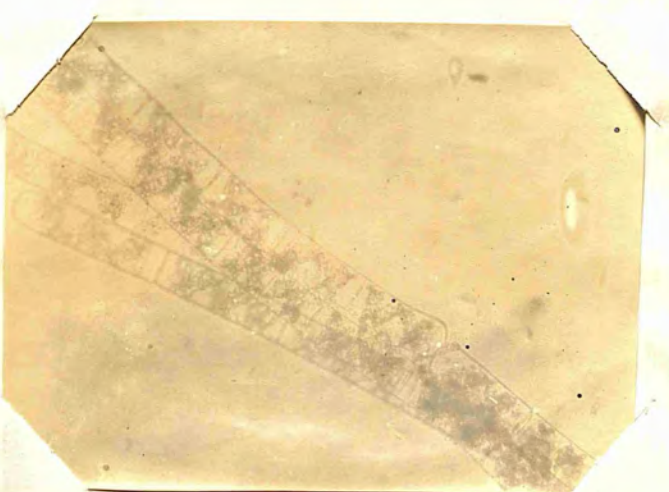


Photo 11.

PLATE. 5.

Fig. 1.

Fig. 2.

Fig. 3a

Fig. 3b.

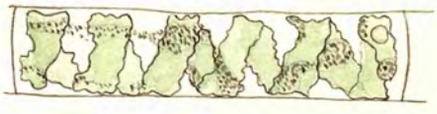


Fig. 4.

Fig. 5.