"SOME OBSERVATIONS MADE ON THE GROWTH OF ŒDOGONIUM SP."

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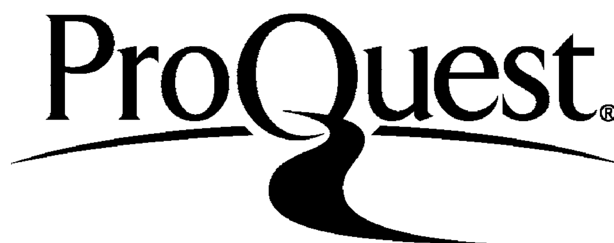

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ABSTRACT

SOME OBSERVATIONS ON THE GROWTH OF ŒDOGONIUM SP.

The growth of an unidentified species of the green fresh water alga Œdogonium has been investigated under natural conditions and under varying conditions of culture.

The material used in the investigation was collected from a pond in the Botany Garden, Royal Holloway College. Cultures were established in the laboratory in a number of different solutions of which soil extract solution proved to be the most satisfactory.

Observations were made on filaments of different ages both in the material grown in the pond and in the material grown in culture, and records were made of:-

1. The variation in the cell size of the filaments by means of measurements of cell length;
2. The distribution and the size of the groups of caps which were found in the filaments.

Attempts were made to discover whether the addition of growth substances to the culture medium affected the growth of the filaments. The substances used singly in the various cultures were as follows:-

1. Yeast extract
2. Thiamine
3. Indole-3-acetic acid
4. α-naphthalene-acetic acid.

Records similar to those made on the filaments from the pond and from soil extract solution were repeated on

/these

these cultures. Attempts were also made to investigate the effect of these substances on the rate of cell division in the filaments.

Cultures were set up with the addition of colchicine to find the effect of this substance on cell division in Oedogonium sp.

An attempt has been made to use these results to interpret the general pattern of growth and development in the species of Oedogonium used in this investigation.

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SOME OBSERVATIONS ON THE GROWTH OF OEDOGONIUM SP.

1. INTRODUCTION

Early in 1950, an opportunity arose to study the growth of certain freshwater algae in their natural habitat and in laboratory culture simultaneously. The scope of the investigation was widened to include a study of the effects of some growth promoting substances and of colchicine on the cultures.

For the purpose of this study Oedogonium sp. was chosen partly because of its abundance at Royal Holloway College, the ease with which it may be cultured and because its peculiar method of cell division makes it possible to determine precisely where growth is about to take place and where growth has occurred. An additional reason in favour of using a filamentous alga was that practically all the work concerning the effects of growth promoting substances has been done on cultures of unicellular algae.

The colchicine produced effects so different from the normal growth in culture or growth in culture with additional growth promoting substances, that the results of the work on the effects of colchicine will be considered separately.

The classic work on the growth of fresh water Chlorophyceae under controlled conditions is that of Klebs (1896) which was concerned primarily with the effect of different physical conditions upon reproduction. Very little work appears to have been done on the effect of culture conditions on vegetative growth in the filamentous Chlorophyceae, although there is considerable literature on the growth of unicellular Chlorophyceae under controlled conditions (Bristol-Roach (1926) (1927), Pearsall and Loose (1937), Pratt (1940) (1941), Bold (1942)).

Work on growth promoting substances in the Chlorophyceae has been confined largely to the unicellular forms (Yin (1937), Leonian and Lilley (1937) (1941), Pratt (1938), Brannon and Bartsch (1939) and Brannon and Sell (1945)). Ulva and Enteromorpha have been used as experimental material by De Valera (1940), Kylin (1941), and Suneson (1942). The results of some of this work are contradictory but the general conclusion reached is that growth promoting substances do not affect the cell size in the algae investigated but that the rate of cell division is increased by their presence.

Some effects of colchicine on the growth of Cladophora, Oedogonium and Hydrodictyon have been observed by Gorter (1943).

There is little known concerning the factors which influence the growth of Oedogonium. According to Tiffany (1936), the genus tolerates a wide range of pH as some of

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the species grow at pH 3.4 - 6.8 while others grow at a pH of 8.8; phosphates, nitrates and perhaps calcium and magnesium salts are important in its growth. An alteration in the conditions of light, temperature or culture medium causes an increase in zoospore formation. These effects are summarized by Pringsheim (1949). Gussewa (1930) states that the amount of free carbon dioxide is the important factor causing zoospore formation. According to Mainx (1931), sexual reproduction is likely to occur if the medium is slightly alkaline.

2. MATERIALS AND METHODS USED IN THE INVESTIGATION

The *Oedogonium* used in this investigation was obtained from a small cement pond in the Botany Garden, Royal Holloway College, the level of which is maintained by tap water during dry periods. It was present at all times of the year on the sides of the pond and as an epiphyte on various water plants including *Typha latifolia*. Growth was largely concentrated close to the water level of the pond. The filaments were in greatest abundance during April to October and were then found in addition on the petioles of an unnamed waterlily. Growth appeared to be perennial since filaments of all ages were found in the pond at the beginning and the end of the winter and there was little cell division in the filaments during this period of the year. Zoospore formation was infrequent during October to April.

It has, unfortunately, been impossible to name this *Oedogonium* specifically, since at no time in the present investigation nor in previous observations have sexual organs been found.

The cells are between 40 - 240 μ long and 24 - 40 μ wide. There are, however, two distinct groups of vegetative cells in the filaments: there are cells of 140 - 240 μ long which rarely exceed 32 μ in diameter and there are cells of less than 140 μ with a diameter of 32 - 40 μ. The shorter cells are a darker green colour and sometimes

/they

they tend to be broader at their upper end. The basal cell of the filament is usually 80 - 120 μ long and has as average diameter of 40 μ . This cell has a holdfast which is a deeply lobed circular plate with an irregularly serrated margin (Fig.1,1(a)). The apical cell of the filament does not differ appreciably from the other cells of the plant.

Certain isolated cells in either an apical or intercalary position in the filament may become greatly enlarged (Fig. 2). These cells always have a group of caps and measure as much as 144 - 200 μ in length and 52 - 92 μ in diameter. The contents of these cells are dark green. Generally the two cells immediately beneath the enlarged cell show a smaller increase in diameter. It is believed that these cells are akinetes. Occasionally the contents of the enlarged capped cells become rounded and a wall is formed around them. These cells appear to have degenerated and may be unfertilized oogonia although no pore or split has ever been seen in the cell wall.

Normal growth in the pond was investigated in two ways. In the first method, groups of filaments were removed from the pond. These were measured for cell size and were examined to find the distribution of caps in the filaments. During April to October, it was possible to distinguish the long filaments of the previous season on the pond wall and the filaments of the current season on the water-lily plants.

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In the second method, Oedogonium was induced to grow on slides placed just beneath the surface of the water in the pond. Each slide had a hole bored in it at one end and was attached to a piece of string across the pond. The slides were temporarily removed at intervals and the filaments on them were examined without disturbing the holdfasts.

Preliminary cultures were set up using Knopp's solution, an inorganic tissue culture solution (White (1943)), solution 42 (Reynolds (1950)), pond water and soil extract solution. The best growth was produced in soil extract solution and this was used in all the subsequent culture work. Mainx's method of obtaining the soil extract and of making up the soil extract solution was used (1927).

Although the soil solution extract proved to be the best culture medium for Oedogonium filaments, it is not possible to repeat the exact conditions of the previous extract when a fresh one is made. This is a disadvantage in controlled experiments.

The pond water had a pH 7.0 and this pH was used for the culture medium. The soil extract only needed a slight adjustment with dilute sodium hydroxide or dilute hydrochloric acid to bring it to this value. The pH adjustment in the early part of the work was done with a comparator and later electrometrically.

Attempts were made to produce the experimental material

/as

as a clone but these were not very successful. Single filaments were washed several times in distilled water and placed in sterile soil extract solution. Zoospores were formed by these single filaments but they only produced a small number of filaments in each culture and their growth was very slow in sterilized solution. It was not practicable to use any of the small clones to set up the cultures. Since this material has only been found in the vegetative state, it seemed likely that the genetical constitution of the individual filaments would not differ greatly.

Cylindrical pyrex vessels (approximately 2¾" diameter by 2" deep) were used for the cultures. 100ccs of culture solution were placed in each vessel and this quantity came to within an inch of the top. Oedogonium filaments growing on leaves of Typha latifolia were placed in each vessel. Pieces of waterlily petioles were unsatisfactory to use as they became mucilaginous and the solution became brown. Two glass slides were placed back to back and were put diagonally in each vessel. The vessels, covered with a glass lid were placed in a window facing north-east and were illuminated continuously from above by a 40 watt lamp. The lamp was 16 inches above the vessels. It was not practicable to introduce temperature control for the greater part of the experimental work.

Following this change of growing conditions, zoospore

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formation was usually very rapid and within a few days zoospores had become attached to the vessel walls and to the slides just beneath the level of the culture medium. The greatest concentration of attached cells was towards the source of greatest light, although some filaments developed on the base of the vessels.

When the cultures were established, the propagating filaments were usually removed. The well established cultures were aerated for two hours every few days by means of a small pump. The culture solutions were changed every ten days. This renewed the nutrients and eliminated some of the other filamentous and unicellular algae which were introduced by the original filaments and which grew quickly in soil extract solution. Some of the contamination remaining on the filaments was removed by a small camel hair brush. This was done as little as possible because the filaments broke very easily. The aeration of the cultures decreased the contamination in the filaments.

When growth substances were used in the culture work they were dissolved in soil extract solution. Yeast extract (Difco), thiamine, indole-3-acetic acid and a-naphthalene-acetic acid were used in the following concentrations:-

Yeast extract	100 mgs. per litre	(Reynolds 1950)
Thiamine	0.1 " " "	(White 1943)

/indole

Indole-3-acetic acid	0.1 gms	per litre
	0.05	" "
	0.025	" "
	0.0125	" "
	0.00625	" "
a-naphthalene-acetic acid	0.4 gms	" "
	0.3	" "
	0.2	" "
	0.1	" "
	0.05	" "
	0.025	" "
	0.0125	" "
	0.00625	" "

Soil extract solution was used in the control cultures.

The yeast and thiamine were easily dissolved but the indole-3-acetic acid and the a-naphthalene-acetic acid were difficult to dissolve completely, even by boiling, unless a small quantity of 95% alcohol was first added to the crystals. Heat does not destroy the activity of these substances: Brannon and Bartsch (1939) found that they could autoclave indole-3-acetic acid without altering its activity. The solutions of indole-3-acetic acid and a-naphthalene-acetic acid were made up in the strongest concentration required in a series of cultures and diluted to give a range of concentration. These solutions needed considerable adjustment to bring them to pH 7.0 before the

/dilutions

dilutions were made. The solutions containing yeast extract and thiamine were approximately pH 7.0.

Cultures were grown for several months in indole-3-acetic acid and a-naphthalene-acetic acid but the cultures in yeast extract and thiamine could only be maintained for a few weeks because zoospore formation occurred in so many cells over this short period that the filaments disintegrated: moreover the bacterial contamination became excessive. The yeast extract and thiamine solutions were changed more frequently than at 10-day intervals if they became badly contaminated.

The colchicine cultures were set up using 1.0 gms and 0.1 gms in a litre of soil extract solution: the pH of these solutions did not require adjustment. The filaments used to set up the cultures were not removed from the vessels and were kept under observation for two months. In addition, filaments of 2 - 3 cells growing on slides were placed in the colchicine solution for a week and were then fixed.

When observations were made on the filaments from the pond and from the cultures, records of the cell size and the distribution of the groups of caps within a filament were made simultaneously: 10 filaments were examined in each group. Since the cells varied but little in diameter, measurements of cell length were used to show cell size. These measurements were taken in order from the base of the

/filament

filament and are recorded in the accompanying tables to the nearest 4μ . As single caps were often difficult to distinguish, records of cells with only one cap are not included in the tables.

Cell shape was recorded by means of a reflex drawing apparatus.

Oedogonium filaments proved to be very sensitive to environmental changes and when continual observations were being made, care had to be taken to cut down the illumination and to prevent any pressure of the coverslip on the filaments. Even so, entire filaments or some cells in the filaments were often killed in the process of observation.

The cell walls, the groups of caps and the rings were easily seen in the living condition but not so the nucleus. Cytological techniques were principally used on the colchicine treated material but difficulties were encountered in making permanent preparations of the filaments. The most satisfactory results were obtained by fixing the material in weak chromo-acetic fixative or in Navaschin's solution. The material was mordanted in 4% iron alum before staining it with aceto-carmine. The preparations were mounted in glycerine jelly.

3. EXPERIMENTAL DATA AND RESULTS

A. Normal Growth in the Pond

Long filaments which had developed in the previous season and filaments developing in the current season on slides and on waterlily petioles were used to obtain measurements of cell size and to find the distribution of the caps. The pattern of cell division was observed in young filaments on the waterlily petioles.

(a) Long filaments developed in the previous season.

Many of the long filaments taken from the pond wall consisted of 70 to 100 cells. The appearance of these cells is shown in Fig. 3 and 3a. Enlarged cells as described on page 5 and shown in Fig. 2 were found in the filaments throughout the year. Variation in cell size was obvious in these filaments and the measurements of cell length ranged from 40 - 240μ and the cell width from 24 - 40μ. Although the range in cell length and in cell width was continuous, it appeared that there were two types of cells in the filaments. These cells were distinguished by their shape and by their colour. There were longer, pale green cells which were usually greater than 140 μ long and were about 28 μ wide and there were shorter, darker green cells which were less than 140 μ long and were about 36 μ wide. A length of 140 μ was used to distinguish long and short cells as it was found that the majority of the cells in

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the young filaments were longer than 140μ and that the shorter wider cells only developed as the filament aged. The longer cells were usually grouped in the basal part of the filament and the shorter cells were in isolated groups in the apical part of the filament. The distribution of cell length measurements in the filaments taken from the pond in July 1951 is shown in Table 1. and in Fig. 4. The mean cell length in 10 of these filaments was 160.1μ and the standard deviation was $\pm$34.1.

Intercalary groups of 2 - 6 caps were frequent in the filaments. Groups of more than ten caps were rarely found, although as many as 20 caps were counted in a single group. Abnormalities were found in these large groups and these are described on page41 and are shown in Fig. 28 (8, 9, 10 and 11). It was difficult to distinguish every single cap present in the filament because of epiphytic growth but groups of two caps were easily seen. Small groups of caps or single caps were seen on some of the apical cells but in the majority of the filaments the apical cell was devoid of caps even though ring formation was common at the apex of the filaments. The apical cells were frequently seen to lose single caps or groups of caps. (Fig. 5).

Cell division was not confined to definite regions in the filament. It was not unusual to find contiguous cells each with a number of caps or to find small groups of caps between two larger groups. Several adjacent cells were seen to form

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their first ring at the same time. In many-celled filaments little cell division occurred in the long cells at the base of the filament: there were only a few small groups of caps found in these cells.

Theoretically, the number of caps in the filaments should be one less than the number of cells. The number of cells counted in these filaments always exceeded the number of caps. This discrepancy was accounted for by the loss of caps from the apical cells, by the inability to count all the single caps and by the disintegration of the filaments following zoospore formation.

The examination of the fully developed filaments from the pond raised two problems which required further investigation. There was, firstly, the problem of the great variation in cell size and secondly, that of determining the pattern of cell division in the filaments. Both these problems were investigated further by using filaments which were developing in the first growing season.

(b) <u>The development of filaments in the first season of growth</u>

Filaments of one, two or three cells which had been grown on slides in culture were placed in the pond November 17th, 1950. These filaments were started in culture because an earlier attempt to establish zoospores on slides in the pond in October was unsatisfactory. Filaments from the slides were examined at intervals until September 1st, 1951 so that any changes in the cell size of the filaments could

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be observed throughout the growing season. It was impossible to measure the same filaments each time the slides were removed from the pond. These examinations were made at monthly intervals because filaments often died after temporary removal from the pond. Until April, it was possible to recognize the filaments which had been growing on the slides since November. When zoospore formation began in the pond during April, the identification of the original filaments on the slides was less certain. In the subsequent examinations the longest filaments were assumed to be the older filaments.

During December and January, the surface of the water was often frozen and there was no cell division in the filaments on the slides. The first measurements were made on February 17th. The filaments then averaged four cells in number and the cell length ranged from 80 - 140μ. Several rings were found and these were mostly in the apical cells. One ring was found in a basal cell of a filament. By March 21st, the cell number had increased to five or six cells but there was no alteration in the cell length. By April 19th, when the filaments were 10 - 20 cells long, isolated cells of 140 -168μ long had developed and intercalary groups of 2 - 4 caps had formed.

The filaments examined on May 9th were on a slide which had not been previously removed from the pond. When compared with the previous record these filaments showed no

/increase

increase in cell number but the newly formed cells showed an increase in cell length. The newly formed cells measured as much as 208 μ long and they were a paler green colour than the first formed cells in the filaments. Their distribution is shown in Fig. 6. Zoospores became plentiful in the pond during April and filaments of 2 - 4 cells were covering the slides at the time when these measurements were made. The cell length in these young filaments was the same as that of the cells then forming in the older filaments (Fig. 6(a)). The cells which formed in the original filaments on the slides during February and March were smaller than those then developing in the young filaments as they had been formed before normal cell division began in the pond filaments.

On the slides which were examined on June 18th, the filaments measured were approximately 30 cells long and the majority of the cells were 140 - 200 μ long. Measurements made on July 17th, when filaments were 25-42 cells long, showed for the first time isolated intercalary cells of 60 - 140 μ in length. By September 1st, the numbers of these shorter cells had increased to such an extent that they formed the greater proportion of the filaments. These shorter cells were found singly or in groups in these filaments composed of 32 - 68 cells. Isolated groups of enlarged cells were found in these filaments and they

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resembled those shown in Fig. 2.

Before the first growing season was ended, the longest filaments growing on the slides showed the same variation in cell length measurements as was found in the long filaments which are described in the previous section. The two types of cells were present in both these groups of filaments of varying age although the filaments from the slides were younger and much shorter.

Cell lengths of filaments at different times of the year are recorded in Fig. 7 and in Table 2. Changes in the distribution of cell length in the filaments are indicated by the mean values for cell length and by the Standard deviations (Table 3). The larger deviations correspond with the increased proportion of the shorter cells in the filaments.

TABLE 3		Mean cell length	Standard deviation
17.11.50	- 17.2.51	109.0 μ.	$\pm$ 17.9
"	- 21.3.51	113.6	$\pm$ 17.8
"	- 19.4.51	119.0	$\pm$ 21.2
"	- 9.5.51	142.0	$\pm$ 43.2
"	- 18.6.51	177.4	$\pm$ 35.6
"	- 17.7.51	157.2	$\pm$ 35.2
"	- 1.8.51	153.8	$\pm$ 46.9

A more satisfactory indication of this change in the composition of the filaments during the time of the observations is given by dividing the range of cell length

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into units of 20 μ and calculating the percentage number of cells in each group at each examination (Table 4).

Similar changes in filament composition were observed in filaments growing on the waterlily petioles. These filaments had been left undisturbed in the pond: a series of them was measured late in May and another in the middle of July. In May they were 12 - 20 cells long and were at the most six weeks old. The cell length was 140 - 220 μ (Table 5). The mean cell size in individual filaments was as shown in Table 6.

TABLE 6	Mean cell length	Standard deviation
	158.8 μ	$\pm$ 26.2
	177.0	$\pm$ 20.3
	156.6	$\pm$ 19.3
	172.0	$\pm$ 23.6
	169.2	$\pm$ 31.5

By July filaments of 40 cells were found and the shorter cells of 60 - 140 μ long were evident in the apical part of these filaments. The average cell length of ten filaments was 153.3 μ and the standard deviation was $\pm$ 27.4. The variation in cell length was as great, after three months, as that in the long filaments from the pond wall (Table 7).

(c) <u>The effect of the time of year on cell length and Zoospore formation.</u>

It seemed possible that the shorter cells had formed

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in the filaments developing in the first season of growth because of changes in the environment. A second series of slides was therefore placed in the pond for short periods between June 18th, 1951 and October 29th, 1951 to investigate the effect of the time of year at which a zoospore was formed on the cell lengths of the young filament developing from it. The slides were placed in the pond for the following periods:-

June 18th, 1951	-	July 18th, 1951
July 6th	-	" 18th
" 17th	-	August 6th
" 30th	-	" 6th
" 30th	-	" 22nd
August 6th	-	" 22nd
" 30th	-	October 3rd
October 3rd	-	" 29th

Zoospore formation was frequent until early October and filaments developed on all the slides placed in the pond. When these filaments were examined at the end of each of the above time intervals, it was found that practically all of their cells were 140 - 220 μ long (Table 8). This indicated that the length of cells forming in the developing filament was not influenced by changes in the external conditions of the pond during June to October. The formation of the smaller cells of 60 - 140 μ long in the filaments which had been growing in the pond for three months, was not caused by the

/decrease

decrease in the temperature of the pond water.

Cells of 140 - 220 μ long were formed in the young filaments during the growing season irrespective of the time of their origin. The formation of the shorter cells in the filaments appeared to depend on the stage of development in the filament rather than on the external conditions of growth.

(d) The pattern of cell division in the developing filaments.

Young developing filaments were investigated to discover the position of the cell divisions. Filaments of varying age were scraped from the waterlily petioles in July 1951. The cells in these filaments numbered from 1 - 40 and the cell length ranged from 140 - 200 μ . An attempt was made to find the pattern of cell division in these filaments by closely examining each cell to locate either a cap or the apical break in the cell wall formed by a stretched ring (Fig. 8). There was little epiphytic growth on the filaments and single caps were easily recognized as a slight swelling at the apex of the cell.

Ring formation was often seen in the apical cells of the filaments, but few of these cells accumulated groups of caps. Single caps were frequently seen to separate from the apical cell. Occasionally, in filaments of approximately five cells, an apical group of caps was found with the cap number one less than the cell number.

In the majority of the filaments of 3 - 12 cells no

/intercalary

intercalary groups of caps were found ~~but as the cell~~ but as the cell number increased above 10 or 12, small groups of caps were present (Fig. 8). The apical cell continued to divide even after intercalary growth began. At first intercalary growth tended to occur in isolated positions in the filament but very soon, the divisions spread to neighbouring cells. This was evident in filaments with approximately 20 cells.

In spite of the absence of intercalary growth until the filaments were 10 or 12 cells in length, the majority of the filaments had no groups of apical caps. Approximately ten caps had therefore been lost from the apical cell before intercalary growth began. For example in Fig. 8 in filaments of 20 and 38 cells there were only 7 and 25 caps, respectively, instead of the expected 19 and 37. If it is assumed that approximately ten caps had been lost from the apical cell before the intercalary groups were formed, a closer agreement between the observed number of caps and the theoretical number is obtained. The correlation of the cap number and the cell number in even longer filaments is even more difficult because the apical cell continues to lose caps after intercalary growth begins. The correlation of the two numbers in very long filaments is impossible because of the continued loss of caps from the apical cell and because of zoospore formation.

B. Growth of the Filaments in Culture

Two types of Oedogonium cultures were set up. There were those in which the filaments had begun their development in the pond and those in which the filaments had entirely developed in soil extract solution. Both types of filament were examined to find the effect of the conditions of culture on the cell size and the distribution of the groups of caps. Some observations were made on the order of cell division in the young filaments and on the cell size and cap grouping in filaments of a clone. Limited observations were made on the effects of external factors on the growth of the cultures.

(a) Cell size and cap grouping in filaments from the pond grown in culture.

Oedogonium filaments, which had started development on leaves of Typha latifolia in the pond, were placed in soil extract solution and also in pond water. These filaments were approximately 20 celled when placed in culture and they had few caps. The cell length varied between 140 - 220μ. After two months in culture the cell number in these filaments had doubled but the majority of these new cells, in both soil extract solution and in pond water, were less than 140μ in length (Figs. 9, 10, 11 and 11(a)). The shorter cells were usually confined to the apical end of the filaments. The range in cell length of these filaments

/after

after they had been in culture was similar to that of filaments grown completely in the pond (Table 9).

(b) Cell size and cap grouping in filaments entirely grown in culture.

The range in the cell length in the filaments which developed entirely in culture was not as great as in the filaments developed entirely or partly under natural conditions (Table 10). Very few cells of the longer length group were found. Most of the cells were in the group of 40 - 140 μ long. Sometimes the longer cells were entirely lacking from the basal part of the filaments. The mean cell length in the filaments from several cultures grown throughout the experimental period was not constant. The lowest value found was 86.5μ and the largest value was 139.8μ. Approximately half of the filaments examined had small groups of 2 - 4 caps on the apical cell and occasionally apical groups of 10 or 12 caps were counted; the rest had no apical caps at all. The filaments seemed to retain their caps for a longer time in culture than they did under natural conditions. When intercalary caps occurred the number of caps in each group was small.

Filaments developing in culture were never as long as those of comparable age growing on slides in the pond.

(c) The order of cell division in filaments developing in culture.

To make possible the mapping of the order of cell

/division

division a method was devised whereby identified filaments could be examined at frequent intervals. Narrow glass rings, approximately ¾" diameter and ¼" deep, were suspended in cultures of Oedogonium so that zoospores would become attached to them. The ground end of the glass ring was divided into segments by pencil markings so that the position of the attached cells could be recorded (Fig. 12). A double line was placed in one position on the ring so that it could be orientated. The glass rings were placed in culture and the identified filaments were examined daily. Records were made of cell length, of cell shape and of the position of cell division. During the examination, the glass rings were placed in a small petrie dish of soil extract solution and the filaments projected from them.

Observations were continued until the filaments became overgrown with other algae or until zoospore formation caused them to disintegrate.

The records of cell length were constant from day to day unless a ring had formed and stretched in a cell. Once a ring stretched to form a cell there was no further increase in the size of the new cell: there was a decrease in length of approximately 4 μ in a cell which had divided. Cell division often took place during the night but rings were seen to stretch during the daytime. Usually it was possible to locate the position of the new cell by the record of the formation of a ring in previous observations. If, between

/observations

observations, the cell number increased without a ring being recorded, the position of the upper daughter cell could be located by comparison with the records of cell size and cell cell shape on the previous day and recognised by its brighter green colour. The formation of a new intercalary cell was easier to recognise than the formation of a new apical cell because the latter frequently lost its cap between the observations.

These filaments did not form more than nine cells before they became overgrown or before they completely disintegrated through zoospore formation but it was evident that apical growth alone or apical and intercalary growth was taking place in the young filaments. This was in contrast to the order of cell division in the majority of the young filaments developing in the pond.

The order of cell formation in some of the observed filaments is recorded in Fig.12(a). The cells of the filaments are represented diagrammatically and the Figure is a combination of two sets of data. When the initial cell of a filament divided the upper daughter cell was labelled 1 and when this in turn divided the upper daughter cell formed was labelled 2, so that a series of numbers placed beside the filaments represent the order in which the cells were formed in the filaments. The date when the cell formed is recorded beneath it. The arrows placed beneath the filaments indicate the cells which were lost in zoospore formation ; the

/ date

date of zoospore formation is also recorded. When cells have been lost from the filament, brackets are placed round the numbers representing the order of the formation of these cells.

If the apical cell alone was responsible for cell formation in the filament, the numbers representing the order of cell formation in that filament would be in a series of ascending order. For example, in a filament showing such apical growth, the order of cell formation is represented in Fig. 12(a) in the following way:- 1, 2, 3, 4, 5, 6, 7. In several of these filaments the apical part was lost in zoospore formation. After this happened the cell in the apical position frequently divided and apical growth was continued. An example of this is shown in the following series in which brackets indicate where the cells have been lost:- 1, 2, 3, 4, (5, 6, 7,) 8, 9. Where there is intercalary as well as apical growth, the numbers representing the order of cell formation are not in an ascending series. An example of this is the series 1, 5, 2, 3, 4, 6. If apical and intercalary cell division was taking place simultaneously there is duplication of some of the numbers representing the order of cell formation in the filaments. Examples of this are seen in the following:- 1, 2, 3, 6, 7, 5, 4, 7. and 1, 2, 4, 6, 3, 5, 4, 5.

One celled filaments did not generally form a ring until

/approximately

approximately ten days after the development of the holdfast but there was variation in this respect. The rate of cell division in the individual filaments over the period of the observations differed. Cell division occurred at regular intervals in some filaments and in others it occurred at irregular intervals. Sometimes a ring formed in a cell several days before it was seen to stretch. The variation in the rate of cell division in the developing filaments during 31 days is represented in Fig. 13. The decrease in cell number caused by zoospore formation is also indicated. When the final disintegration of filaments took place, the basal cell was left attached to the glass ring and it did not form a zoospore.

(d) Cell size and cap grouping in a clone grown under controlled conditions.

A series of clones were set up in an incubator designed to keep the cultures under constant conditions of temperature ($20^{o}C \pm 1^{o}$) and of illumination. The light was supplied by a 60 watt lamp from behind a glass screen. These conditions were not available until the later part of the experimental work was in progress.

Clones were established by placing a long filament in a vessel of sterilized soil extract solution. The solution was only changed infrequently to avoid loss of zoospores and the remains of the original filaments were discarded when the solution was first changed.

/Growth

Growth was very slow under these conditions and the filaments of the clones did not become very long. After six weeks, the largest clone was one with 17 filaments. These filaments were of different ages because some of the first formed filaments of the clone had produced zoospores.

The cells of these 17 filaments grown under controlled conditions showed the same variation in cell length as did the filaments grown in the laboratory with no control of temperature or light (Table 11).

There was no group of caps on the apical cell of the filament which gave rise to the clone. Of the 17 filaments of the clone two showed intercalary growth and nine had apical caps. Five of these nine filaments had apical groups of 3, 4 or 5 caps and the other four had single caps. These observations seem to indicate that the presence or absence of a group of caps on the apical cell is not genetically determined.

(e) The effect of temperature, light and pH on the growth of the filaments.

There was no temperature control available for most of the experimental work but since the same variation in cell size occurred in the filaments grown in the incubator as in those grown in the laboratory, it was inferred that the temperature did not effect cell size. It has been shown that the smaller cells developing in the fully grown filaments in the pond at the end of the growing season were

/not

not formed because of a decrease in temperature. The cells of the filaments withstood freezing+when the cultures were taken from the refrigerator zoospores were quickly formed.

It is possible that illumination, which in a pond shaded by waterlily leaves must be weak, exerts an influence on cell division and cell size and this may account for the differences observed between growth in culture and growth in the pond. At the time, it was not possible to carry out experiments to investigate the effects of illumination on growth. There was no difference seen in the growth of filaments in daylight or under the continuous light from a 40 watt lamp (Table 12).

When light intensity was further increased by the use of a 100 watt lamp there was a decrease in the rate of cell division: very few rings were seen in these filaments. This effect does not agree with the observations made by Freund (1928), who reported a decrease in the time interval between cell divisions in increased light in Oedogonium pluviale.

Increase in light intensity caused increased zoospore formation and a large number of the zoospores developed abnormal holdfasts. The contents of many of these cells were set free again as a zoospore: the zoospores escaped by means of a lid at the apical end of the cell. The

/formation

formation of these abnormal holdfasts was seen in the cultures illuminated by a 40 watt lamp but the numbers of these cells were relatively few.

The filaments growing in high light intensity were short because of the combined effects of increased zoospore formation and decreased rate of cell division (Table 13).

Table 13 High light intensity (100 watt lamp)

Cell numbers: 4, 3, 4, 5, 6, 4, 4, 2, 4, 4

Low light intensity (40 watt lamp)

16, 10, 10, 13, 12, 9, 10, 8, 10, 18.

Although the filaments in culture were grown in continuous light, cell division was not entirely confined to the night-time as rings were found to be stretching in filaments taken from the vessels during the day. Kretschmer (1930) examined *Oedogonium pachyandrium* and found that cell division usually occurred at midnight and that division could be induced at 2 - 3 p.m. in artificial light. Cell division frequently took place in filaments of *Oedogonium sp.* during the day, under natural conditions.

Attempts to investigate the control of pH on the growth of *Oedogonium sp.* were not successful. When series of cultures were set up with a range of pH of 4, 5, 6, 6, 5, 7, 8 and 10, the soil extract solution behaved as a buffer and the pH values gradually reverted to approximately 7.0. The filaments which had been placed in pH 6.5 and 7.0 were the only filaments which appeared normal when the cultures were

/examined

examined after a week: those in solution of pH 4, 5, 8 and 10 were dead. A series of phosphate buffer solutions ranging between pH 5.7 and 8.2 were made up according to Sorenson (Clarke 1927) using soil extract solution as the basis. The filaments were killed in all the solutions.

C. Growth in Culture With Additional Growth Substances

Observations of growth in yeast extract and thiamine cultures were only possible during short periods because of contamination by bacteria and increased zoospore formation, whereas growth in cultures with indole-3-acetic acid and a-naphthalene acetic acid was observed over a period of several months. Because of this, the effects of yeast extract and thiamine and of indole-3-acetic acid on growth were considered separately.

1. The effect of yeast extract and thiamine on cell size, rate of cell division and cap grouping.

The cultures were set up using soil extract solution and then, when they were established, the propagating filaments were removed from the vessels and the culture solution was replaced by soil extract solution containing yeast extract (100 mgs. per litre) or by soil extract solution containing thiamine (0.1 mg. per litre). This method of setting up the cultures helped to reduce the bacterial contamination in the yeast and thiamine cultures. Control cultures were set up using soil extract solution.

Filaments were removed from each culture after 14 days and again after 20 days. The cell length was measured and compared with that of the cells in the filaments in the control (Table 14). The shape of the cells which was recorded with a reflex drawing apparatus is shown in Figs. 15 (a), (b) and (c) and the appearance of the filaments after 20 days is seen in Figs. 16 (a), (b) and (c).

/The

The addition of yeast or thiamine to the solution had a definite effect on the size of the cells of the filaments. The cells were smaller than the cells in the control and cell lengths of 40 - 60 μ were frequently recorded. The cells were somewhat barrel shaped and this gave an unusual appearance to the filament. The cells were very dark green with a light patch indicating the position of the nucleus. The filaments in yeast extract had a peculiar jointed appearance and they broke easily into segments (Fig. 15(a)).

The mean cell length in the filaments from each solution was as follows (Table 15).

Table 15

	Mean cell length & standard deviation	
	after 14 days	after 20 days
Culture + yeast extract	94.0μ ± 20.0	80.0μ ± 18.8
" + thiamine	97.2 ± 23.6	76.4 ± 21.2
control	101.0 ± 14.0	107.2 ± 27.2

In yeast extract and in thiamine culture the formation of very small cells in the filaments was obvious after 14 days' growth and the numbers of these small cells increased with time.

The rate of cell division was increased by the addition of yeast extract and thiamine to the culture solution. During 14 days, growth was most active in the yeast extract and the number of cells in these filaments was from 10 to 19 (Fig.15(a), (b) and (c)). The number of cells in the filaments in thiamine varied from 5 to 12 and those in soil extract solution from

/5 to 8

5 to 8. The length of some of these filaments is shown in Table 16.

Table 16

	Cell number after 14 days									
yeast extract	16	19	12	10	11	13	12	12	14	14
thiamine	8	9	7	9	10	5	12	7	8	11
soil solution	7	6	6	8	7	5	6	5	7	7

After 20 days, zoospore formation had become so frequent in yeast extract and in thiamine cultures that it was impossible to determine the effect of the growth substances on the rate of cell division.

Very few groups of caps were found in any of these filaments and there was no increase in the number of apical cells which were accumulating caps.

2. The effects of indole-3-acetic acid and a-naphthalene-acetic acid on cell size, rate of cell division and the distribution of the caps.

When preliminary cultures of indole-3-acetic acid and a-naphthalene-acetic acid (0.2 gms. per litre) were set up, the filaments in indole-3-acetic acid were killed within a few days but those in a-naphthalene-acetic acid continued to grow. The concentration of indole-3-acetic acid was then reduced to 0.1 gms. per litre and that of a-naphthalene-acetic acid was increased to 0.3 gms. per litre. The filaments in these solutions were healthy and zoospores formed. Later the concentration of a-naphthalene-acetic acid was increased to 0.4 gms. per litre. Zoospores were formed and developed

/into

into filaments in this concentration.

Two types of culture were set up in both indole-3-acetic acid and a-naphthalene-acetic acid. In one, the propagating filaments were removed from the vessel when the culture was established and in the other, the propagating filaments were left in the culture vessel. A series of concentrations of indole-3-acetic acid or a-naphthalene-acetic acid was used for both types of culture. The concentrations used were:-

Indole-3-acetic acid	0.1	gms. per litre
	0.05	
	0.025	
	0.0125	
	0.00625	
a-naphthalene-acetic acid	0.4*	gms. per litre
	0.3	
	0.2	
* not used in the first series of experiments.	0.1	
	0.05	
	0.025	
	0.0125	
	0.00625	

Control cultures were set up in soil extract solution.

(a) The effect on cell size.

The propagating filaments which had cells of 140 - 220 μ long when placed in culture, were grown in these different solutions of indole-3-acetic acid or a-naphthalene-acetic

/acid

acid for three months. After this period the cell length in filaments taken from all the indole-3-acetic acid and a-naphthalene-acetic acid cultures was measured and was compared with that in the control culture.

The range in cell length of the filaments growing in all concentrations of indole-3-acetic acid and a-naphthalene-acetic acid was similar to that in the control cultures. Cells of 60 - 140 μ had formed in all cultures so that the addition of the growth substances in any of the concentrations used had not prevented the smaller sized cells from forming in culture. The cell lengths of filaments grown in a-naphthalene-acetic acid (0.3 gms. per litre) are compared with those of the control in Table 17. The distribution of cell length measurements in some of the filaments is seen in Fig. 17.

A more detailed examination was made of the filaments which had grown from zoospores in varying strengths of indole-3-acetic acid or ~~0.00625~~ a-naphthalene-acetic acid. These cultures were grown over a period of three months.

Measurements of cell length in a few of the filaments grown in varying concentrations of indole-3-acetic acid and a-naphthalene-acetic acid are given in Tables 18 and 19. The means and the standard deviation for cell length in ten filaments grown in the series of cultures using both these substances are shown in Table 20.

/Table 20

Table 20

Indole-3-acetic acid cultures (0.1 culture became very contaminated.)	Mean Cell Length	Standard Deviation
.05 gms. per litre	104.68 μ	± 20.12
.025 " " "	121.48	± 20.44
.0125 " " "	108.80	± 30.0
.00625 " " "	100.8	± 18.92
Soil solution	113.0	± 18.28
a-naphthalene-acetic acid cultures		
0.4 gms. per litre	122.16 μ	± 25.2
.2 " " "	119.04	± 18.8
.1 " " "	117.08	± 16.8
.05 " " "	113.04	± 19.6
.025 " " "	112.04	± 17.2
.0125 " " "	97.80	± 18.4
.00625 " " "	104.80	± 18.0
Soil solution	105.04	± 19.2.

The range of the measurements of cell length found in the filaments grown in each concentration of indole-3-acetic acid and a-naphthalene-acetic acid was divided into groups of 20 μ and the percentage number of cells in each of these groups was estimated in the filaments examined in each of the cultures. These figures are recorded in Tables 21 and 22. In all but the two weakest concentrations of a-naphthalene-acetic acid the largest proportion of the cells in the filaments was in the group 104 - 120 μ . In the two other

/concentrations

concentrations the largest proportion of the cells was found in the group 84 - 100 μ . In the indole-3-acetic acid series the group containing the largest proportion of the cells varied from concentration to concentration.

Statistical analysis was applied to the cell length measurements in order to find if there was any correlation between the concentration of the two substances used in the cultures and the mean cell length (See Appendix I). The correlation coefficient was calculated for the indole-3-acetic acid series and for the a-naphthalene-acetic acid series of cultures. A correlation coefficient of ± 0.286 which was obtained for the indole-3-acetic acid series was shown by the variance ratio test not to be significant; whereas a correlation coefficient of ± 0.865 in the a-naphthalene-acetic acid series of concentrations was shown to be significant at the 5% level. A tendency for the mean cell length to increase with increase in the concentration of a-naphthalene-acetic acid is indicated in Table 20.

(b) The effect on the rate of cell division.

It was not possible to estimate the effect of indole-3-acetic acid and a-naphthalene-acetic acid on the rate of growth over long periods as zoospore formation made comparisons of the rate of cell division inaccurate.

(c) The effect on the distribution and grouping of the caps.

When filaments from the pond had been growing in indole-3-acetic acid and a-naphthalene-acetic acid for three months,

/an

an accumulation of large numbers of caps was found in many of the filaments. These large groups were more frequent in the filaments in a-naphthalene-acetic acid than in indole-3-acetic acid. The majority of these large groups in a-naphthalene-acetic acid numbered 15 to 20 caps but groups as large as 21 to 30 were often found. Large groups were common on the apical cell of filaments in a-naphthalene-acetic acid (Figs. 19, 20 and 20(a)). In the control filaments the cap number rarely exceeded ten.

Comparable amounts of material were taken from each of the concentrations of a-naphthalene-acetic acid and from the control, and records were made of the groups of caps in the filaments. The position of the caps was recorded as intercalary, intercalary which had become apical or apical. The results obtained in this manner were plotted as a series of histograms in Fig. 21. These records only indicate the general effect of a-naphthalene-acetic acid on cap grouping since unequal numbers of filaments of varying ages were examined: they do show, however, that there was an accumulation of caps to form large groups in a-naphthalene-acetic acid. This increase was not so obvious in the examined filaments grown in the two weakest solutions. Groups of caps were infrequent in the control material.

Similar records were also made of filaments which had been produced and grown in indole-3-acetic acid and a-naphta-

/lene-

lene-acetic acid for three months. These results are recorded in figs. 22 and 23. The number of caps recorded for the filaments in a-naphthalene-acetic acid (0.2 gms. per litre) were not representative of the culture because the growth of diatoms and other algae made it difficult to count the number of caps in a group.

Since these filaments were shorter than those which had been taken from the pond and grown in culture, the number of caps in each group was smaller: the largest group recorded numbered 22. The formation of large groups of caps in the filaments was more obvious in a-naphthalene-acetic acid than in indole-3-acetic acid and these large groups in a-naphthalene-acetic acid were usually apical. Although the records of cap grouping in these figures cannot be expressed quantitiatively there is no doubt that large groups of caps had formed in both indole-3-acetic acid and a-naphthalene-acetic acid. There were usually only one or two large groups of caps found in a filament which suggested that cell division had been concentrated in a few positions in the filament. a-naphthalene-acetic acid caused many of the apical cells to retain their caps and the formation of these large groups showed that apical growth was common in the filaments. In contrast indole-3-acetic acid did not cause the caps to accumulate on the apical cells, so that it was not possible to decide how much apical growth had occurred.

The large groups of caps which formed in the filaments

/treated

treated with indole-3-acetic acid and with a-naphthalene-acetic acid were conspicuous because of the increased cap number and because of the abnormalities within the cap group which accompanied this increase. Some of these abnormalities are shown in Figs. 19, 25, 26 and 27. The increase in the thickness of the caps caused a decrease in the size of the protoplast of the cell.

As the number of caps in a group increased beyond ten, the group became thickened on the inside towards the apical end of the cell. The thickening did not appear to extend along the transverse wall of the cell (Fig. 24). In many of the cells, the thickening was uneven and the group had the appearance shown in Fig. 27. In large groups of caps, the cap material became so extensive that it appeared to cut off the apical part of the protoplast from the rest of the protoplast. In apical groups of 15 to 30 caps it was frequently found that the cap material completely filled the apex of the cell (Fig. 25). Abnormalities which were still more complex were found in some cells as shown in Fig. 26. In such cases it was often found that the portion of the protoplast adjacent to the upper transverse wall of the cell appeared to be cut off by a solid ingrowth of cap material and that a second portion of the protoplast appeared to be isolated by the formation of another ingrowth of cap material further towards the base of the group of caps (Fig. 26). More than two such ingrowths of thickening were

/sometimes

sometimes found within the group of caps as in Fig. 28(3) and 28a(1). When these groups of caps were examined it was not possible to discover if the cap material completely traversed the cell so that the protoplast was actually divided into two or more parts or whether the protoplast was only constricted by the cap material. When the protoplast is plasmolysed in dilute glycerine, the lower portion may separate from the thickening at the base of the group of caps leaving the upper portion clearly isolated as in Fig. 26. This suggests that the thickening in some cases at any rate, may have been continuous across the cell.

Abnormal ring formation was frequently recorded in the large groups of caps. Single rings were often formed within a group of caps and not at the base of the group (Fig. 19, 28(7)), and occasionally in the filaments treated with a-naphthalene-acetic acid, more than one ring was formed in the group (Fig. 28a(2)(3)). The majority of such rings did not stretch; many of these appeared to be embedded in the cap material which extended across the cell. (Figs. 26, 28(3), 28a(1), 29(2)).

Similar abnormalities in wall thickness and ring formation were occasionally found in the long filaments from the pond when a group of more than ten caps was found and these are illustrated in Fig. 28(1)(8)(9)(10)(11). Indole-3-acetic acid and especially a-naphthalene-acetic acid had the effect of increasing the frequency of the occurrence of these abnormalities by increasing the number of caps in many of the groups.

/Examples

Examples of very abnormal cell structure found in filaments from the pond and in filaments from the indole-3-acetic acid and a-naphthalene-acetic acid cultures are shown in Figs. 28(1), 28(2) and 29(1), 28a(4) respectively. In these cells with large groups of caps, normal cell division appeared to have been hindered and an increase in cell length had probably taken place by the stretching of abnormal rings formed inside the group of caps. Part of the latest formed caps had separated from the group and was attached to the wall of the cell.

Horizontal striations are apparent in the surface view of any group of caps and have always been used to determine the number of caps present. When the large groups described here were examined in optical section the material of the group of caps appeared as if it was laminated. These layers were not evident in the small amount of thickening on the transverse wall at the cell apex (Fig. 24). In a later section these facts will be considered in relation to the normal process of cell division.

D. Growth in Cultures Containing Colchicine.

A series of cultures were set up using soil extract solution to which colchicine had been added in the two concentrations of 1.0 gms. per litre and 0.1 gms. per litre. Control cultures were set up in soil extract solution. Filaments which had been growing in the pond were placed in the cultures and were left for two months and filaments of two to three cells which were growing on slides in culture were placed in colchicine culture for a week.

At the end of this time in solutions containing colchicine of concentrations 1.0 and 0.1 gms. per litre, the cells of the filaments were bright green and in the younger filaments the chloroplasts were distinctly reticulate. The appearance of the chloroplasts may have been caused by unfavourable conditions of growth although the filaments all appeared healthy. Zoospore formation and ring formation was frequent in the filaments in all the cultures. Abnormal cell divisions ~~with a swollen apical end~~ had caused many cells to become an unusual shape.with a swollen apical end.

Observations were mainly confined to the effect of colchicine on cell division. Similar observations on colchicine treated filaments of a species of Oedogonium were made by Gorter (1943) using a colchicine concentration of 1.0 gms. per litre.

The observations made on the filaments in the two concentrations of colchicine are treated separately.

(a) Growth

(a) Growth in cultures containing colchicine (1.0 gms.per litre).

Most of the observations made on the material from these cultures agree with those made by Gorter (1943).

Normal cell division was entirely suppressed by this concentration of colchicine. Although apparently normal ring formation continued and the rings stretched there was no division of the nucleus and no cross wall formation in the cells. The stretched ring became swollen under the influence of colchicine and these swellings gave an abnormal appearance to the filaments (Figs. 30, 31). Frequently several rings formed and stretched in succession in a cell, when t he cell became even more abnormal in shape. Very few abnormalities were found in the basal part of the older filaments: the abnormal cells were usually confined to the apical part of the filaments (Fig. 31).

The colchicine caused rings to form in abnormal positions in some of the cells of the filaments and frequently more than one ring was found in a cell. These abnormal rings which did not stretch were found in the following positions in the cell:-

1. in the swollen apical part of an abnormal cell
2. in the swollen apical part of an abnormal cell and at the base of the swelling
3. at the apex and at the base of a cell (Fig. 36, 37).
4. at the apex and in the central region of a cell (Fig. 30).

The nuclei in cells in which a ring had stretched were large

/and

and their outline was indefinite. The position of these nuclei varied: sometimes they were in the swollen apex of the cell and sometimes they were in the original part of the cell.

The zoospores rounded off and secreted a wall but none of these cells developed a holdfast. A series of rings formed at one end but none of these rings was found to have stretched (Fig. 32). There was no indication that nuclear division had begun in these cells.

(b) Growth in cultures containing colchicine (0.1 gms. per litre).

After two months, it was found that the effects of the weaker concentration of colchicine on cell division were more varied. Both normal and abnormal divisions had taken place in this material.

Many of the zoospores produced in these cultures developed into apparently normal several-celled filaments on the walls of the vessels: these filaments were similar to filaments developing in the control (Fig. 33). Similar growth was also seen in the filaments which were developing on slides placed in the cultures for a week. The filaments were 2-3 celled when placed in colchicine and observations after a week showed that the cell size and the rate of cell division in these filaments was similar to the cell size and the rate of cell division in the control (Figs. 34, 35, 35(a)). However, many of the apical cells in these young filaments became expanded at the tip and the nucleus was often found in this expansion (Fig. 34, 35).

/When

When abnormal cell division took place the cell had a similar appearance to that already described for the stronger concentration of colchicine. Successive rings had stretched in some of the cells without any cross wall formation: this was frequently the case in the germlings developing from zoospores, although strong holdfasts were developed (Fig. 33).

Ring formation was very obvious in the filaments and many of these rings were abnormal in position and number in the cell. These abnormalities were similar to those found in the stronger concentration and representative examples of them are shown in Figs. 36, 37, 38.

When the filaments were stained with aceto-carmine, variations were found in the behaviour of the nucleus. Nuclear division was generally normal in the filaments which had been formed in colchicine and it had also taken place in some of the cells in the filaments which had been placed in the culture. In some of these cells the nucleus had divided and a ring had stretched without any cross wall formation, so that a nucleus was found in both parts of the cell (Fig. 37). In others, the nucleus had divided but no rings had formed in the cell (Fig. 39). Frequently the nucleus had divided in the cells in which both apical and basal collars were formed (Fig. 36, 37).

Occasionally abnormal nuclear behaviour was seen in cells in which otherwise normal cell division had occurred.

/Binucleate

Binucleate cells were found with an adjacent cell lacking a nucleus (Fig. 40); other cells had a single nucleus although the adjacent cell only contained a nucleolus.

Abnormal nuclear behaviour was more obvious in cells in which other abnormalities were found. In many of the abnormal cells, the nuclei were similar to those in the abnormal cells in the stronger concentration of colchicine: they were large bodies which had no clearly defined outer limit. Occasionally the nucleus appeared to have disintegrated into patches of deeply staining material in the cytoplasm of the cell. The large nuclei were found either in the apical swelling of the cell (Fig. 39) or in the position where the cross wall should have formed or below it.

These observations made on the effects of colchicine on cell division in _Oedogonium sp._ show that the processes of nuclear division and ring formation and stretching, normally closely related, may take place independently under abnormal conditions of growth. A concentration of 1.0 gms. per litre inhibits nuclear division and cross wall formation but it does not prevent ring formation and ring stretching. A lower concentration of colchicine (0.1 gms. per litre) may have the same effect on some dividing cells in the filaments but no obvious effect at all on other similar cells in the same filament.

5. DISCUSSION

Oedogonium sp. proved to be satisfactory material for use in a growth study because the frequency of vegetative reproduction ensured an unlimited supply of material in all stages of development under natural and under cultural conditions and because the peculiar method of cell division in the genus facilitated observations on the pattern of development of the filaments. The nature of the material, however, was not well suited to quantitative comparisons of the effects of the varying conditions on growth. The continual disintegration of the filaments after zoospore formation made it impossible to compare the rate of cell division under varying conditions of growth over long periods and made it difficult to obtain quantitative measurements of the distribution of caps and the size of the cap groups. Because of unequal rates of cell division in the filaments and the loss of cells in zoospore formation, filaments of equal length were not necessarily of equal age.

Soil extract solution is not an ideal medium to use in experimental work carried on over a long period because it is impossible to reproduce the exact conditions of the previous solution in successive extracts. Neither is it an ideal solution to use in control cultures in the work concerned with the effects of additional growth substances, as this solution possibly already contains traces of growth substances extracted from the soil. Soil extract solution was used in

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the control cultures because it produced the best growth of Oedogonium and it was expected that the effects of the additional experimental growth substances on cell size and on the rate of cell division would be so great that any effects due to the growth substances in the soil extract solution would be negligible.

An interesting feature of the growth of Oedogonium sp. under natural conditions was the loss of caps from the apical cell of the filaments. Caps were frequently observed flaking off the apical cell of the filaments either singly or in groups. A loss of cap material has been referred to by Fritsch (1902), (1904) and by Van Wisselingh (1908). This loss of caps generally occurred after the division of the first cell of the filament, although it was also found to occur in some of the apical cells of many celled filaments. Instead of a ring of additional material forming in these cells the new cell wall material formed as a dome shaped structure at the cell apex, the rim of the dome being thickened. In cell division, the apical thickening became divided into two layers and the outer layer was lost as the cap. The inner layer stretched to become the wall of the new upper cell of the filament. In the present study neither the structure of the cap material nor the stages in its loss from the apical cell of the filaments were investigated.

Normal ring formation certainly occurred in apical cells

/of older

of older filaments although unfortunately no detailed study of the first division of the zoospore was made.

Most of the available literature reporting the effects of growth substances on the growth of algae deals with work on cultures of unicellular algae. The references to this work have been given in the Introduction. Some of these results are contradictory, possibly on account of variations in the culture conditions, but in general, growth substances have been found not to affect cell size in such algae but to increase their rate of cell division. A similar effect for multi-cellular algae was found when indole-3-acetic acid and thiamine were added separately to cultures of Ulva and Enteromorpha. (Kylin, 1941).

The observations reported here on the rate of cell division in filaments of Oedogonium sp. in yeast extract and thiamine cultures are in agreement with these general conclusions but the observations on cell size are not. The addition of these substances to the culture caused a decrease in the cell size in the filaments. The records of mean cell length in filaments growing in varying concentrations of indole-3-acetic acid and a-naphthalene-acetic acid were subjected to statistical analysis to determine whether the addition of these growth substances in varying concentration made a significant difference to the mean cell size of the filaments. This showed that the variation in the mean cell

/length

length in the different concentration of indole-3-acetic acid was not significant but that in the different concentrations of a-naphthalene-acetic acid mean cell length was correlated with concentration. These results cannot, however, be regarded as conclusive; cell size may have been affected by the partially uncontrolled conditions of culture and a better estimate of cell size would possibly have been obtained if at each sampling, a definite number of cells, rather than a definite number of filaments, had been taken at random.

The most significant effect of indole-3-acetic acid and a-naphthalene-acetic acid is that of increasing the number of caps found in some of the large groups of caps in the filaments and of causing abnormalities to form in these groups. The possible interpretation of these abnormalities will be referred to in the section which now follows.

The unusual method of cell division in Oedogonium in which a ring of additional material forms in the upper part of the cell and stretches to become part of the lateral wall of the upper daughter cell has attracted the attention of many Botanists. The stages in cell division, the structure of the wall of the cell and the structure and formation of the ring have been investigated by several workers including Van Wisselingh (1908) and Ohashi (1930). The wall of the cell is usually represented as consisting of two layers, the outer layer being cuticular and the inner being made of

/cellulose.

cellulose. Some workers claim that the wall consists of three layers, the outer layer being chitinous while the middle layer is pectose and the inner layer cellulose (Smith 1938).

According to Van Wisselingh (1908) the ring is formed in the inner layer of the cell wall by intussusception. When the ring stretches it is intercalated between the small part of the original cell wall at the cell apex and the remainder of the old cell wall which is referred to as a "sheath". Later, after cross wall formation is complete a cellulose wall is laid down in both the daughter cells inside the existing wall layers. The cap, which is evident in the apical part of the cell following cell division,is caused by the splitting of the outside layer of the cell wall to enable the ring to stretch.

Under natural conditions of growth, the groups of caps in the material investigated here were usually small and there was no indication of additional wall thickening in the vicinity of the caps : with added growth substances, the groups of caps were often large and very thick walled. If a new cellulose wall is laid down after the formation of the two daughter cells, an increase in thickness in the wall beneath the caps is to be expected.This increase in thickening might become obvious as the number of caps greatly increase so that the abnormalities observed in the groups with a large number of caps in indole-3-acetic acid and a-naphthalene-acetic acid and in the occasional large groups from the pond may possibly have been

/caused

caused by the laying down of this additional cell wall material.

The abnormal thickening in the large groups of caps appears to be laminated (Fig.24). Each of the layers begins in the apical part of the cell and extends downwards: the inner layers extending further towards the base of the group of caps. Each of the layers in the thickened wall material is associated with one of the areas between the striations on the surface of the group representing a cap. The laminated appearance of the thickening suggests that it has been laid down at successive intervals of time. As the thickness of the wall beneath the caps increases with each successive cell division, the area available for the formation of additional layers would decrease so that the abnormalities associated with the large groups of caps might then result. In many of the cells with large apical groups and in some of the cells with large intercalary groups of caps the apex frequently appears to be filled with cell wall material(Fig. 25).

As the walls of the cell beneath the group of caps became thickened laterally, the apical part of the protoplast would become restricted and then with further increase in the number of cell divisions a continuous layer of cell wall material might be formed across the cell so that the apical part of the protoplast became cut off from the rest of the protoplast of the cell. The successive cell wall layers would then be formed beneath the continuous layers of cell wall material. If further cell division continued to increase the /lateral

lateral thickening, a second portion of the protoplast might become isolated by the formation of a second continuous layer across the cell. A condition which is possibly similar to this is illustrated in Fig. 26.

The occurrence of localized cell division, indicated by the large accumulation of caps, seems to interfere with the efficiency of cell division and is associated with the formation of many abnormal rings. The formation of wall material around non expanding rings would account for the fact that the rings appeared to be embedded in the abnormal material in the apical part of a cell with a large group of caps(Fig 26).

The formation of abnormal caps in both apical and intercalary position was observed in an unnamed species ~~by~~ of Oedogonium by Fritsch (1904). Cap material accumulated on the apical cells of some filaments and to a lesser extent in some intercalary cells. The apical addition of this cap material gave an abnormal rectangular appearance to the apex of the filament. This material was formed between the outer and the inner layer of the cell wall and probably represented successive non stretching dome shaped layers of ring material formed in the cell apex. These abnormal accumulations of cap material were ultimately seen to drop off the apical cell. Their formation was probably due to unfavourable conditions of growth as they were only found in filaments which were exposed above the level of the culture solution; the normal division of the cell was suppressed by these unfavourable conditions. A similar interpretation given to

/the

abnormalities described here in the large groups of caps in both apical and intercalery position could account for the accumulation and lamination of the thickening in the cells but it would not account for the fact that each of the layers was associated with a single cap.Since normal ring formation and stretching has often been observed during the accumulation of these groups of caps, it seems much more likely that the abnormalities here are due to the number of cell divisions which have occurred in a localized position.

In normal cell division the processes of nuclear division followed by cross wall formation and ring formation and its subsequent stretching to form part of the lateral wall of the upper daughter cell seem to be coordinated, the ring forming as the nuclear division proceeds.

Nuclear division was suppressed in all the cells in a culture containing colchicine in the concentration of 0.1 gms. per litre, ring formation, however, continued and many of these rings stretched to give an abnormal appearance to the cells. Sometimes rings continued to form in these cells but the additional rings did not stretch. The weaker concentration of colchicine may have been close to the critical concentration necessary for any effect to be apparent because normal and abnormal cell division took place in the cells of the filaments treated with colchicine in the concentration of 0.1 gms. per litre. In this concentration some of the cells had two nuclei and although nuclear division had occurred there was no formation of the ring

/required

required for cell division. In other cells, with more than one ring, the nucleus had divided but none of the rings had stretched.

The observations made on the colchicine treated material indicate that although the processes of nuclear division and of ring formation and ring stretching seem to be coordinated in normal cell division, they may be made to occur independently when the filaments are grown under abnormal conditions in culture containing colchicine.

The observations reported on the growth of Oedogonium sp. are of a preliminary nature and much more work needs to be done. In further work, it is necessary to discover the conditions of culture which will produce material similar to that growing under natural conditions in the pond, and then to re-investigate the possibility of producing the experimental material as a clone. The experimental work concerned with the effects of varying conditions on cell size needs to be repeated using the controlled conditions of light and temperature now available and it would be more satisfactory statistically to take a definite number of cells at random in each observation rather than to make measurements on a definite number of filaments. It would be interesting to extend the investigations reported here to other species of Oedogonium to find if a common plan of filament development exists in the genus and if all species of Oedogonium react in the same way to the varying experimental conditions of culture used.

5. SUMMARY

The growth of an unidentified species of Oedogonium growing in a pond in the Botany Garden, Royal Holloway College has been investigated under natural and cultural conditions with and without the addition of various organic substances. Records were made of the cell size, the rate of cell division and the grouping of the caps in the filaments. The observations made on this growth are summarised here.

(1) Long filaments growing in the pond showed great variation in cell size. Two types of cells were recognized in these filaments, longer narrower pale green cells and shorter wider dark green cells. The shorter cells formed in the filaments as they aged but their formation was not a response to changes in environmental conditions.

(2) Although the growth of the young filaments in the pond was usually apical for the first ten or so divisions, the apical cell rarely accumulated groups of caps. Single caps and groups of caps were frequently observed flaking off the apical cells.

(3) Groups of caps occurred in the filaments in intercalary positions and each group generally numbered fewer than ten caps. Larger groups were rare and when they occurred they were abnormal in form.

(4) It was impossible to correlate the number of caps and the number of cells in the filaments because of the loss of

/caps

caps from the apical cell and because of the disintegration of the filaments when zoospores were formed.

(5) Cell size was less variable in the filaments grown for long periods in culture in soil extract solution than in the long filaments from the pond. The longer cells, when present, were few in number and more often were completely absent.

(6) In culture, growth was often intercalary in the very early stages of the development of the filaments.

(7) There was no increase in the number of the caps in the cap groups in the cultured filaments but the proportion of the filaments with groups of apical caps was increased, possibly because caps were retained for a longer time before flaking off from the apical cell.

(8) The addition of yeast extract or thiamine to the cultures decreased the size of the cells but increased the rate of cell division.

(9) No satisfactory conclusions can be drawn as to the effect of indole-3-acetic acid and a-naphthalene-acetic acid on cell size or on the rate of cell division.

(10) The addition of indole-3-acetic acid and a-naphthalene-acetic acid to the cultures increased the size of the groups of caps; groups numbering 15 to 20 were common and groups numbering as many as 20 to 30 were found in cultures with additional a-naphthalene-acetic acid. This also increased the proportion of the filaments with large groups of apical caps.

(10) contd:

The increase in cap number in indole-3-acetic acid and a-naphthalene-acetic acid was accompanied by abnormalities in the large groups of caps.

(11) The addition of colchicine (1.0 gms. per litre) caused the suppression of nuclear division while ring formation continued; such rings stretched in the absence of cross wall formation. A weaker solution of colchicine (0.1 gms. per litre) produced the same effects on some of the cells in the filaments but apparently normal cell division continued in many of the cells. In other cells the nucleus had divided in the absence of ring formation. These observations show that the processes of nuclear division and ring formation can be made to take place independently of one another.

In conclusion I wish to thank both Professor F.W.Jane and Dr. M.A.P.Madge for the help they have given me. I am especially grateful to Dr. Madge for suggestions made during her supervision of my work. I also wish to thank Mr.R.Brinsden who is responsible for the photographs.

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APPENDIX 1.

The method of calculating the correlation coefficient.

Correlation coefficient is represented by r

$$r = \frac{xy - \frac{(Sx)(Sy)}{n}}{\sqrt{\left[\left\{Sx^2 - \frac{(Sx)^2}{n}\right\}\left\{Sy^2 - \frac{(Sy)^2}{n}\right\}\right]}}$$

The variance ratio of the correlation coefficient was found from the following formula.

$$\frac{(n-2)\ r^2}{1 - r^2}$$

In Table 20 in the indole -3- acetic acid cultures;-

x	=	value of the concentration
y	=	mean cell length.
n	=	number of observations = 5.
Sx	=	0.094
Sy	=	548.8
Sxy	=	11.26
$S(x)^2$	=	0.0103
$S(y)^2$	=	60,393.0

In the a-naphthalene-acetic acid cultures;-

n	=	8
Sx	=	0.794
Sy	=	891.12
$S(x)^2$	=	0.213
$S(y)^2$	=	99,673;0
Sxy	=	94.73

Key to Figures.

Filaments grown under natural conditions

Fig. (1) Microphotograph of the structure of the holdfast in Oedogonium sp. Material fixed and stained with aceto-carmine. Holdfast is attached to a slide. x 600.

(1)a Camera lucida drawing of the structure of the holdfast.

(2) Microphotograph of the enlarged cells which are possibly akinetes. x 50. Natural condition.

(3) Microphotograph of filaments from the pond. Natural condition. x. 70.

(3)a Microphotograph of filaments from the pond. Fixed and stained with aceto-carmine. x 160.

(4) Graphical representation of the distribution of cell length and cap grouping in long filaments from the pond. Cell length is recorded in micrometer units (10 units = 40μ.).

(5) Diagrammatic representation of the loss of caps by the apical cell of filaments taken from the pond. November 17th, 1950 (Drawing apparatus).

(6) The variation in cell length in filaments growing on slides in the pond in May 1951. The filaments were placed in the pond November 17th, 1950. (Drawing apparatus).

(6)a Cell lengths in filaments developing in the pond May, 1951. (Drawing apparatus).

(7) Graphical representation of the cell length measurements in filaments developing on slides in the pond during November 1950 to September 1951. The figure represents filaments taken from the pond at intervals during this time. Cell length is recorded in micrometer units (10 units = 40 μ.).

(8) Diagrammatic representation to show the lack of correlation in cap and cell number in young filaments from the pond.

Filaments grown under cultural conditions

Fig. (9) Microphotograph of the smaller sized cells formed in culture in soil extract solution. x 70. Filaments were mounted in dilute glycerine.

(10) Variation in cell size in a filament grown in culture in soil extract solution. (Drawing apparatus).

(11)&(11)a Formation of smaller cells in filaments in culture in soil extract solution and in pond water. (Drawing apparatus).

(12) Diagram illustrating the method used to identify the position of filaments growing in culture.

(12)a Diagrammatic representation of the order of cell division in young filaments grown in culture.

(13) Graphical representation of the rate of cell division in young filaments in culture.

(14) Diagrammatic representation of the irregular holdfasts formed in filaments developing under increased light intensity.

Filaments grown in culture with additional growth substances

Fig. (15)a,b,c. The appearance of filaments grown for 14 days in cultures containing (a) yeast extract (100 mgs. per litre) (b) soil extract solution and (c) thiamine (0.1 mgs. per litre). (Drawing apparatus).

(16)a,b,c. Microphotographs of the appearance of the above filaments after 20 days. x 25. Natural condition.

(17) Graphical representation of cell length measurements and cap distribution in filaments taken from the pond and grown in culture containing a-naphthalene-acetic acid (0.3 gms. per litre) and in culture with soil extract solution. Cell length is recorded in micrometer units (10 units = 40μ.).

(18) The abnormal appearance of the apical cell in filaments grown in culture containing

a-naphthalene-

a-naphthalene-acetic acid (0.4 gms. per litre). (Drawing apparatus).

(19) The appearance of large groups of caps formed in a culture containing a-naphthalene-acetic acid (0.3 gms. per litre). (Drawing apparatus).

(20)&(20)a Microphotographs of filaments growing in culture with a-naphthalene-acetic acid (0.1 gms. per litre) and in soil extract solution illustrating the large groups of caps found in the culture with the growth substance. x 15. Natural condition. The large groups of caps accumulated on the filaments in a-naphthalene-acetic acid are illustrated.

(21) A series of histograms showing the size and the number of the apical and intercalary groups of caps in filaments placed in varying concentrations of a-naphthalene-acetic acid.

(22) A series of histograms showing the size and number of the apical and intercalary groups of caps in filaments produced and grown in varying concentrations of indole-3-acetic acid.

(23) A series of histograms showing the size and number of the apical and intercalary groups of caps in filaments produced and grown in varying concentrations of a-naphthalene-acetic acid.

(24-27) Microphotographs of large groups of caps formed in filaments grown in culture containing a-naphthalene-acetic acid (0.3 gms. per litre). Filaments were fixed and mounted in dilute glycerine. (See below).

(24) Laminated appearance of a group of caps. x 750.
(25) Apical cell showing thickened apical region. Cell contents have plasmolysed. x 600.
(26) Intercalary cell with large group of caps showing possible separation of the protoplast by the formation of a continuous layer of cap material. A ring appears to be embedded in this material. Cell contents slightly plasmolysed. x 600.
(27) Unequal lateral thickening in a large group of caps. x 700.

Fig. (28) Diagrammatic representation of abnormalities formed in large groups of caps in the filaments in the pond and in culture containing various amounts of indole-3-acetic acid.

(28)a Diagrammatic representation of abnormalities formed in large groups of caps in filaments from cultures with a-naphthalene-acetic acid (o.5 gms. per litre).

(29) Diagrammatic representation of abnormalities formed in large groups of caps in filaments from cultures with indole-3-acetic acid. (0.1 gms. per litre).

Filaments grown in culture with additional colchicine

Fig. (30)&(31) Abnormal cell division in cells of filaments treated with colchicine (1.0 gms. per litre). (Drawing apparatus). Rings have formed and stretched without cross wall formation. Abnormal rings have also formed in the cells.

(32) Abnormal development of zoospores in colchicine culture (1.0 gms. per litre). A series of wall thickenings have formed in the apical part of the cell, cell divisions being suppressed. (Drawing apparatus).

(33)&(34) Normal and abnormal cell division in filaments treated with colchicine (0.1 gms. per litre). (Drawing apparatus).

(35)&(35)a Microphotographs showing comparable growth in filaments grown on a slide for a week in colchicine culture (0.1 gms. per litre), and in soil extract solution. x 25. Material was fixed and stained with aceto-carmine.

(36)&(37) Abnormal ring formation and nuclear behaviour in cells in filaments treated with colchicine (0.1 gms. per litre). (Drawing apparatus).

(38) Microphotograph of abnormal ring formation in a cell treated with colchicine (0.1 gms. per litre). Rings have formed in both the apical and central part of the cell and the nucleus is a patch of deeply stained material. Material fixed and stained

/with

with aceto-carmine. x 300.

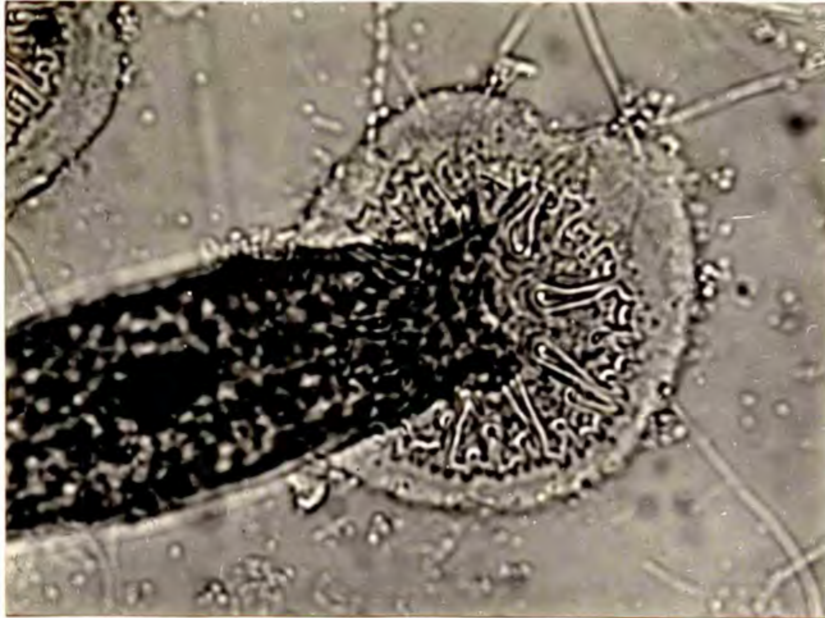
Fig. (39-41) Microphotographs of nuclear behaviour in cells treated with colchicine (0.1 gms. per litre). Material fixed and stained with aceto-carmine.

(39) Abnormal cell division in colchicine. The ring has stretched but the nucleus has not divided and also the nucleus in the apical cell has divided without a ring being formed. x 320.

(40) One cell of the filament has two nuclei and the other cell in lacking a nucleus. x 320.

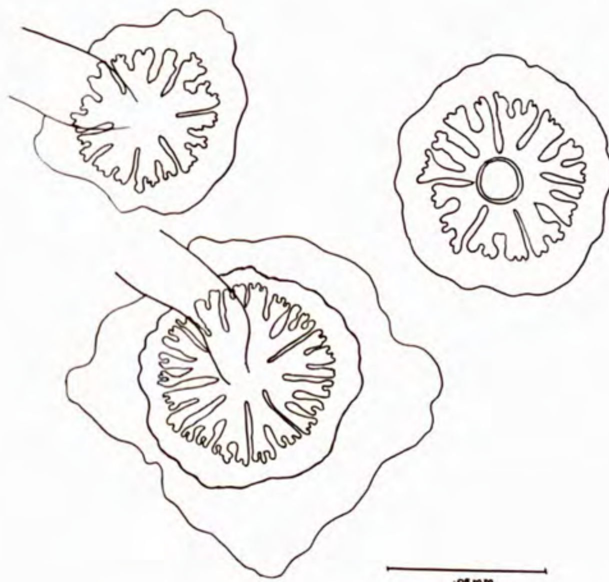
(41) The nucleolus is shown to have separated from the nucleus and is found in the apical part of the cell formed by the stretched ring. x 320.

Figs. 1 and 1a. The structure of the holdfast in filaments of *Oedogonium sp.*



1.

x600

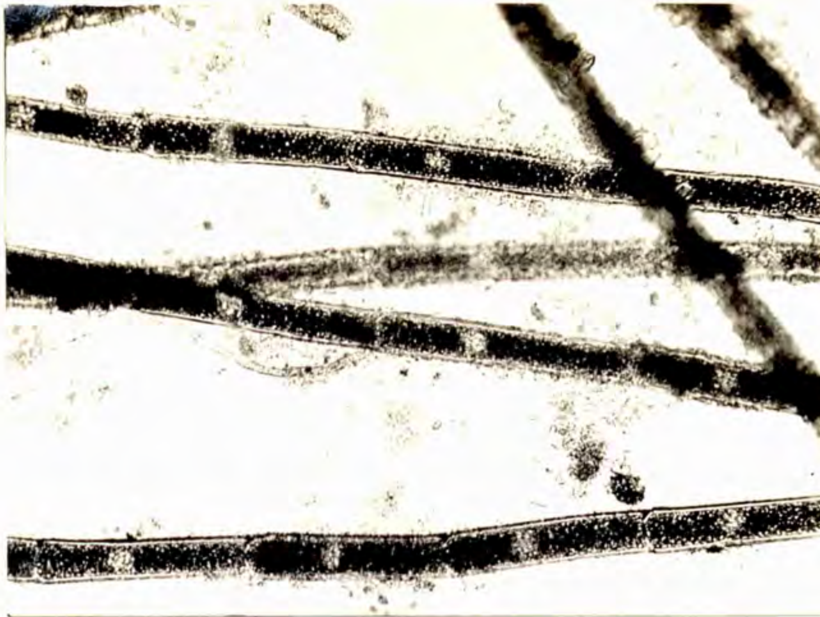


1a.

Fig 2. Enlarged cells in filaments from the pond which resemble akinetes.

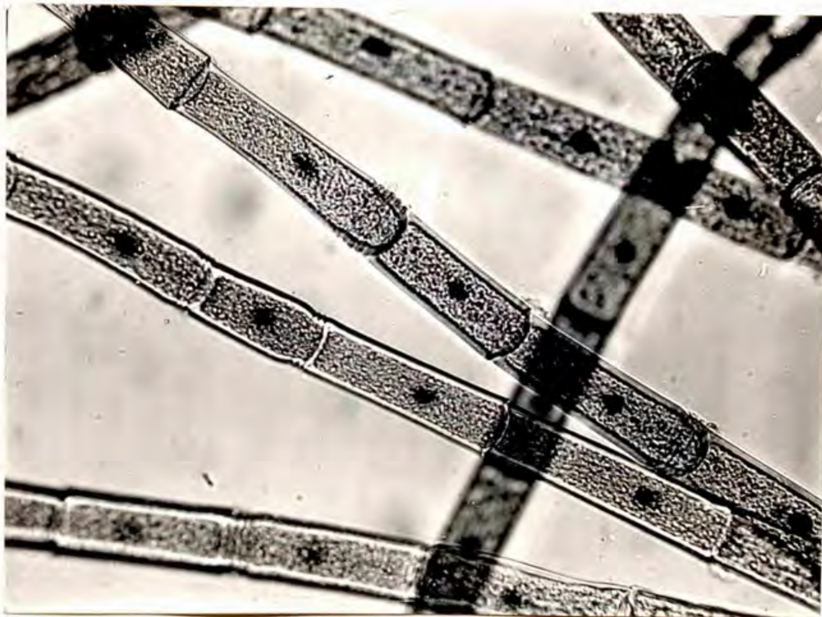
x50

Figs. 3 and 3a. Filaments of *Oedogonium sp* growing under natural conditions.



3.

x 70.



3a.

x 160.

Fig.4. Distribution of cell length and cap grouping in six fully developed filaments from the pond. The basal cell is represented first in each filament. (IO units equal 40 μ.)

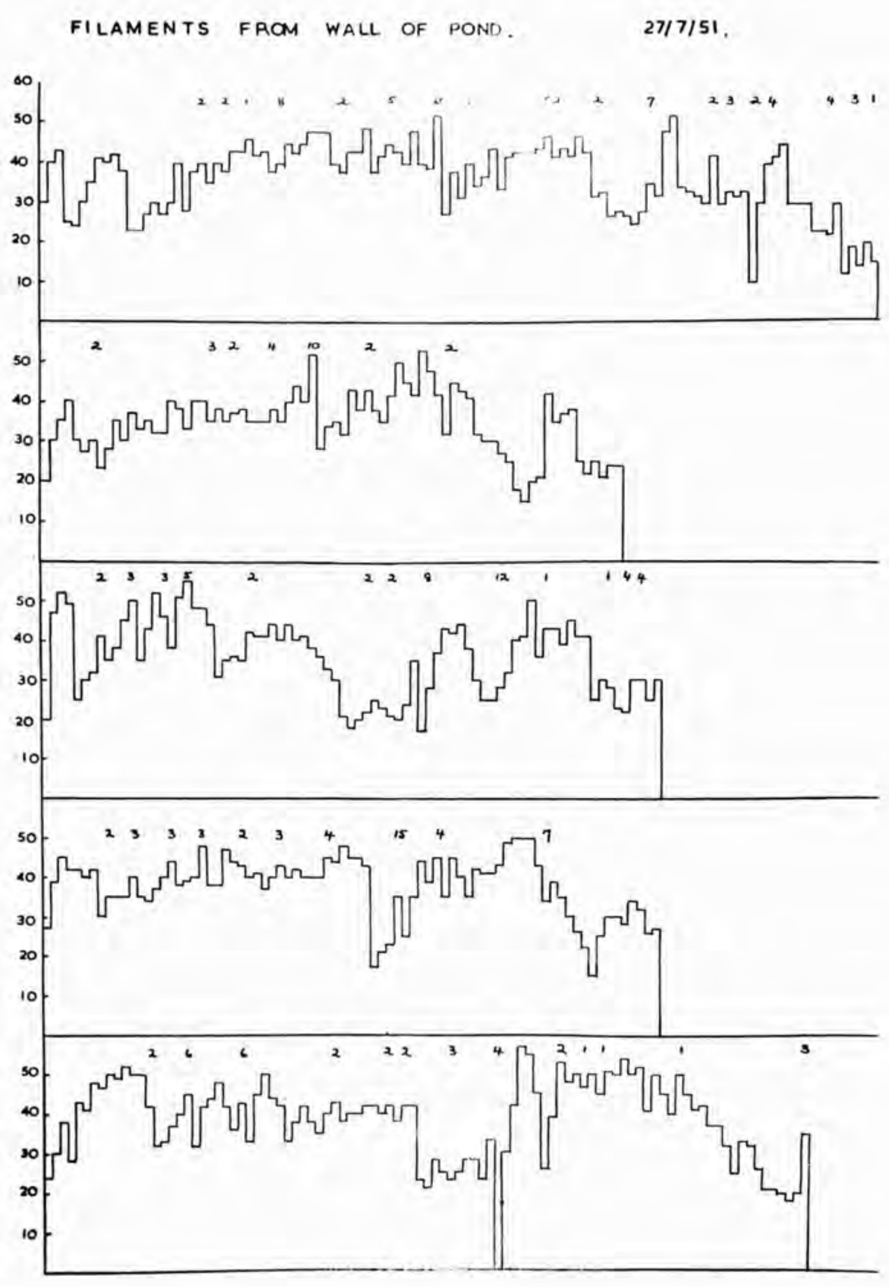


Fig. 5. The loss of caps from the apical cell of a filament. (diagrammatic.)

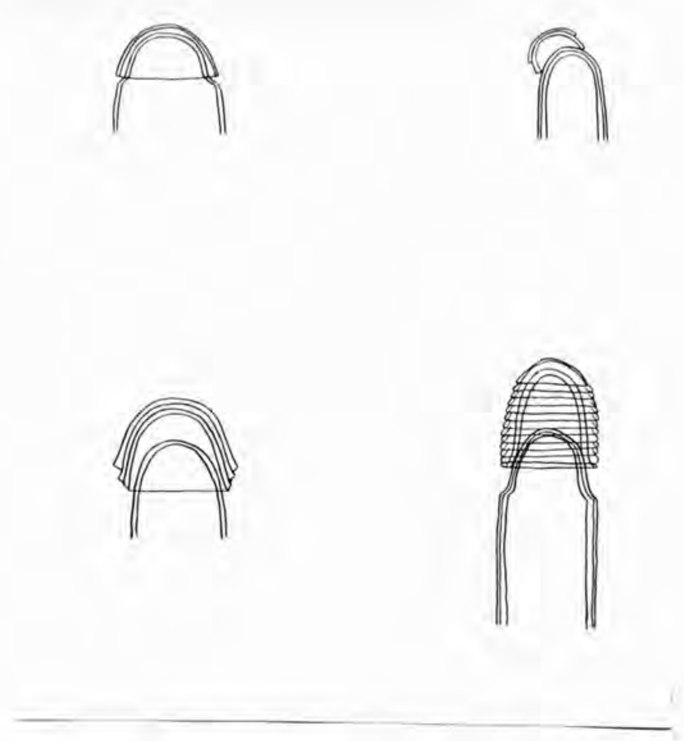


Fig. 6. Filaments developing on slides in the pond. The hatched cells represent the small dark green first formed cells. Filaments were placed in the pond November 17 th. 1950.

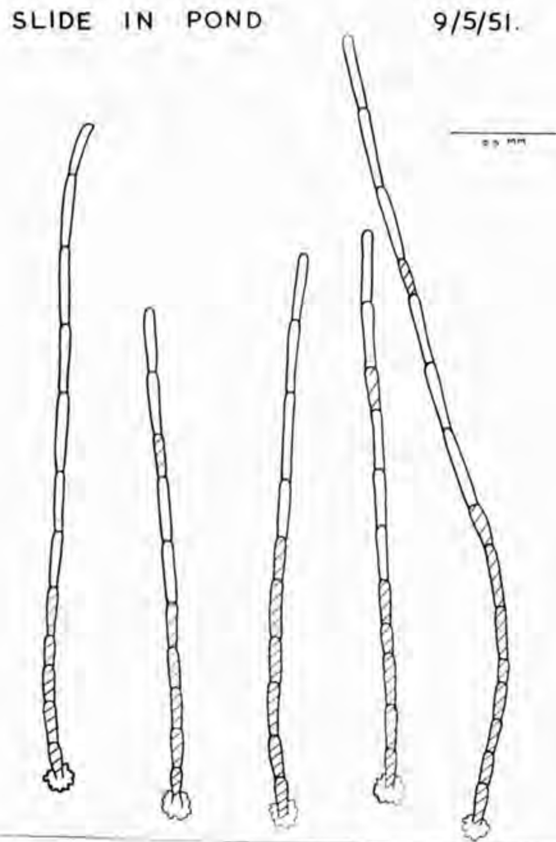


Fig. 6a. Filaments developing on slides from zoospores formed in the pond.

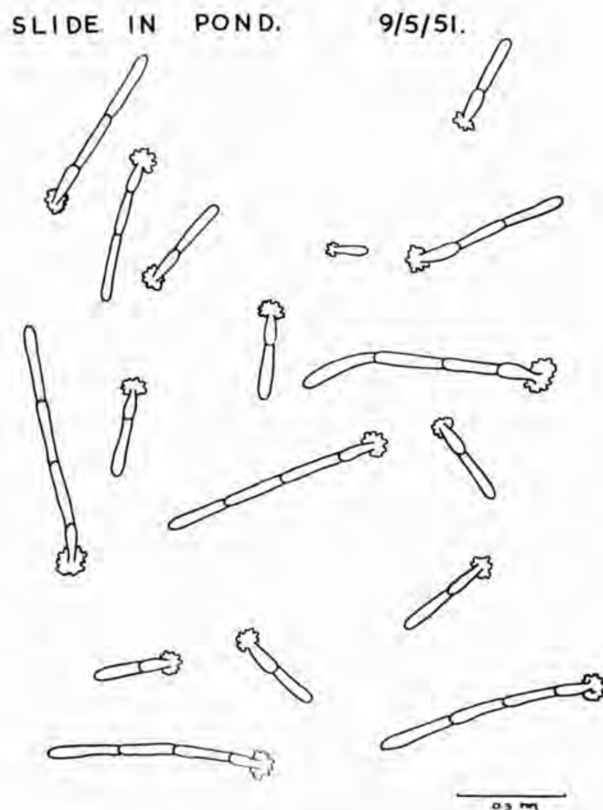
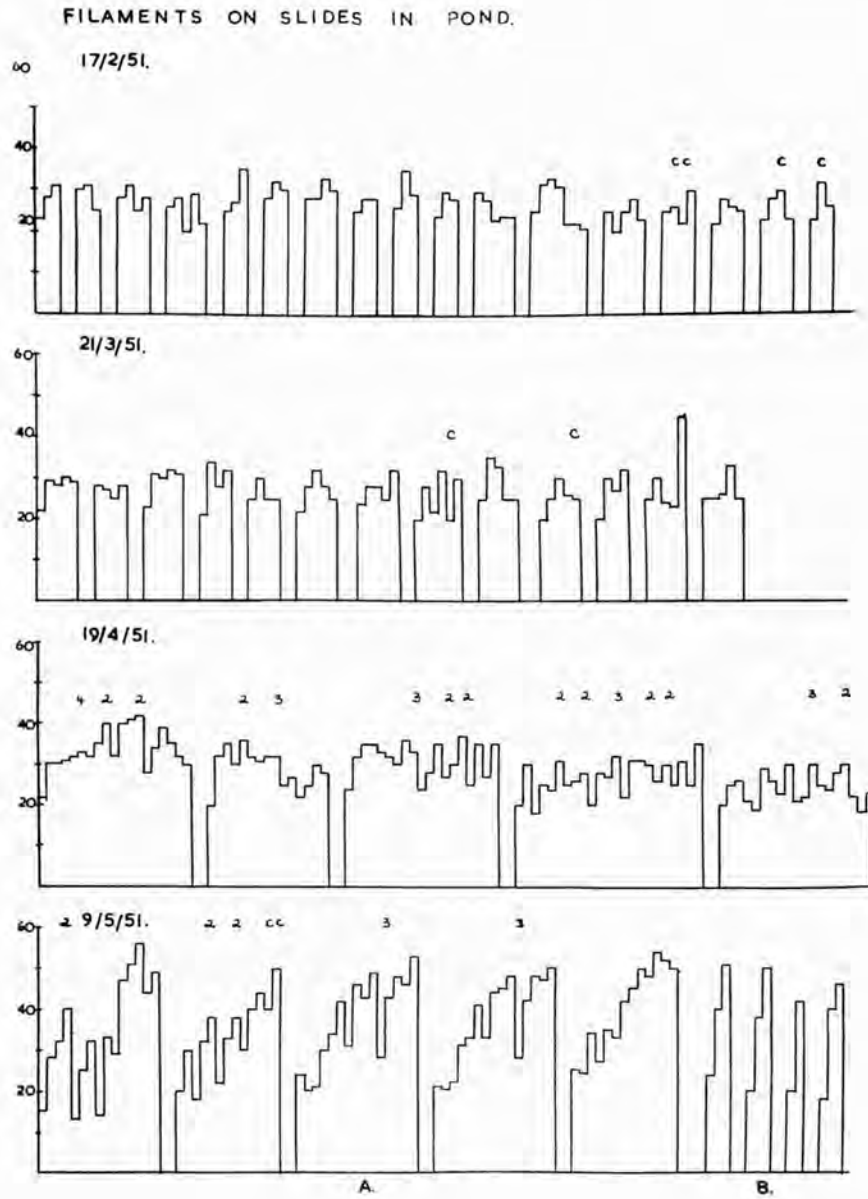


Fig. 7. Cell size measurements in filaments which were grown on slides in the pond. The measurements were made at intervals from November 17. 1950 until August 1. 1951 (10 UNITS EQUAL 40μ)



B (9/5/51) FILAMENTS DEVELOPING ON SLIDE FROM ZOOSPORES.

(continued on the next page.)

Fig. 7. continued.

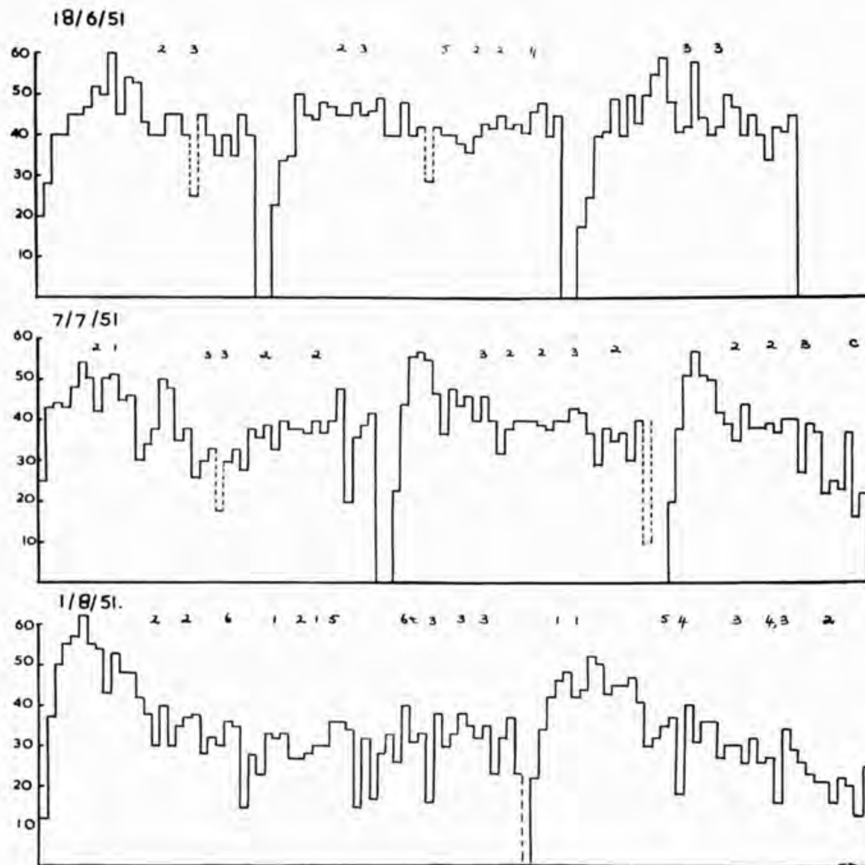


Fig.8. The lack of correlation of cap and cell number in filaments developing in the pond.

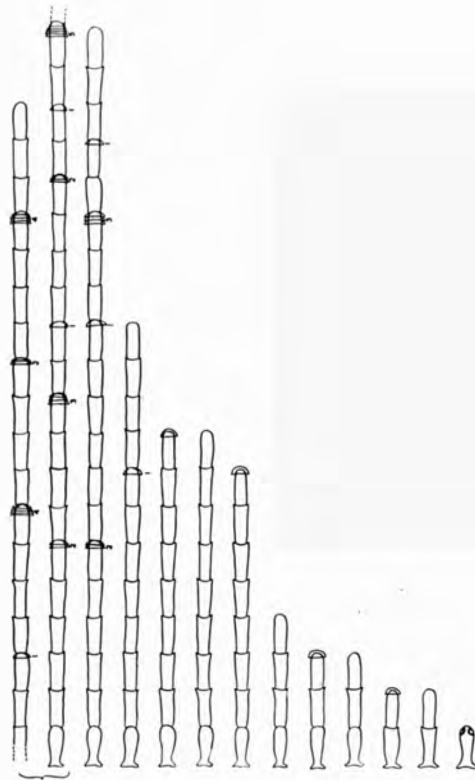


Fig.9. The formation of shorter cells in the filaments grown in culture in soil extract solution.

Fig.10. The cell size variation in a filament grown in culture in soil extract solution. The shorter cells formed in culture.

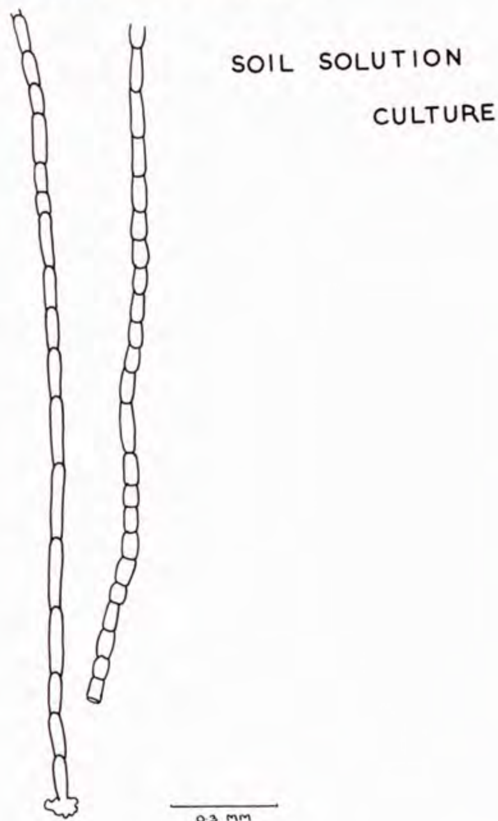


Fig.11. Filaments from the pond which were grown in culture in soil extract solution.

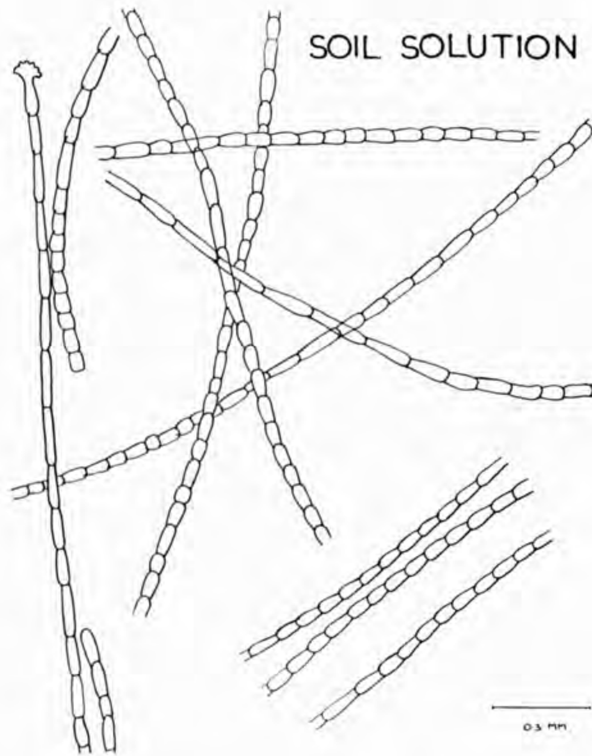


Fig.11a. Filaments from the pond which were grown in culture in pond water.

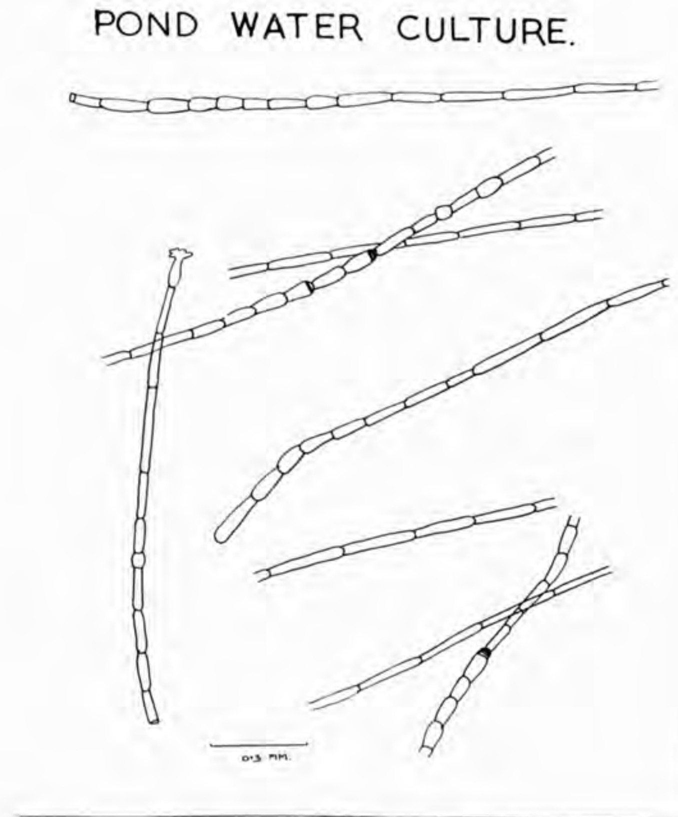


Fig.12. Method used for identifying individual filaments. The end of a glass ring was divided into segments by pencil markings and the double line made the orientation of the ring possible.

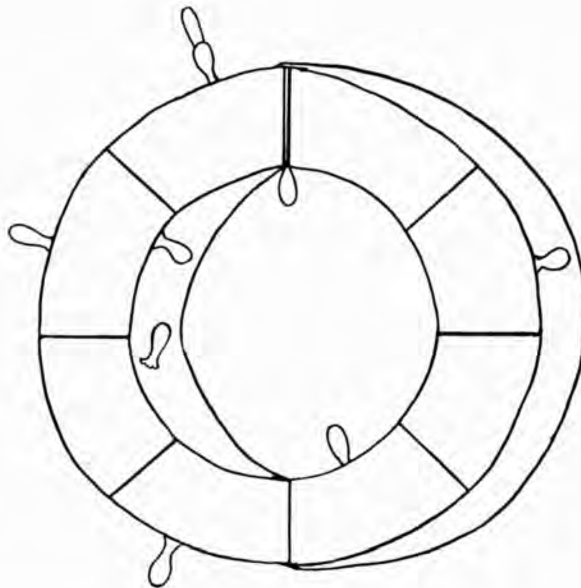


Fig.12a. The order of cell division in young filaments grown in culture. The date of cell formation is recorded by the day and month of the year beneath each cell and the order of cell formation is recorded beside each filament. The arrows underneath the filaments indicate the disintegration of the filaments by zoospores.

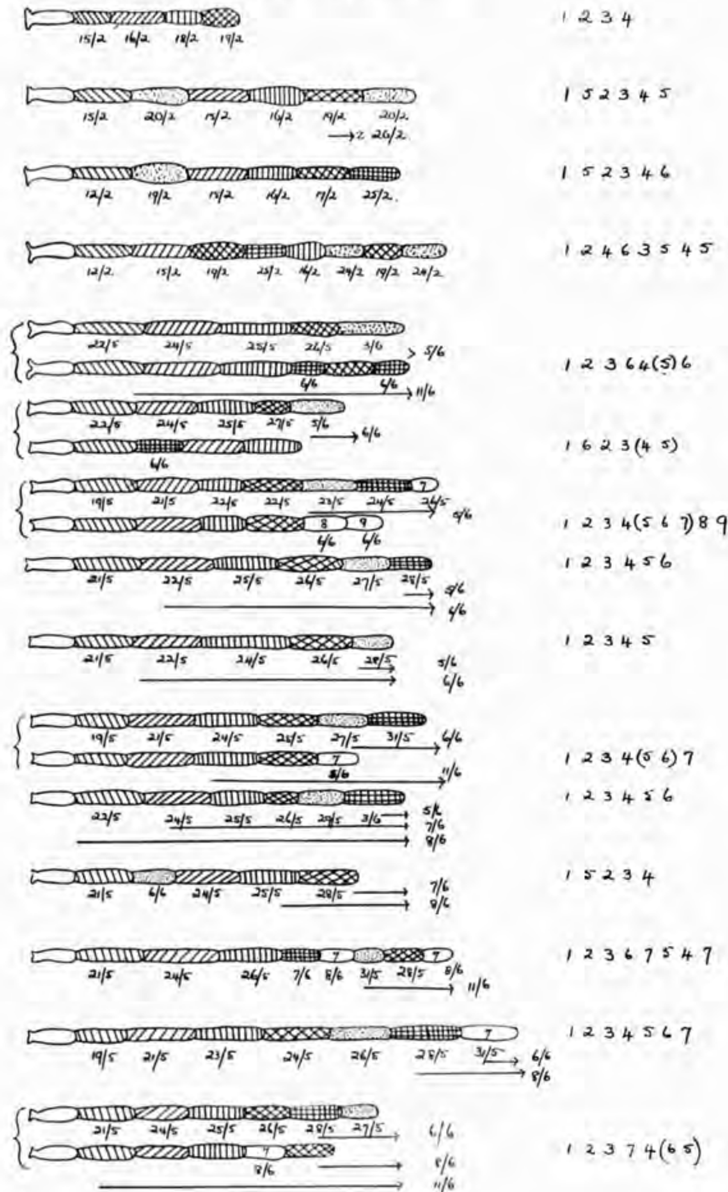


Fig. 13. The rate of cell division in young filaments in culture. Zoospore formation is indicated by hatching.

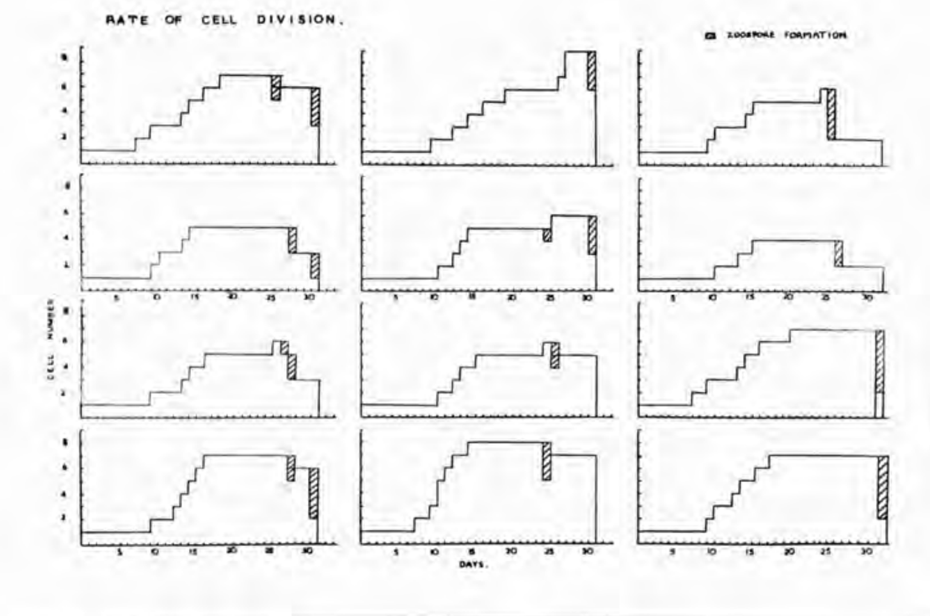
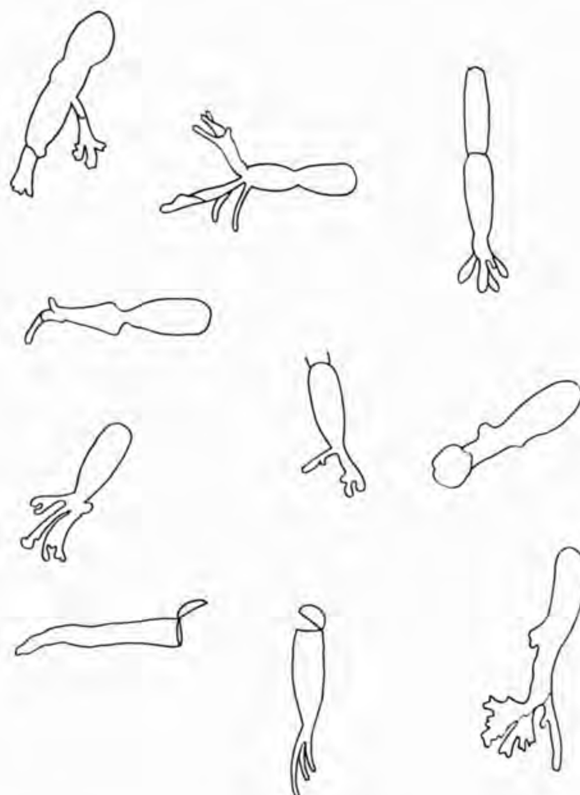
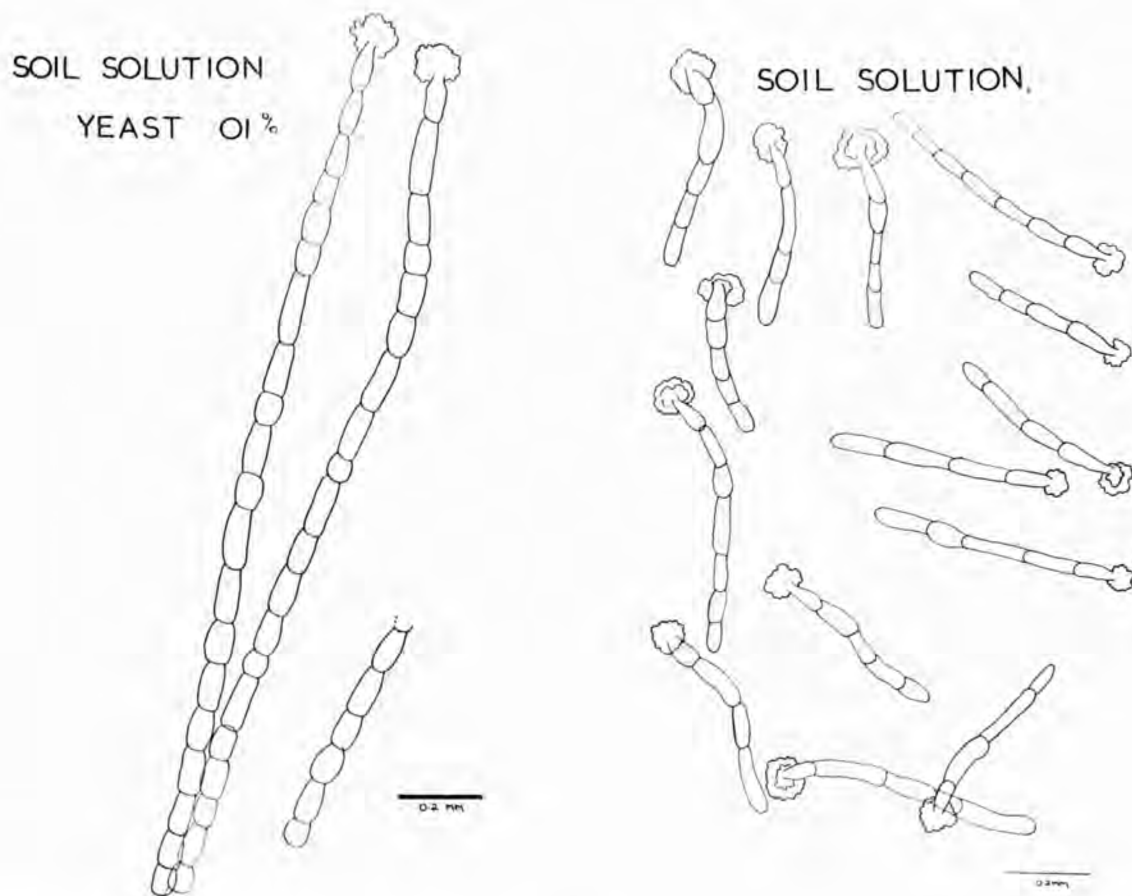


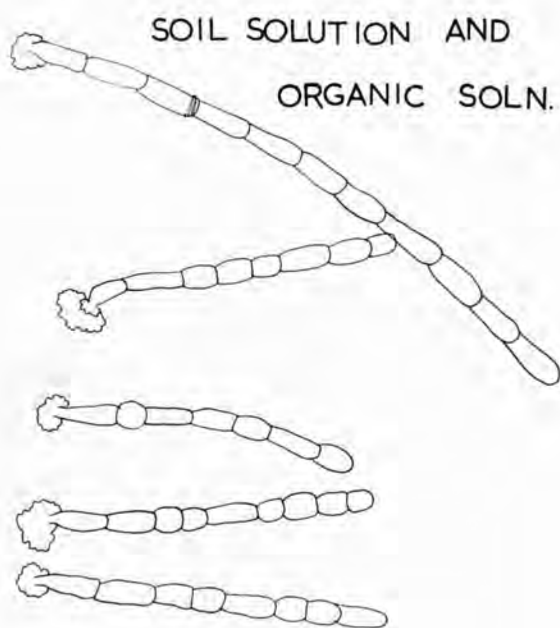
Fig.14. The formation of irregular holdfasts in increased light intensity.(not drawn to scale.)



Figs. 15. a,b and c. Filaments grown for 14 days in a)yeast extract (100 mgs. per L.) b) soil extract solution and c) thiamine (0.1 mgs. per L.).

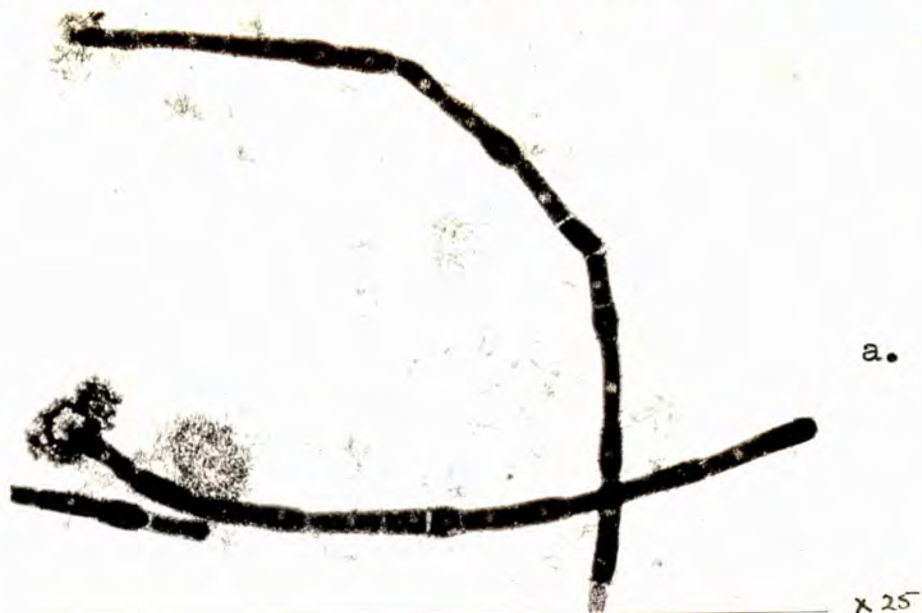


a. b.



c.
(thiamine.)

Figs. 16 a,b and c. The appearance of filaments grown for 20 days in a) yeast extract (100 mgs. per L.) b) thiamine (0.1 mg per L.) and c) soil extract solution.



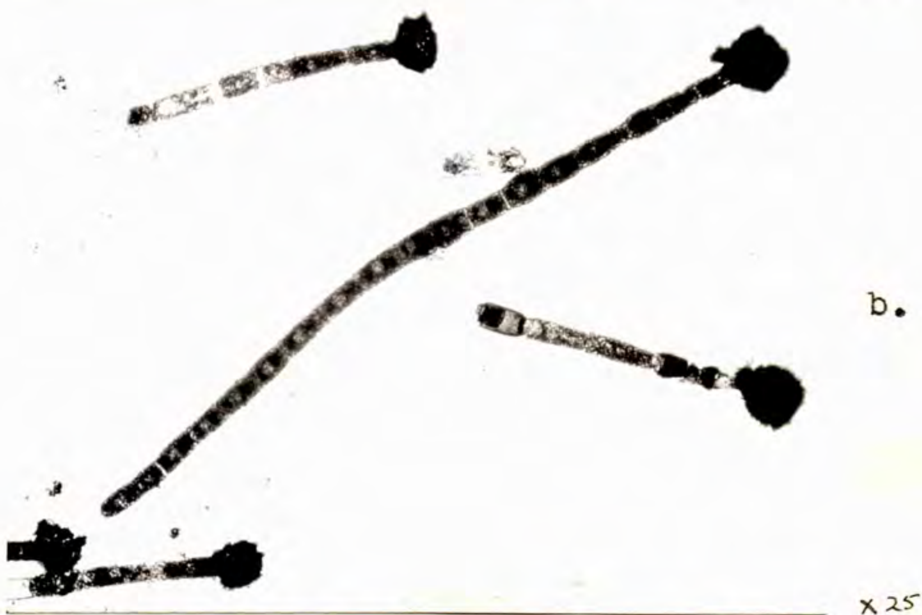


Fig.17. Cell length measurements and cap distribution in filaments taken from the pond and grown in culture WITH ~~in~~(0.3gms. per litre)naphthalene-acetic acid and in soil extract solution.(10 units equal 40 μ.)

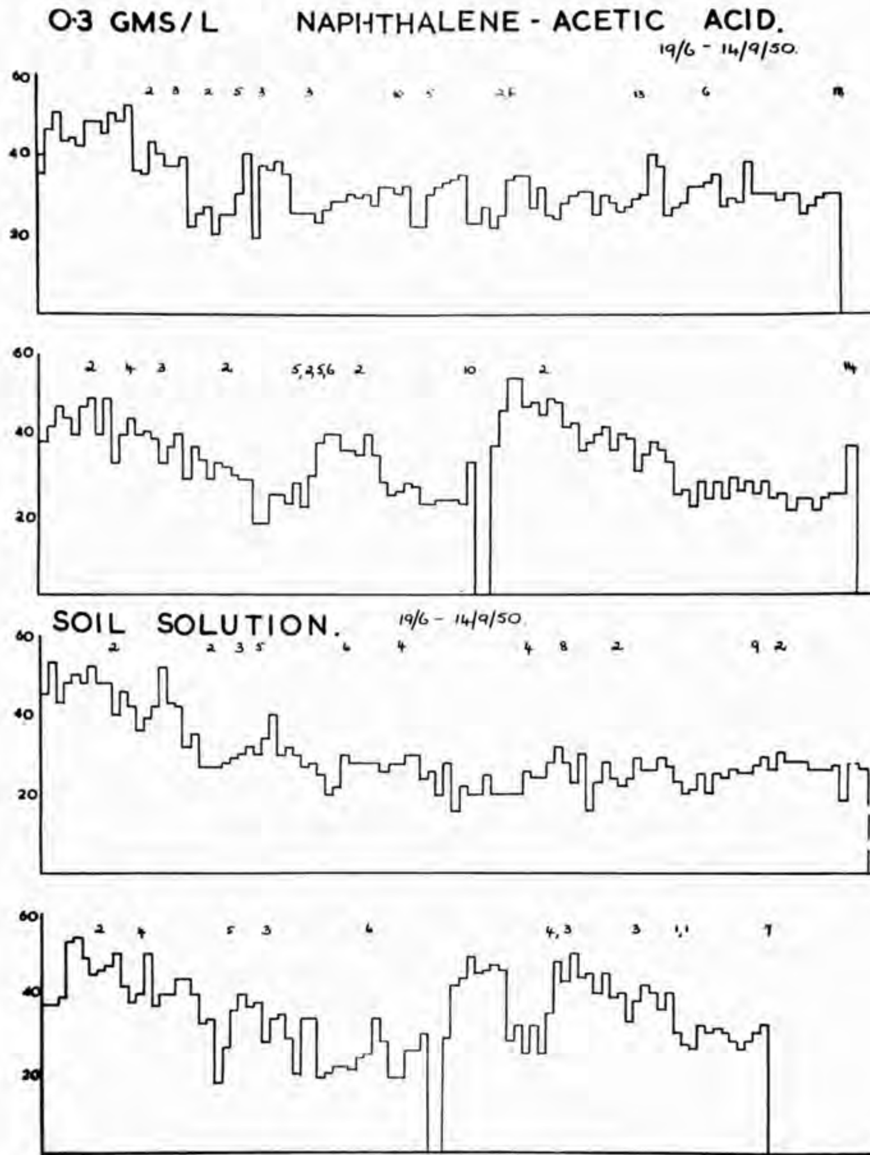


Fig. 18. The appearance of filaments which were produced and grown in culture containing naphthalene-acetic acid (0.4 gms per litre).

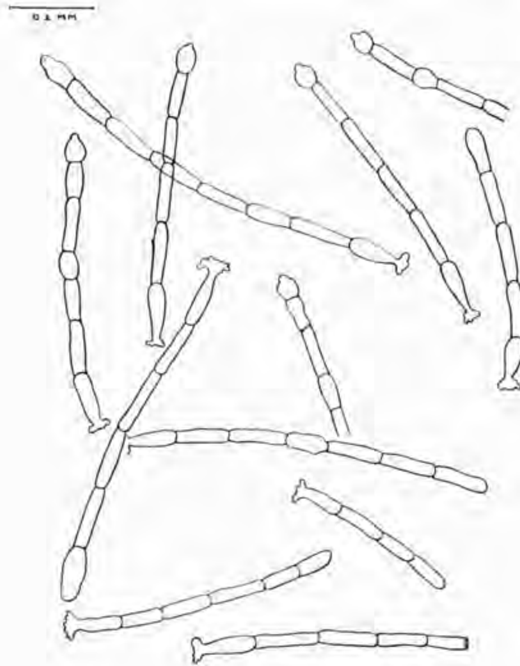
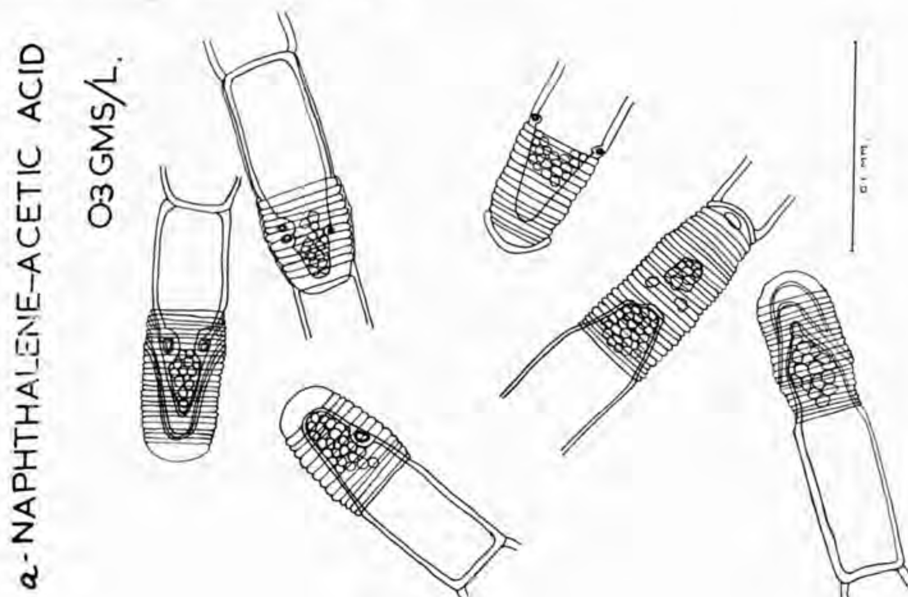


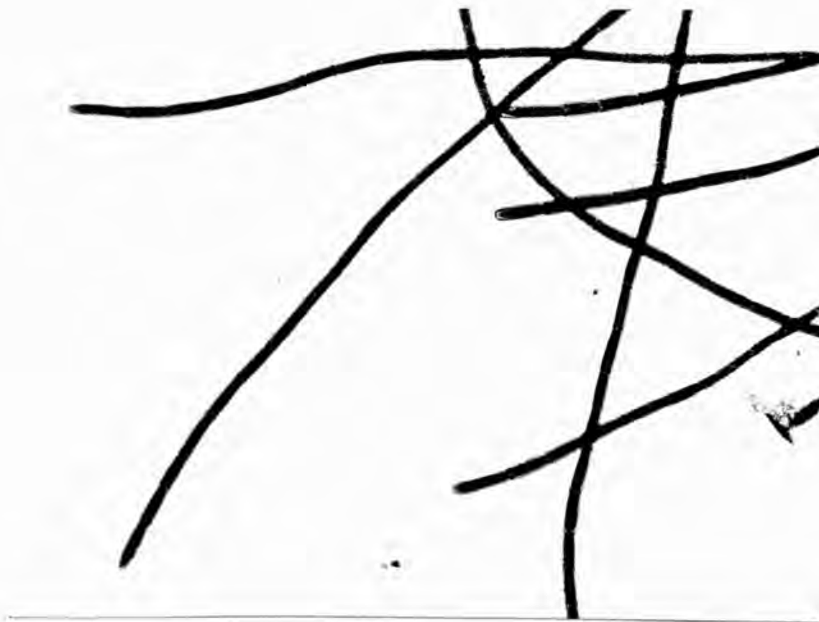
Fig. 19. The accumulation of large groups of caps in filaments grown in a culture containing naphthalene-acetic acid (0.3 gms. per litre).



Figs. 20 and 20a. Cap grouping in filaments grown in (0.1 gms. per litre) naphthalene-acetic acid (20) CULTURE and in soil extract solution.

20.

x15



20a.

x 15

Fig.21. Histograms showing the number and the size of apical and intercalary groups of caps in filaments which were taken from the pond and grown in varying concentrations of naphthalene-acetic acid.

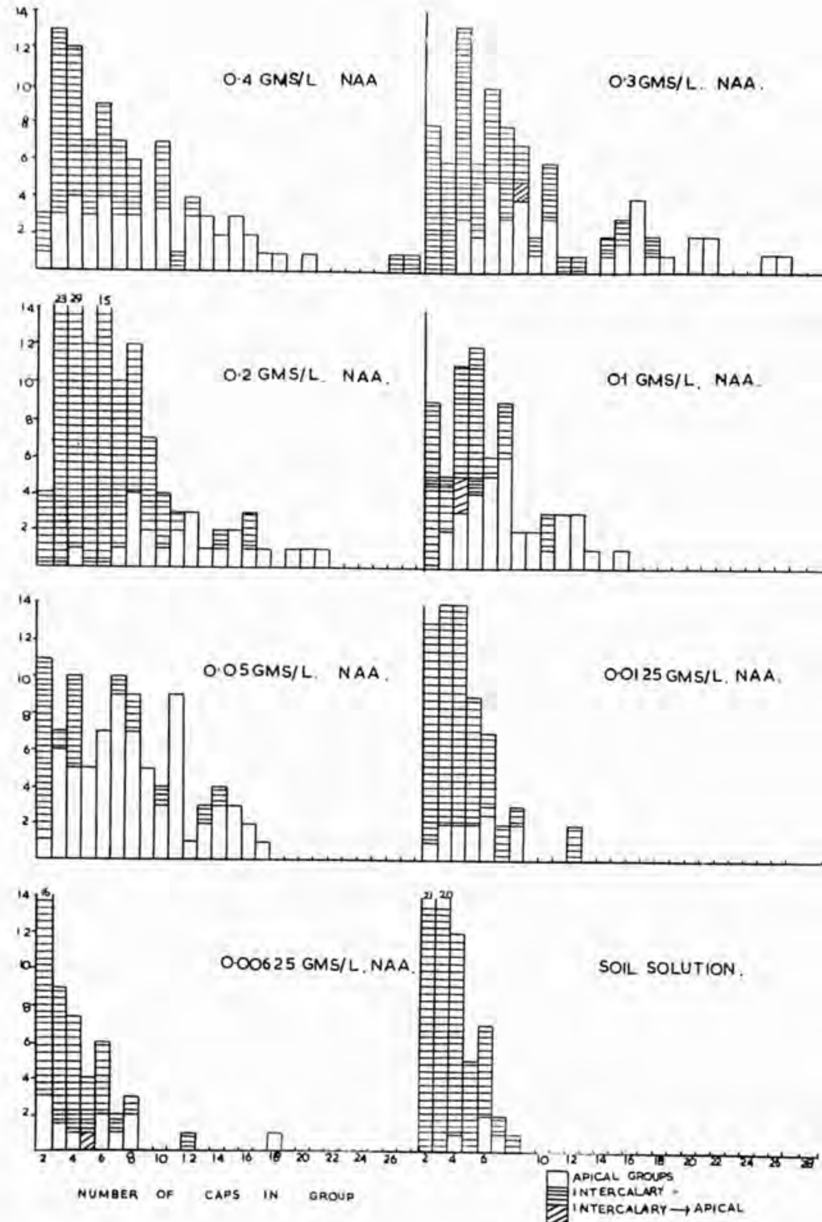


Fig.22. Histograms showing the number and the size of groups of apical and intercalary caps in filaments which were grown in culture in varying concentration of indole-3-acetic acid.

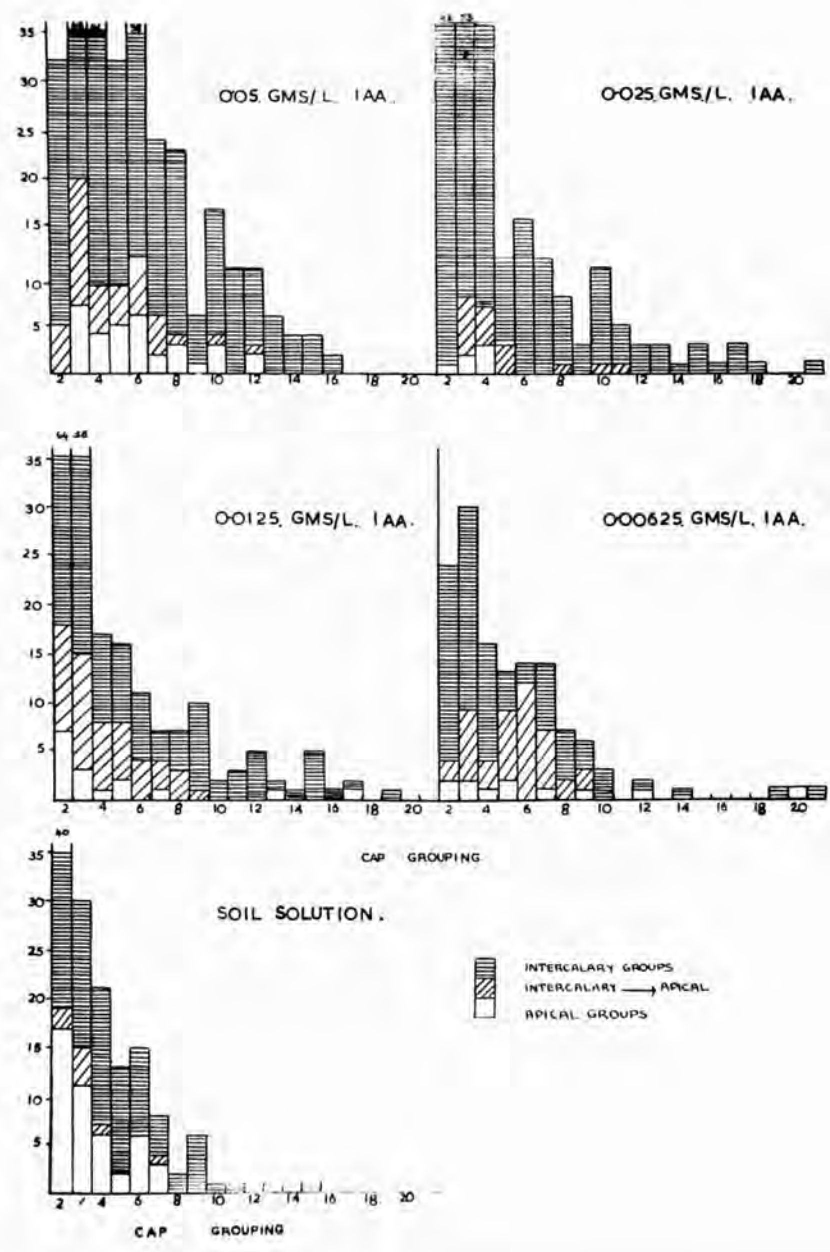


Fig 23. Histograms showing the number and the size of apical and intercalary groups of caps in filaments which were grown in culture in varying concentrations of naphthalene-acetic acid.

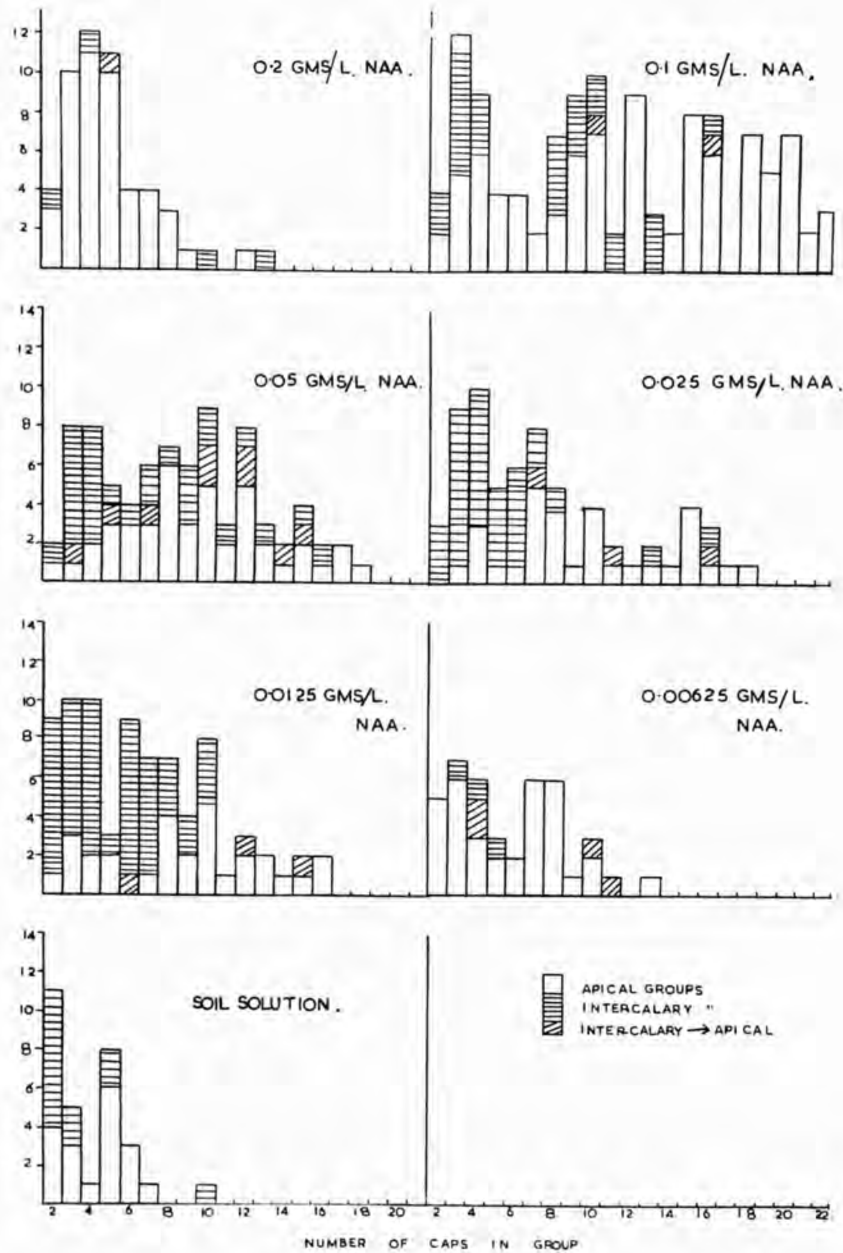
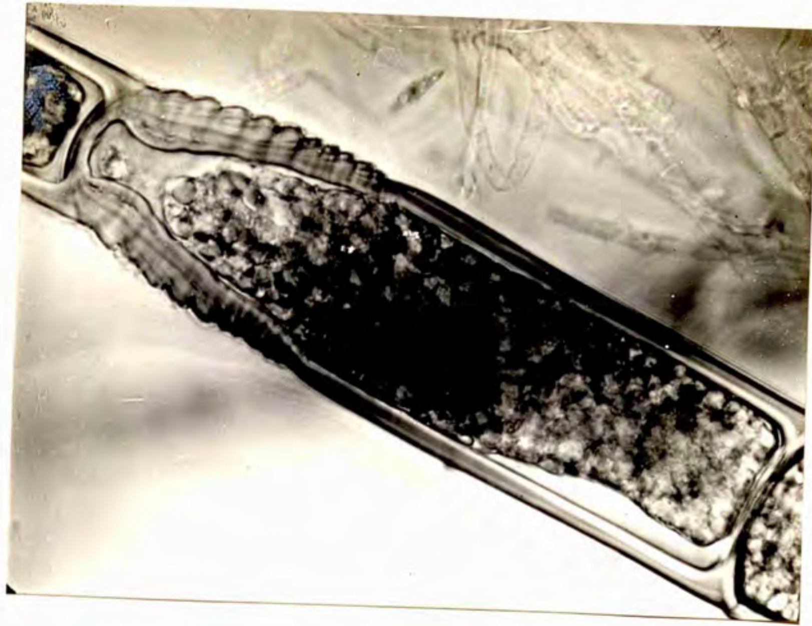


Fig. 24. Fig.25. Groups of caps found in filaments which were grown in(0.3 gms. per litre)naphthalene-acetic acid, culture.

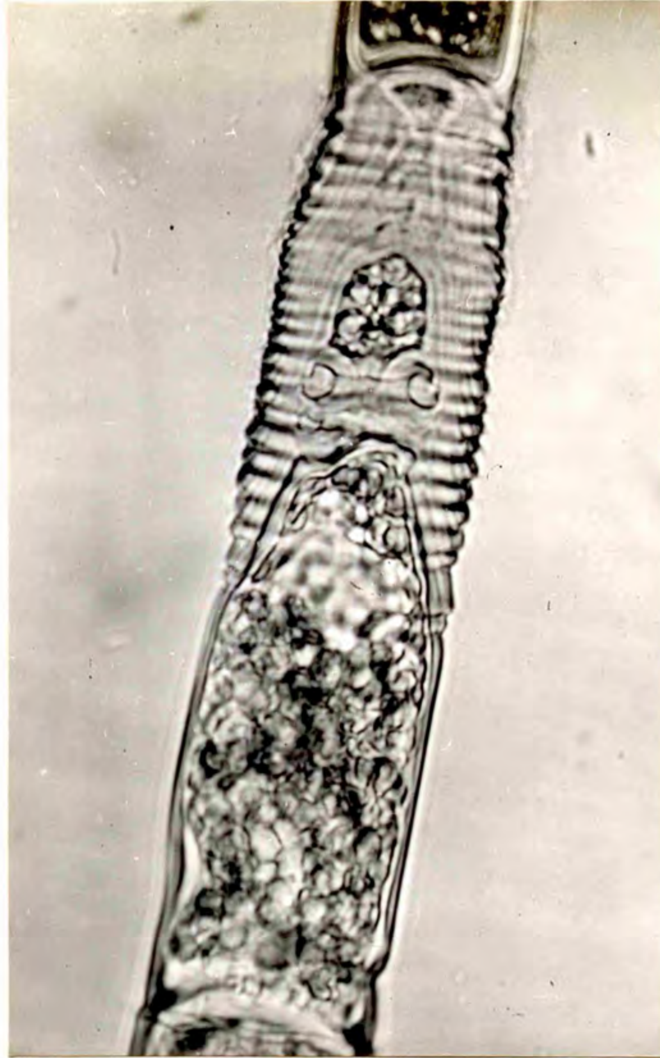


24.
x 750

25.
x 600.

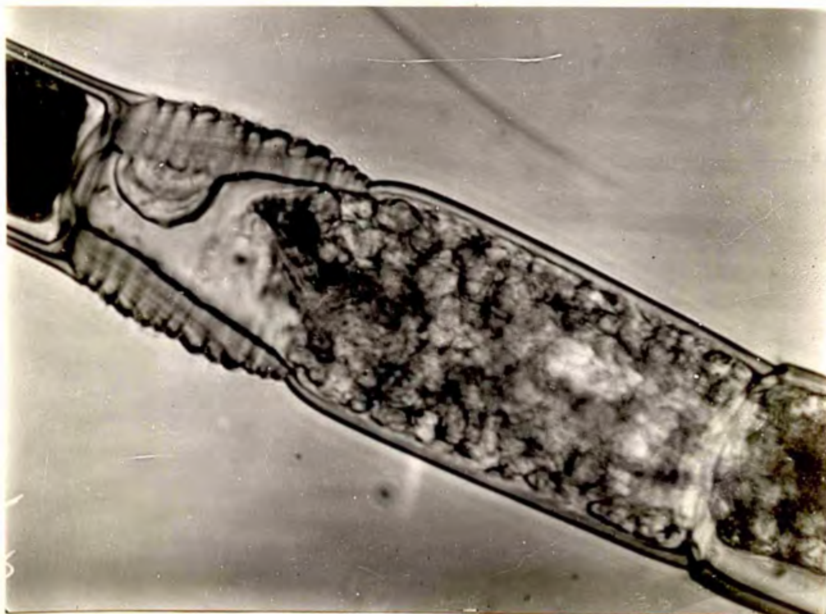
Fig.26. Groups of caps in filaments which were grown in(0.3 gms. per litre)naphthalene-acetic acid, culture.

Fig.27.



26.

x 600.



27.

x 700.

Fig. 28. Abnormalities formed in large groups of caps.

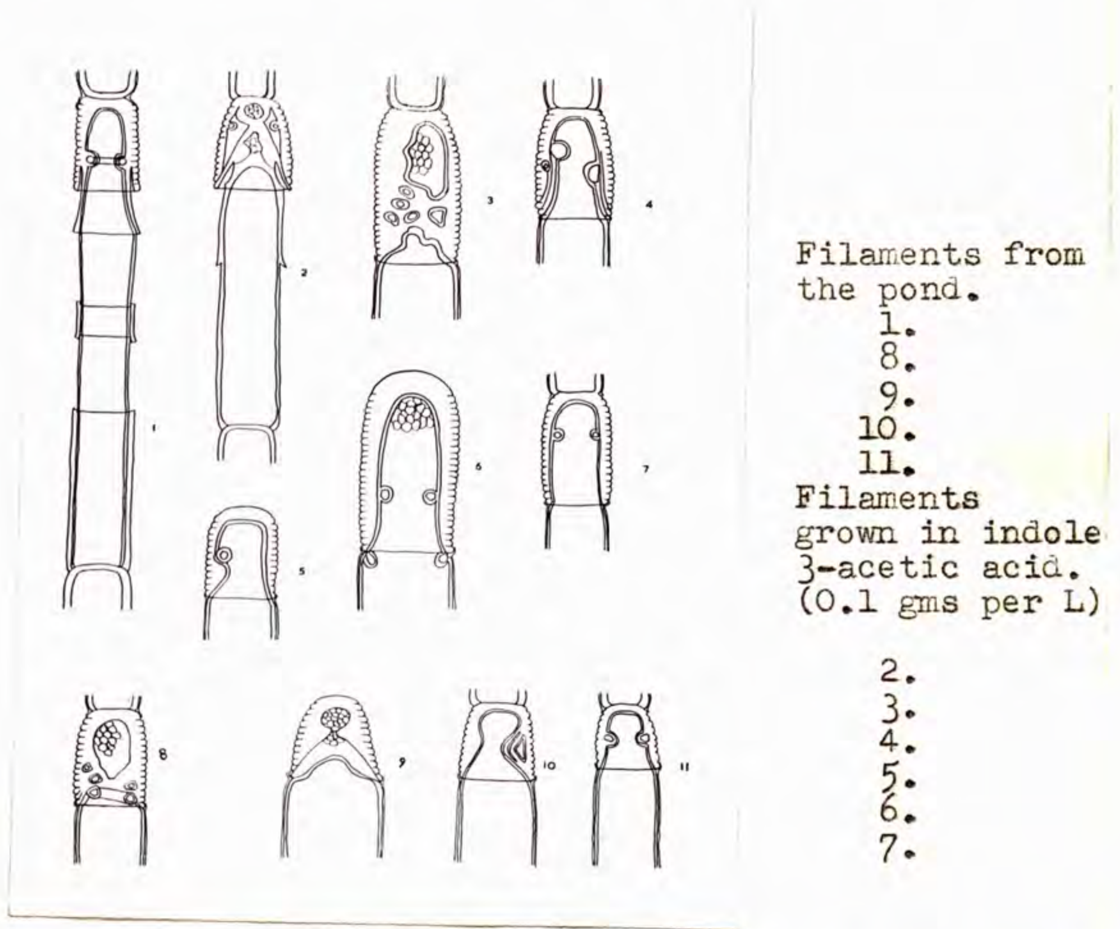


Fig. 28a. Abnormalities formed in large groups of caps found in filaments grown in(0.05 gms per litre) naphthalene-acetic acid culture.

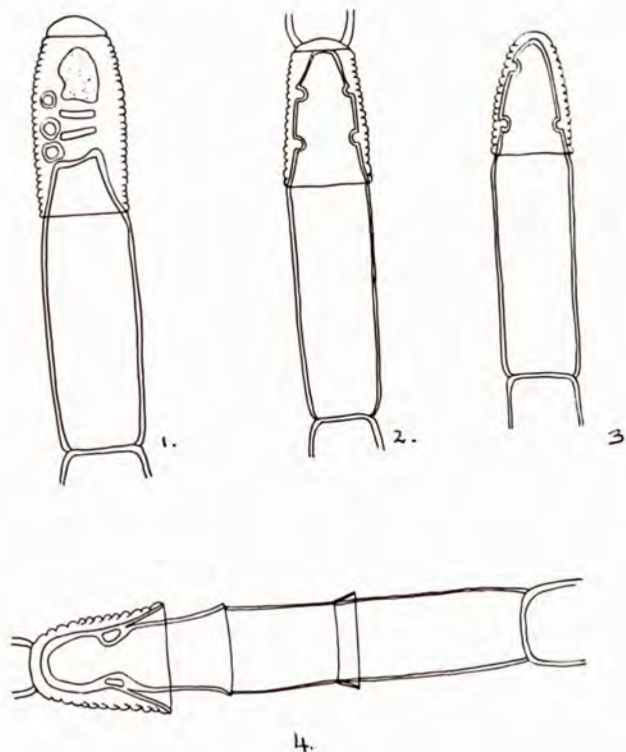


Fig. 29. Abnormalities found in large groups of caps formed in filaments grown in (0.1 gms. per litre) indole-3-acetic acid culture.

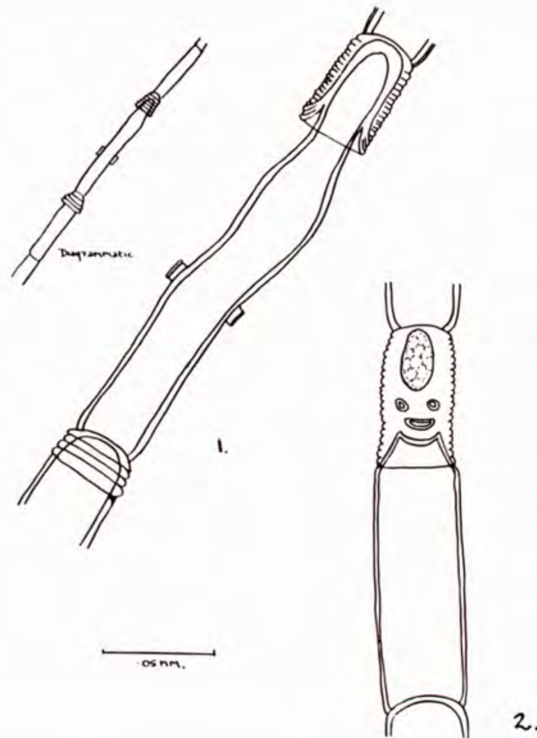
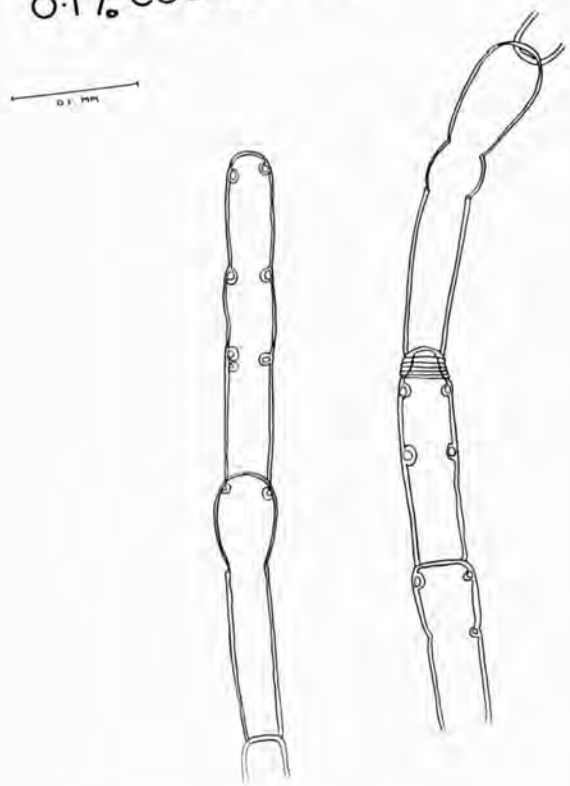
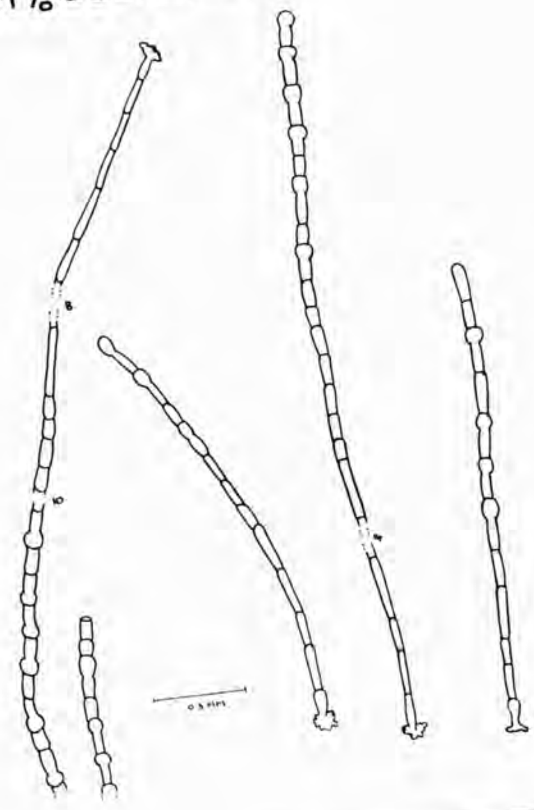


Fig. 30. Filaments grown in colchicine 1.0 gms. per litre of SOIL EXTRACT SOLUTION.

Fig 31.



30.



31.

Fig. 32. Abnormal development of zoospores formed in (1.0 gms. per litre) colchicine culture.

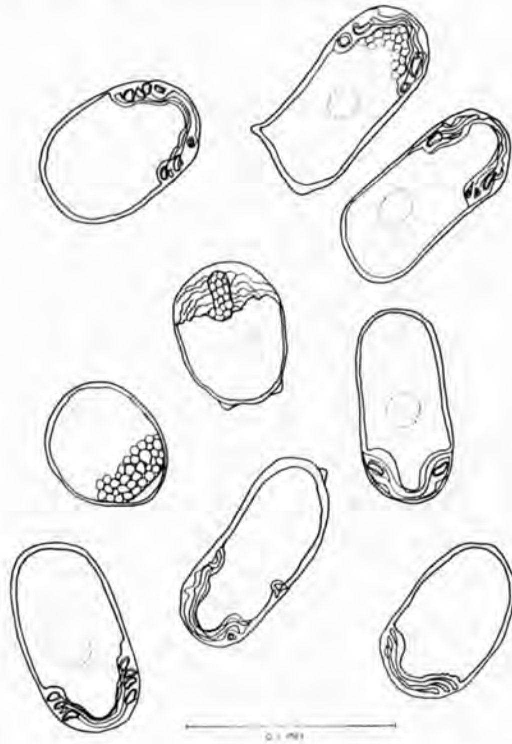


Fig. 33. Normal and abnormal development in filaments produced in (0.1 gms. per litre) colchicine culture.

·01% COLCHICINE.

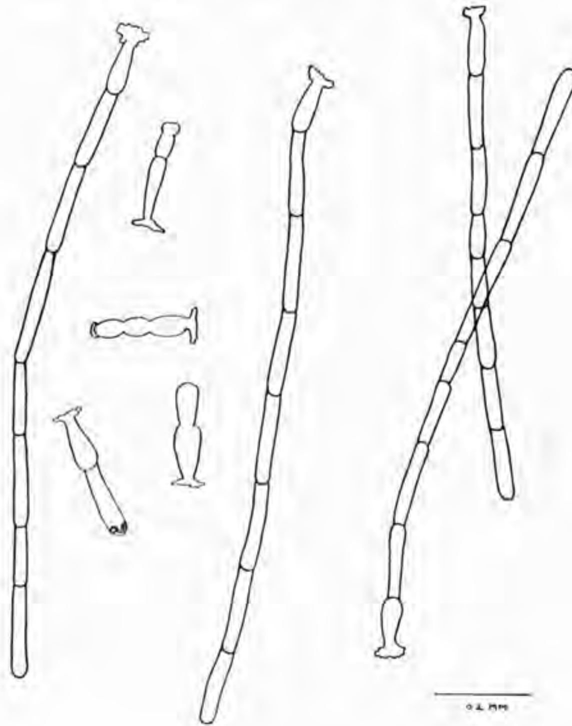
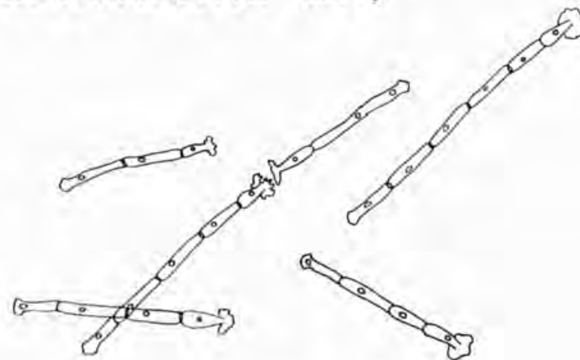


Fig. 34. Normal and abnormal development in filaments growing on slides after a week in (0.1 gms. per litre) colchicine culture.

COLCHICINE ·01%



SOIL SOLUTION.

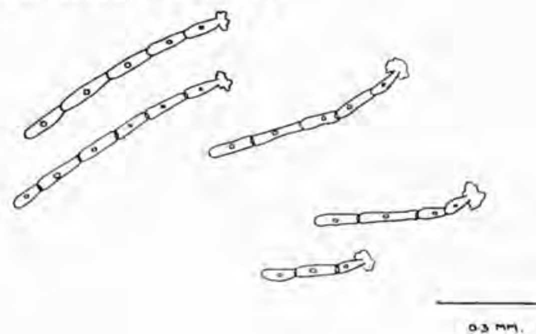
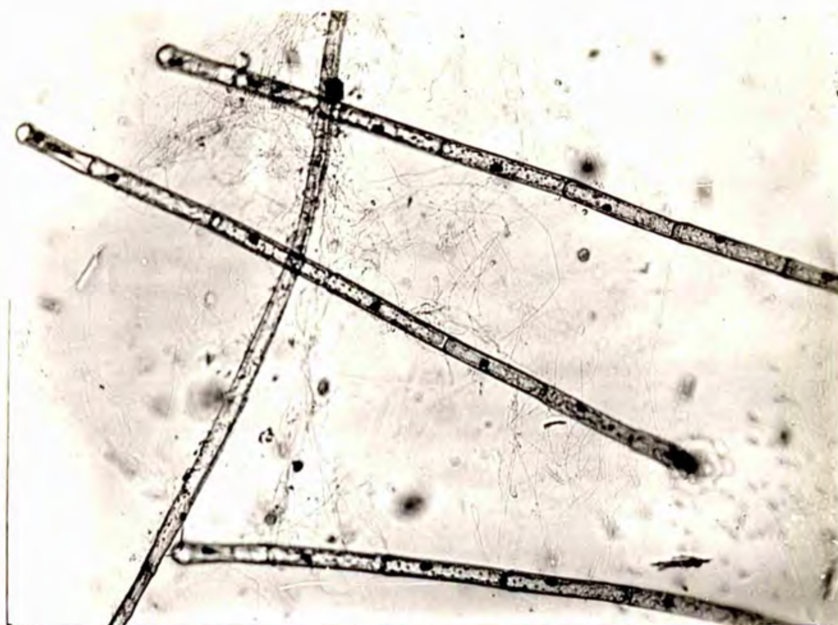
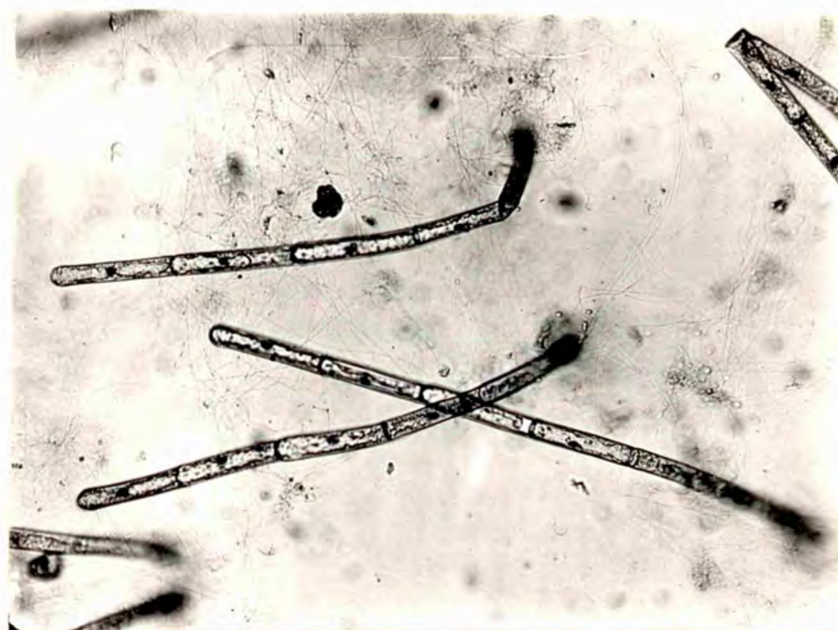


Fig. 35. and 35a. Filaments grown on slides for a week in soil extract solution and in 0.1 gms per litre colchicine. Control is Fig. 35a.

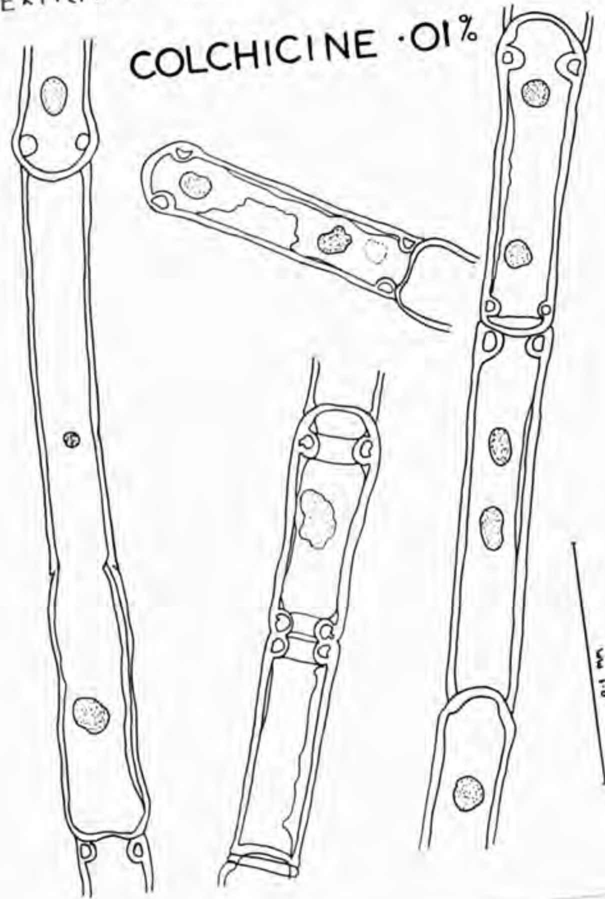


35.
x 25.

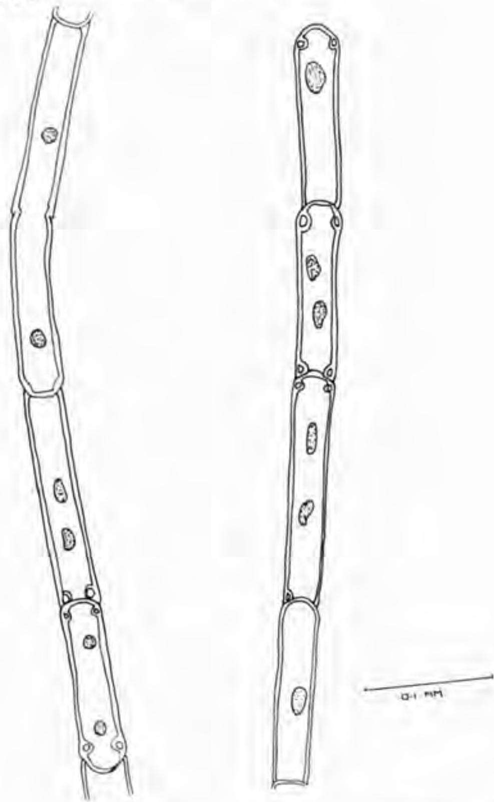


35a.
x 25.

Figs. 36 and 37. RING formation in filaments grown in colchicine(0.1 gms per litre of SOIL EXTRACT SOLUTION.)

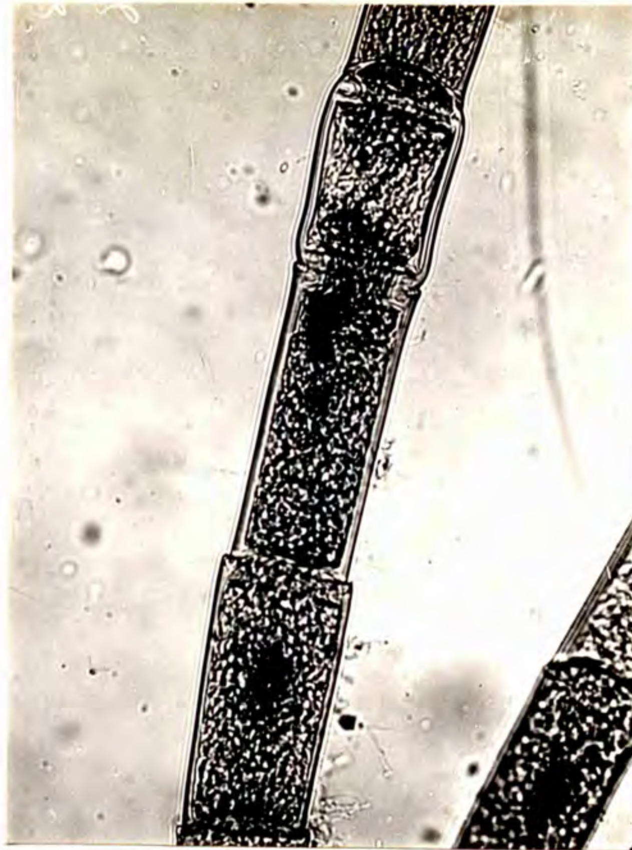


36.



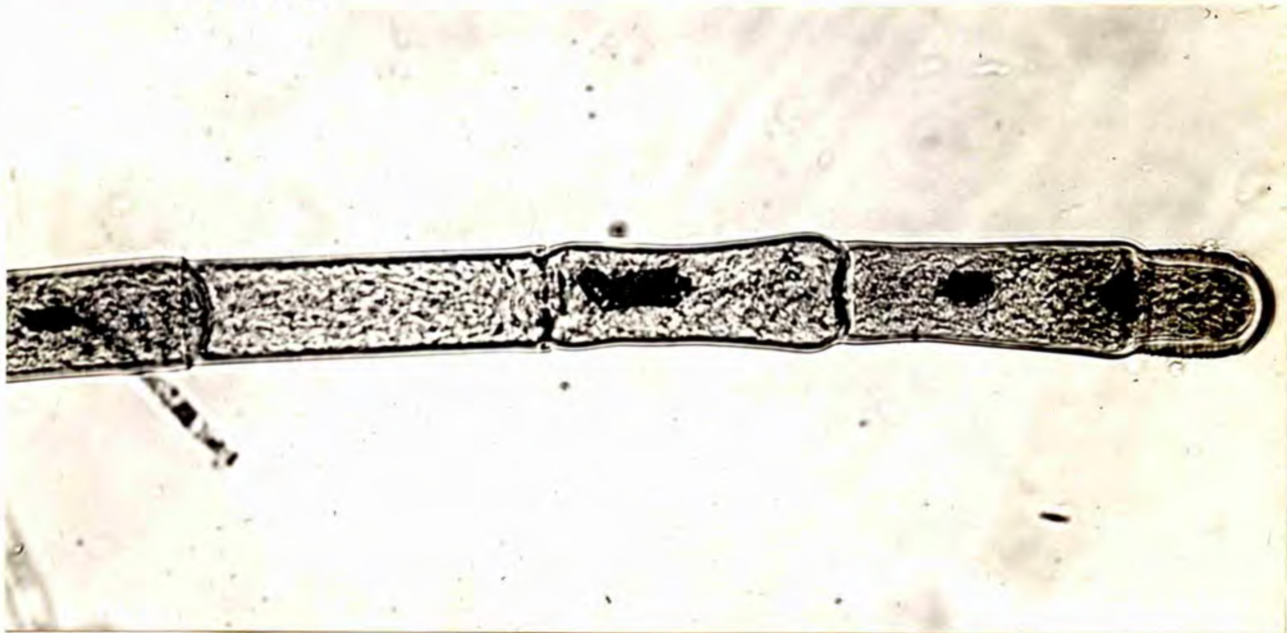
37.

Fig. 38. Abnormal RING formation in a cell of a filament grown in(0.1 gms. per litre) colchicine culture.



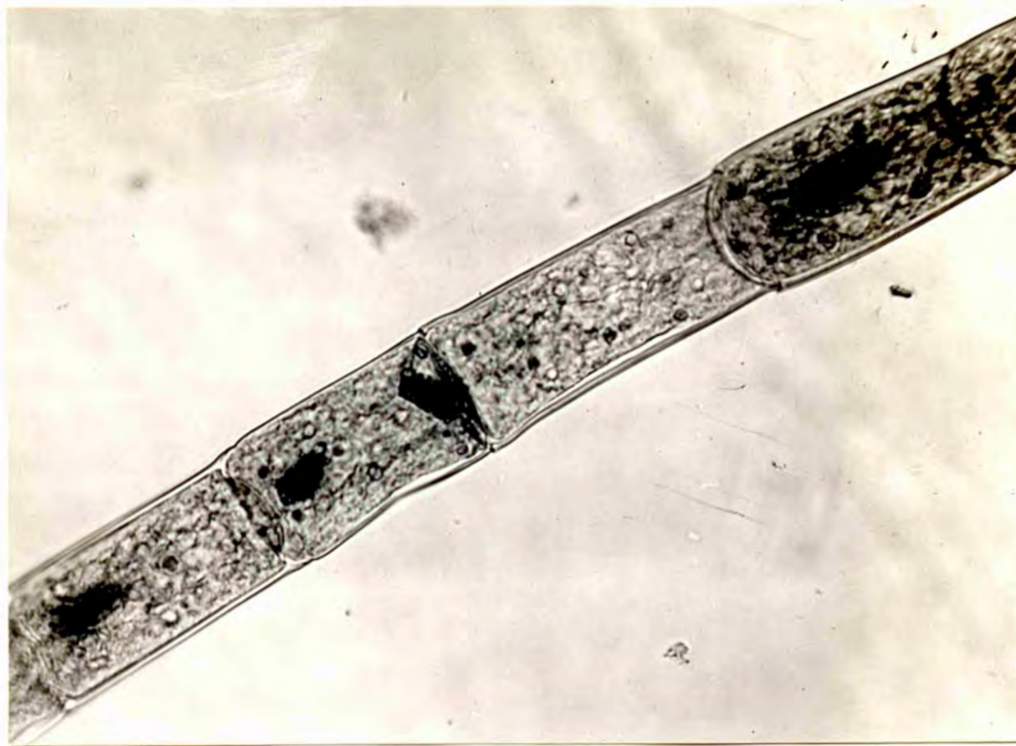
x 300.

Fig. 39. The behaviour of the nucleus in a cell of a filament grown in(0.1 gms. per litre) colchicine culture.

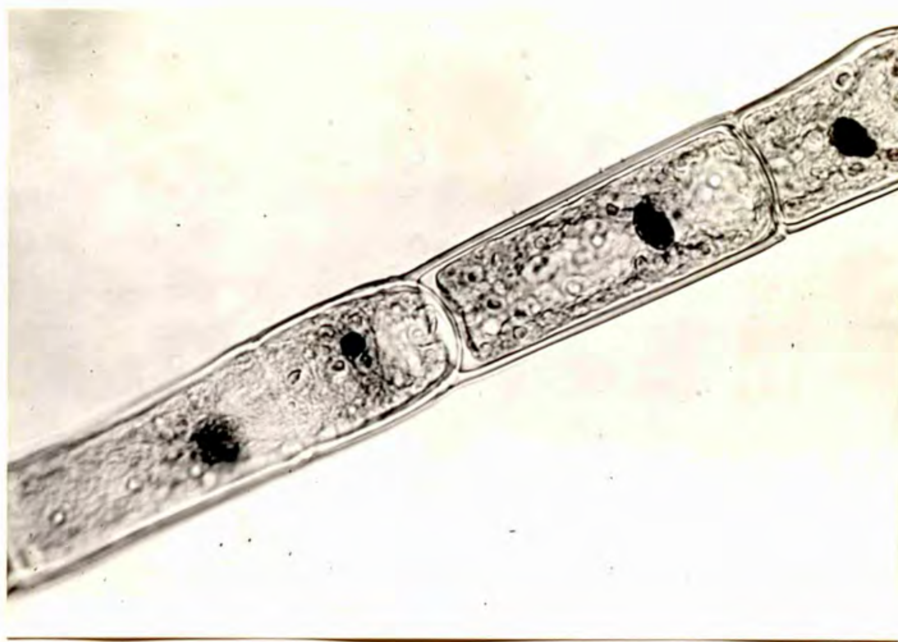


x320.

Fig. 40. Nuclear behaviour in a cell of a filament
Fig. 41. grown in(0.1 gms. per litre) colchicine culture.



40.
×320.



41.
×320.

Table 1. Cell length measurements in fully developed filaments from the wall of the pond.

120 μ	112	88-4	80 μ	112-8	80 μ	168	88 μ	164
160	152	200	188	148	120	212	128	136
172	128	48	208	172	140	192	112	160
100	160-2	72	196	168	160	168	128	144
96	140	56	100	176	120	128-2	132	124
120	148	80	120	152	108	180	152	160
140	176	60	128	120	120-3	172	188	124-2
164	136	100	164-2	100	92	164	204-2	112-10
160	168	40-3	140	100	112	128	160	160-4
168	172	100	152	112-12	140	120	176-2	164
152	172	100	180	128	120	120	120	212-5
92	172		200-3	160	148	108	128	136
92	172		140	164	132	100	160-6	140
108	176		172	200	140	72	164	152
120	188		208	144	128	60	172	200
108	168-2		184-3	172	128	80	124	192
120	176		152	172	160	84	156	156
160	168		204	156	152	168	160	152
112	188		220-5	180	132	140	168	152
152	172		192	164	160	148	176	144
160-2	128-2		192	164	160	152	164-4	140
140	132		176	100	140-3	100	116	
160	108		124	120	152	88	136	
152-2	112		140	112	140	100	188-2	
172	108		144	92	148	84	128	
184	100		140	96	152	96	176	
168	112		168-2	120	140	96	172	
173	140-7		164	120	140		160	
152	128		160	100	140		156	
160-8	192		160	120	152-4		160	
180	208		176		140		176	
172	136		160		160		172-2	
180	128		176		176		140	
192	132		160		160		160	
192	120		164		208-10		160	
192	168-2		152		112		160	
160	120		144		136		172-2	
152-2	132-3		132		140		164	
172	128		120		128		168	
172	132		84		172		164	
196	40-2		72		152		140-5	
152	120		80		160-4		180	
168	160-4		88-2		120		164	
180-4	168		100		152		180	
172	180		92		172-2		160	
160	120		84-2		152		136	
192	120		80		140		160-4	
160	120		96		168		152	
156	92		140		200		168	
208-20	92		68		180		180	

Table 2. Cell length measurements and cap grouping in filaments grown for a long period on slides in the pond. The filaments were examined at intervals from November 17 th. 1950 until September 1st. 1951.

Nov.17-Feb.2.	Mar.21.	April.19.		May.9.		June 18.	
92µ	88µ	88µ	108-2	60µ	84µ	80µ	cont.
112	116	120	120	112	80	112	160
124	108	120	108	128	88	160	180
	120	124	100	160-2	124	160	160
120	116	128	140	52	132	180	136
124		132-4	108	100	164	180	168
100	92	128	140	128	132	188	164
	124	140		56	164	208	180
112	120	160-2	80	132	176	200	
124	128	128	140	116	180	240	100
100	124	160	72	188	192	180	136
112		164	96	204	112-3	216	140
	84	168-2	100	224	168	212	200
104	136	112	96	216	192	172	180
112	112	136	124-2	176	188	160	176
80	128	156	100	196	200	160-2	192
116		144	104			180	188
88	88	128	112-2	80	80	180	180-2
	112	120	80	120	112	100-3	180
112	128		112	72	144	180	184
112	112	80	108	128	132-2	160	188-3
140	100	128	128-3	152-2	80	140	184
140		140	88	88	140	140	196
	96	120	124	132	132	160	160
120	112	144-2	124	152-2	160	140	160
112	112	128	120-2	120	152	180	192
92	100	124	104	160	156	160	160
96	128	128	120-2	176	172		160
96		128-2	100	160	200	72	192
	80	100	124	200	156	100	160
100	112	108	100		200	160	168
128	88	88	140	96		164	112
132	128	100		80	80	196	168
124	80	120		84	112	160	160-5
88	120	112		120	144	200	160
88				136	132-2	172	152
84	100	96		168	80	200	144
	140	128		124	140	220	160-2
104-c	132	140		184	132	236	172
116	100	140		172	160	192	168
120	100	132		196	152	164	172
		128		116-3	156	168-3	164
		120		172	172	232	184-4
		144		192	200	176	192
		132-4		184	156	160	160
		96		212	200	168-3	180
		112				200	
		140				188	

Table.2. (continued)

Nov.17th.-
July 17th.

September 1 st.

100μ	92μ	cont.	96μ	cont.	cont.	cont.
172	176	88	120	112	120-3	152
176	224	140	180	128-8	88	112
192	228	92	220	100	60-2	128
216	220	64	224	80	92	120
200-2	188	88	248		72	120
168	147		240	88	80	144-6
200	192	100	232	128	80-4	140
204	172	152	216	168	96	60
180	184	88	196	192	80	112
184	160	92-2	188	196	88	92
120	184-3	200	170	240	116-4	132
136	160	208-2	160	228	112	128
152	128	224	132-2	216	100	132
200	152-2	212	180	236	100	108
192	160	192	168	188	120-6	108
140	160	200	132	184	120-2	112
152	156-2	180	80	184-2	140	120
104	152	140	112	196	132	144-5
120-3	160	128	112	212	120	144
132	160	128	120-5	188	136-3	136
120-3	172-3	144-6	160	88-2	104	60
132	148	148	100	180	120	128
112	116	136	112	92	140	68
152	152	140	80-4	116	112	112
165-2	144-2	128	128	128	148	132
132	148	128	100	108	120	104
120	124	80	120	72-2		160-6
152	160	152	120	132	88	124
152		160-6	120	120	148	132
148	80	136	92	120	200	64-3
120-2	152	152	88	112	220	152
148	208	168	80	80-5	228	120
120-2	228	160	120-3	48	248	132
148	204	160	84	104	220	152-3
120	200		72	120	216	120
192	168		116	96-5	172	132
80	156		120	120	212	152
144	140-2		100	100	192	140-3
156	176		112-5	120	192	132
168	152		120	112	168	92
	152		128	112	152	132
	148		92	100	120-2	148
	156-2		112	100-3	160	92
	160		88	120	120	
	160		120	100	140	
	108-3		116-2	80	148-2	
	156		80	128	152	
	148		100	112	112	

Table 4. The changes in the cell size composition in filaments growing on slides in the pond from November 1950 to September 1951. The percentage number of cells in each group of cell length measurements of 20 μ are shown.

	Feb.2.	Mar.21.	Apr.19.	May.5.	June.18.	Aug.17.	Sept.1.
0- 20μ	0	0	0	0	0	0	0
24- 40	0	0	0	0	0	0	0
44- 60	0	0	0	0	1	1	2
64- 80	7	2	4	9	2	3	7
84-100	33	28	24	10	4	5	14
104-120	*41*	*43*	32	13	2	5	*27*
124-140	19	25	*33*	*18*	5	13	18
144-160	2	0	7	13	20	*36*	9
164-180	0	2	2	13	*28*	15	7
184-200	0	0	0	16	22	14	7
204-220	0	0	0	4	8	6	5
224-240	0	0	0	2	7	3	5

Table 5. Cell length measurements in filaments growing on waterlily petioles in May.

100µ	96µ	100µ	88µ	100µ	100µ
152	80	192	152	136	164
168	152	220	176	168	172
176	176	204	180	192	192
180	192	184	172	200	176
200	192	168	200	196	128
216	216	168	216	192	156
200	224	192	200	160	172
196	208	172-4	208	176	140
200	184	220	120	160	172
192	192	208	196	188	168
160	192	192	160	196	204
176	188	196	172	192	180
	160	156		176	192
	188	168		188	196
	140	164			144
	140				160-3
					180
					184

Table 7. Cell length measurements in filaments growing on waterlily petioles in July.

84µ	96	100µ	100µ	88µ	cont.	88µ	cont.
152	100µ	148	120	116	200	120	140
184	112	184	140	176	80	140	160-4
160	160	160	152	184	80	184	160
140	140	160	160	192	100	200	156
164	128	168	192	176	120	196	132
156	128	172	192-2	92	120	200	152
160	176	160	196	152		196	136-3
132	168	112	204	120		180	128
144	168	160	180	60-2		200	152-2
172-3	140	88	180	152		192	152
188	128	72	168	120		192	140
164	152	100	128	120		156	160
156-3	152-2	128	144	120-2		112	152
124	160	128	140	112		128	
128	152	156	140	128		132	
128	132	160	80-3	120		120	
140-3	172	168	168	88		132	
132	120	152	160	96		140-4	
124	140		160	72-3		172	
144	148		132-3	112		140	
140	144		140	128		144	
120	112-2		152	80		152-3	
140-3	136		128-2	100		140	
124			140	112		128	
140			120	140-2		144	
168			152	112		136-3	
			132	120		144	
				120		120	

Table 8. Cell length measurements in filaments growing on slides in the pond for short periods of time. The measurements were made during June to October 1951.

June.20-July.18.	July.6-July.18.	July.17-Aug.6.	July.30-Aug. 22.	Aug.6-Aug.22.	Oct.3-Oct.29.
96μ	112μ	80μ	92μ	112μ	112μ
112	144	120	132	100	100
156	144	160	184	132	176
160	176	168	184	160	208
152	192	144	180	184	
180	192	140	180	180	112
188	188		204	172	156
168		80	176	192	192
180	104	120	192	188	168
192	120	144	192	160	
	160	128	200	200	112
112	156	160	176	192	152
120	168	172	180	180	180
172	172	80	192	160	192
180			172	160	
168	120	72		160	104
152	136	120	92		140
180	160	160	128	88	192
180	184	168	164	128	192
176	188	148	160	160	
	192-2		172	192	92
100		100	176	192	172
128	104	132	180	188	92
152	92	128	200	160	152
140	140	140	184-3	200	
152	128	120	160	192	92
172	168		172	188	132
172	208		176	160	160
156	180		180		200
			92	100	112
96	88		152	132	
108	120		168	200	
152	184			200	
172	160			204	
160	196			196	
200	200			192	
168	208			172	
168				192	
188				168	
				180	

Table 9. Cell length measurements in filaments taken from the pond February 19 th. and grown in culture for two months in soil extract solution and in pond water.

Filaments from the pond Feb. 19th.	Soil extract solution culture.		Pond water culture.	
100μ	84μ	cont.	100μ	88μ
204	140	72	204	152
212	144	80	220	180
240	192	72	208	184
224	184	104	216	144
200	188	100	224	160
208	216	96-3	220	164
208	196		216	156
176	220		224	100
160	184		220	124
176	200		212	132
160	188		204-3	124
136	172		204	132
152	192		220	124
132	104		184	128
144	104		164	120
128	120		104	96
112	112		88	128
112	120		84	120
112	104		80	92
116	120		124-7	108
	132		180	128
120	124		136	120
152	112		152	92
212	144		100	108
208	140		88	128
121	152		84	104
192	148		84	60-5
224	48		92	108
216	72		60	104
216	80		200-7	120
192	80		140	84
204	84		180	120
196	112-3		80	128
176	64		72	104
168	80-3		152	156
152	64		64	124
160	80		84	88
164	64		80	84
152	80		160	72
144	72		84	72
172	84		60	64-5
	112		76	152
	96		80	120
	112		40	104
	72		80	120

Table 10. Cell length measurements in filaments developed from zoospores in soil extract solution.

July 20-Oct. 23 1950			Nov. 3-Dec. 12.		May 5-June 20 1951.	
128 µ	cont. 140-5	156 µ	100 µ	cont. 92	88 µ.	100 µ
128	116	148	120	100	144	132
144	136	140	72	132	192	128
124	136	120	112	96	140	120
160	152	112	108	112	80	120
160	132	108	84	88	120	120
164	156	140	68	96	152	160
148	160	136	22	92	140	120
160	120	124	116	96	112	128
128	60	148	96	160		140
160		160	92	96		160
132		132	80	108		144
120		160	92	112	100	128
152	160	128	80	104	152	152
120	180	156	84	112	200	144
120	148	136	93	72	200	
112	144	104	80	104	192	
112	100	112	88	120	80	
152	108	128-2	112	112	128	108
140	116	144		128	128	148
140	128	60			128	152
136	112	108			128	120
140	140	140	100		80	160
120	132	112	88	88	92	144
132	156		88	92	140	152
	156		72	108	120	136
	148		112	100	120	172
152	60	140	92	92	112	160
156	112	160	112	112	140	156
180	148	140	80	108	120	120
132	128	120	120	64		152
148	128	124	100	96		
168	128	152	112	88		
144	120	132	80	96	80	
160	120	132	112	104	152	
152	120	140	80	108	120	
152	136	140	128	100	168	
160	128	140		76	168	
152	140	136		84	152	
140	144	148		80	120	
148	108	120	112	92	148	
128		116	136	112	168	
132		116	108		160	
140		136	100		152	
144		128	94		152	
116		120	72			
116-2		128	88			
108		116	80			
112		128	96			
		116	80			
		104	88			
		128				

Table II. The variation in cell length in filaments of a clone grown under controlled conditions of light and temperature.

100µ	80µ	80µ	96µ	80µ	112µ
136	112	112	136	112	120
148	152	128	136	136	128
132	128	132	152	120	144
140	104	112	160	152	128
180	108	128	152	160	108
144	120	112	128	160	100
132	132	136	124	172	L00-4
124	124	124	120	176	
120	152-5	140	112	128-2	
132			120	124	92
132			84-3	124	136
132	80	80		124	152
	160	136		144	140
	180	132	84	112-3	136
100	168	132	172	128	152
160	196	152	196	120	96
200	184	116	200	124	152
148	184	128	200		136
176	160	136	196		96
176	172	128	192	100	
168	172	152-1	196	112	
176	168		160	156	100
168	120			160	144
176	128	96		180	164
160	116	140	88	160	168
128	140-3	160	128	120	164
172	120	168	172	128	168
184	132	180	152	140	164
108	160-1	160	152		120
160		136	172		120
132		120	136	80	128
112	88	96	160	120	144
140	132	128	160	172	128
144	160	112-5		160	104-1
	124			160	
	128			180	
	128			140	
	124			168	
	120			160	
	128				
	140-3				

Table 12. Cell length and cell number in filaments grown in normal daylight and under continuous light from a 40 watt lamp. August8 th. until October 18th.

Daylight			Continuous light		
80µ	104µ	cont.	108µ	88µ	112µ
128	132	80	148	180	120
144	144	108	140	156	128
140	140	108	160	108	120-2
116	140		112	180	100
124	136	88	116	152	96
140	160	108	140	152	108
136	144	124	156	140	108-3
160	152	128		144	
156	144	116	100	112	
	140	140	140	160	
92	144	132	160	156	
84	160-5	128	140		
120			128	100	
124	120		92	128	
124	128		136	140	
116	148		160	128	
136	120		152	144	
140	144			136	
120	104		112	128	
136	100		120	148	
160	148		140	148	
172	160		160		
			116	100	
80	Loo		132	80	
100	120		100	112	
116	100		124	132	
120	96		120	116	
108	160		132	140	
124	112			120	
120	116		96	140	
	152-2		80	80-2	
80	124		140	100	
120	100		152	116	
100	80		112	120	
132	152		152	152	
160-3			120	140	
136	100		152	148	
100	120				
120	180				
140	100				
160	112				
140	120				
160	88				

Table.14. Cell length measurements in filaments grown in culture in soil extract solution and in solutions of yeast extract(100mgs.per litre) and in thiamine (0.1mgs.per litre) for 14 and 20 days.

After 14 days.

Soil extract	Yeast extract	Yeast extract (cont.)	Thiamine
100µ	92µ	cont.	88µ
104	·104	84	80
92	100	96	68
112	68	68	100
88	48-3	100	60
88	116	68	60
84	76	100	52
	44	60	88
100	92	60	64
112	64		
124	108	100	112
96	72	100	72
96	48	88	60
100	96	80	44-2
88	56	120	100
84	144	80	60
		108	80
100	100	68	92
108	100	96	80
92	88	76	
128	48	88	100
104	72	60	72
92	64-3	72	48
88	84	40	44
	80		104
100	92	88	44
124	60	120	48
100	72	92	60
124	64-2	108	80
92	92	64	44
104	84	84	36
	72	64	64
96	100	84	
112	88	68	92
116	80	48	96
92	80	56	72
92			92
80	120		64
	100		56
	80		76
	76		104
	120		72
			40
			40

After 20 days.

Soil extract	Soil extract	Yeast extr.	Yeast extr.	Thiamine
96µ	60µ	92µ	60µ	100µ
112	72	100	100	96
104	80-4	84	60	76
52	84	48	80	76
68	48	72	68	104
140-3	80	72	84	96
144	68-5	124	80	68
84	116	56		88
60	84	96	80	56
84	80	52	104	84
68-2	124	68	92	56
88	60	112	52	84
120		56	80	88
60	104	80	80	72
92	84	56	120	48
124	88	68	52	
64-4	116	108	92	44
112	96	72	60	48
72	100	72	72	
140	116-2	88	104	104
52-2	100	68	60	80
104	84	108	80	72
136	88	72	60	88
48	88	72	68	64
100	100-5	88	80	88
140	84	68	72	60
48	84	80	104	84
112	100	72	68	68
	80	60	64	80
100	68	80	64	92
100	100		80	96
140	112	104	80	80
88	92-4	92	60	100
60-3	100	100	80	40
116	104	88-2		56
68-2	132	112		
72	88	80		100
56-3	112	84		100
132	72	108		76
80	112	80		92
88	128	68		104
88		80		76
84		80		64
		92		64
		72		56
		56		72
				92
				32

Table 17. Cell length and cap grouping in filaments taken from the pond and grown for three months in soil extract solution and 0.3 gms. per litre naphthalene-acetic acid.

Soil extract.

95µ	cont.	cont.	cont.
180	104	116µ	100
212	112-4	104	96
168	120	120-2	136-5
192	120	112	144
200	96	112	108
192	104	104	104
208	80	104	128
192	112	L08	116-2
192	64	72	152
160-2	88	112	140
184	80	108	116
168	88		80
144	100		100
156	80	88	
168	80	172	
208	84	188	
172	80	176	
168	104-4	192	
128	96	188	
140	96	208	
108	112	200	
108-2	128-8	180	
108	112	184	
112	92	180	
116	120	160	
120-3	64	160	
128	92	196	
120-5	112	144	
136	96-2	164-2	
160	88	136	
120	96	148	
128	116	152-2	
120	104	128	
108	104	132	
112	116	132	
100	108	80	
80	88	80	
88	80	88	
120-6	84	92	
112	100	116	
112	80	96	
112	100	112-5	
	104	160	
	100	136	
	180	80	
	108-9	68	

naphthalene-acetic acid.

92µ	cont.		cont.	cont.
152	144	100µ	96	108
168	132	168	112-6	100
184	124-2	204	160	96
184-2	132	208	80	120
192	136-4	180	108	112
184	144	180	108	116
180	160	192	120	120
176-2	128	192	108	124
176	140	232	120	128
188	104	232	108	128
152	100	200	120	132
132-2	100	172	112	136
136	100	152	124	120
104-2	104	180	104	92
124	92	176	96	100-3
144	72-2	84	112-2	112
132-3	96	68	140-6	112-6
132	128-12	64	92	136
140	128	96	112	128
144	156	84	108	124
116	152	108	100	140
112	156-2	92	96	100
104	152	96	120	L00
104	128	108	112	124
L04	176	100	116	88
84	136	100	120	120
84	136	120	124	200
96	124	112	128	188-Ø19
108	132	148-11	128	152
128-7	60	144	132	152
84	72	124	96	124
96 -17	72	92	108	124
176	100	84	120	140
152	96	84	108	
140-2	92	112-4	120	
152	100	72	112	
112	96	968	124	
120-3	96	84	104	
132	104	96	112-2	
160	104	100	140-6	
132	100	96	92	
148-7	152-12	104-5	112	

Table 18. Distribution of cell length and cap grouping in filaments grown for three months in varying concentrations of indole-3-acetic acid. (gms. /L). Basal cell omitted.

0.05	0.025	0.0125	0.00625	cont.	control.	cont.
80 µ	120 µ	120 µ	120 µ	120-5	100 µ	108
120	152	176	152	108	128	96
140	128	120	140	96	140	104
112	108	112	124	100	108	104
140	96	92	96	104	100	120
120	96	100	80	100	80	128
92	112-2	112	80	104	100	
88	100	108	96	104	124	
104	112	104	76	92	92	
100	140-2	100	100	116	96	
120-2	100	112	80	88	96	
84	140	100	80	104	136-4	
100	136	140-8	100-4	100-6	124	
128-5	152	88	92		80	
128	136	100	108		120	
80	128	116	108		100	
100	152	112	88-2		80	
112	140	132	88		120-3	
120	144	104	92		136	
100	168-3	116-3	100-3		128	
120		116	92		92	
128		120	88		120	
120		124	80		80	
80		100	80		116-2	
128-7		108	112		128	
84		100			112	
100		120				
100						
92						
112						
104	84	88	100		92	
136	132	164	120		120	
136	136	80	168		132	
72	104	60	96		92	
108	100	80	120		116	
128	88	92	120		80	
136	104	100	104		132	
124	96	120-3	100		140	
116	112	44	100		124	
120	120	96	96		104	
108	128.	108	112		136	
92	136	112-2	120-4		112	
100	124	88	100		96-2	
84	132	108	112		80	
100	112	124-3	100		84	
80	140	44	100		100	
108	100-2	72	96		120	
80	140	80	96		104	
84	140	104	112		128-4	
100	128	108	100			
152-8	60	112				
112	120	120-5				
120	124	88				
136	140	100				
	136-2					

Table 19. Distribution of cell length and cap grouping in filaments grown for three months in varying concentrations of naphthalene-acetic acid. (gms./L.) Basal cell omitted.

0.4 112 µ	0.2 88 µ	0.1 112 µ	0.05 112 µ	0.025 120 µ	0.0125 100 µ	0.00625 96 µ	control 108 µ
140	140	160	104	100	128	144	108
140	116	148	124	152	120	144	124
132	112	120	124-3	120	124	140	120
124	112	120	84	116	128	80-2	116
120	108	124	116	92	112	128	120
120	112-2	96	124-2	104	84	112	116
144	120	108	104	112-5	92	104	128
148-5	104	92-2	100	120	100	104-3	112
	136-5	120	80-3	92	80	116	112
112	120	112	88	72	100	108	128
160	112	100	100	92	136-7	96	108
160	108	104	100-2	96	120	100	120
44	112	120-5	100	136	112	104	128
120	72	120	128-4	112	100	112-6	116
200	100	120	120	104	104-3	120	112-2
112	96-2	112	120	108	124	104	128
120	100	112	112-2	100	76	120	136
112	100	108	96	108-4	140	108	128
72	120	108	112	92	120	104	120
	136-4	116	112	112	96	116	
120	100	116	140	112	116-5	128-3	80
136	124-4	120		112	108	88	88
116		128	108	120	88	112	88
96	100		96			120	72
72	128	100	144	112	120		76
112-3	72	128	136	128	112	104	72
116	112	152	132	124	120	76	60
96-2	112	128-3	128	120	136	112	72-5
	120	124	112	124	128	128	92
	120-3	84	108	120-3	100	96	76
	108-2	132	108	108	88	104	72-3
	120	112	124	120	80	100	80
	104	120	128-4	88-2	72	96-2	72
	100	108	128	80	92-2	96	72-4
	108	124	124	72	96	88	84
	100	112	124	92	120-5	104-4	80
	116	88-2	120	92	128	120-7	80
	108	112	136-3	100-3	120	120	80
		108-2	144	120	128	128	72-3
		72	120	112	112	120	120
		116	128	104	96	92	104
		120	128-2	112-3	112	92	120
		128	136	108	120	112	108
		120	120	104	100-4	88-4	108
			136	120	112	116	120
			116	128	80	132	120
			132	120	88	116	104
				128	104	100	116
				120	112	100	112
				124	92		80
					112-3		112
					120		88
					64		
					96		
					132		

Table 21. The percentage number of cells occurring in each size group of 20 μ in varying concentrations of indole-3-acetic acid. (gms. per litre.)

	Control	0.05.	0.025.	0.0125.	0.00625.
- 20μ	-	-	-	-	-
24- 40	-	-	-	-	-
44- 60	-	1.5	.6	3.1	2.6
64- 80	5.1	11.3	1.4	11.5	12.4
84-100	24.7	37.8	17.7	30.4	43.5
104-120	45.3	34.0	32.0	31.5	34.8
124-140	23.0	12.1	40.0	13.0	4.7
144-160	4.5	3.4	6.8	7.0	14.6
164-180	-	-	1.4	2.3	-
184-200	-	-	-	-	-
204-220	-	-	-	-	-
220-240	-	-	-	-	-

Table 21. The percentage number of cells occurring in each size group of 20μ in varying concentrations of naphthalene-acetic acid. (gms. per litre.)

	Control	0.4	0.2	0.1	0.05	0.025	0.0125	0.00625
- 20μ	-	-	-	-	-	-	-	-
24- 40	-	-	-	-	-	-	-	-
44- 60	0.9	1.14	1.4	0.6	1.22	0.6	7.9	7.45
64- 80	19.0	6.9	1.4	2.4	5.7	4.6	29.9	30.7
84-100	17.2	16.0	14.4	11.2	21.6	23.0	47.8	47.3
104-120	51.5	33.0	42.5	57.5	44.0	49.6	19.5	9.1
124-140	11.7	22.7	33.9	23.7	23.1	20.7	3.5	5.4
144-160	-	18.2	6.5	4.8	4.5	1.7	-	-
164-180	-	3.4	-	-	-	-	-	-
184-200	-	1.1	-	-	-	-	-	-
204-220	-	-	-	-	-	-	-	-
224-240	-	-	-	-	-	-	-	-