EFFECT OF LIGHT AND CHEMICALS ON THE GROWTH AND DEVELOPMENT OF GAMETOPHYTE OF *HAPLOMITRIUM ROTUNDIFOLIUM*

BAO-YU YANG

Leafy gametophytes of *Haplomitrium rotundifolium* developed from spores have been completed in antiseptic culture in the present laboratory (6), (7) & (8). However, no sexuality occurred in these artificially developed gametophytes. This study attempts to detect whether external factors such as light sources and chemicals may bring some morphological or physiological changes, which may, in turn, induce sexuality to this unique liverwort in antiseptic culture. After six months of observation, under chemical treatment including GA, IAA, NAA and Vitamins and the application of red light, at 9 hour exposure daily, under 20°C, showed successful results on the initiation of sex organ on the gametophyte of *Haplomitrium rotundifolium*. GA 10^{-6}M/1 seemed to induce the formation of primary archegonia consisting of few cells only, at the surface of the shoot tip. Red light at 9 hr illumination, showed the occurrence of archegonia, in mature stage on the tip of the shoot (Pl. III, 1-6). These findings, the antiseptic gametophyte completed in this laboratory in former years and the initiation of sex organs in the present research are all first time reports to the biological science.

INTRODUCTION

Leafy gametophytes of *Haplomitrium rotundifolium* developed from spores have been completed in antiseptic culture in the present laboratory, as reported in my previous papers, 1966, 1967 and 1968 respectively. Since spore germination in the genus *Haplomitrium* has been considered difficult and rare by previous workers, evidently, no one has ever succeeded in producing the gametophyte stage other than the present author. This eventual success, rather unexpected to the author herself, brought new knowledge to our understanding of the life of *Haplomitrium*. However, both the gametophytes developed from spores and those arising asexually from material grown in the present laboratory evidence no sexuality, whatsoever. The present study is, therefore, an attempt to detect whether such external factors as light sources and chemicals may bring about some changes, morphological or physiological, which may, in turn, induce sexuality in this unique liverwort, *Haplomitrium rotundifolium*. The results of the present study are herewith reported in this paper.

MATERIALS AND METHODS

1. Preparation of culture materials—Sufficient cultivated material of of *Haplomitrium rotundifolium* was obtained by propagation from original cultures, both game-

tophytes produced from spore germination and those arising from material grown in the present laboratory. Fresh materials from recent collections were also provided for supplemental cases.

2. Application of light sources—Red light was selected for testing while fluorescent light was used for general illumination. Three kinds of cuttings; a. rhizome-like basal portion with one bud, b. erect branch with leaves, and c. creeping rhizome-like basal portion, were selected for propagation as testing material. These cuttings were divided into nine groups according to the duration of their exposure to light, each consisting of three samples, and they were planted into petri dishes, 27 dishes in all. Prior to the planting, the cuttings were washed several times in distilled water so as to avoid any contamination that might occur in the culture. The substratum was Hoagland solution medium containing 50% Hoagland solution solidified by 1% agar and 0.25% activated charcoal. All the cultures thus prepared were placed under 20°C, 10-hr daily illumination and at about 1200 lux of light intensity. The duration of exposures, under red light was in a graded scale: 30 seconds, 1 minute, 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours and 9 hours and one set was kept as a control. After respective exposures the cultures were placed back under fluorescent light and there they remained until the next exposure.

3. Chemical treatment—Chemicals selected for this experiment include:

Group I

GA	10^{-4}M/1	10^{-5}M/1	10^{-6}M/1
IAA	10^{-4}M/1	10^{-5}M/1	10^{-6}M/1
NAA	100 mg/1	10 mg/1	1 mg/1

VITAMINS:

Vitamins: Ascorbic acid, Calcium pantothenate, Riboflavin, Folic acid, Biotin, Pyrodoxine, Thiamine, Nicotinic acid, p-Aminobenzoic acid, Inositol.

Cencentration—0.25 mg/1.

Group II

GA 100 mg/1 + IAA 100 mg/1
GA 100 mg/1 + IAA 10 mg/1
GA 10 mg/1 + IAA 100 mg/1
GA 10 mg/1 + IAA 10 mg/1

Materials selected for this part of the study consisted of 17 groups of young plants including some 340 erect branches, separated from the original cultures. Each group, consisted of 20 erect branches still attached to the rhizome-like basal portion, was planted into a petri dish with Hoagland solution agar medium as described in

the foregoing paragragh. 17 groups of such material were planted into 17 plates that served for the test designed under Group I and Group II, with two plates as control for Group I, and one for Group II. To the cultures thus prepared, were added about 15 cc of the respective solutions as listed above. New solutions were added twice a week. In handling the antiseptic cultures, technique for disinfection of the culture and the other articles involved was carefully practiced in order to prevent any possible contamination that might damage our cultures.

OBSERVATIONS AND RESULTS

1. The effect of red light on the growth rate of 3 types of cuttings of *Haplomitrium rotundifolium* may be summarized as follows:

Table 1

	Types of cuttings	Results	Exposures under red light								
			0	30″	1′	5′	15′	30′	1hr	2hr	9hr
A	rhizome-like basal portion with one bud	remain healthy	14%	20.6%	0%	36%	33%	16%	0%	0%	48.5%
B	erect branch with leaves	formation new buds	5%	53%	52%	50%	56.2%	60.5%	61%	56.2%	100%
C	creeping rhizome-like basal portion	formation new buds	0%	0%	75%	57.6%	41%	45%	45%	54%	57%
Initiation of sex organ			none	none	several in primary stages	primary stages show a few cells	primary stages show a few cells	primary stages show a few cells	primary stages show a few cells	primary stages show a few cells	mature archegonia on shoot tip (PL. III 1-6)

A. Rhizome-like basal portion with one bud—When the cuttings of this group were exposed to red light for respective durations it seemed to indicate that exposures from 30″–15′ brought some favorable effect to bud formation. Vigorous tissure organization developed in the rhizome in the right proportion with the increase in the length of duration of exposures. However, beyond 15′, as shown in Table 1, it seemed to give a negative effect, for about the 11th week after the beginning of the experiment, most of the testing cultures in this group began to turn whitish in color and gradually decayed. The 9 hr illumination of red light maintaining 48.5% in good growth may be interpreted by some other physiological factor.

B. Erect branch with leaves—In this group of cuttings the potential of growth and development is much higher than that of group A, perhaps it is due to the presence of spreading leaves on the erect branches whose position may give better chance for absorbing light, hence its capacity for food manufacture would be better

than under other conditions. From the data given in Table 1, one can readily see that the longer the exposure under red light the higher was the rate of formation of new buds. (Pl. I, 1–5). A comparison between 100% new bud formation under 9 hr exposure to the 5% of bud formation under O exposure of red light obviously illustrates the case. Moreover, the effect of red light upon the regeneration and growth and the appearance of numerous bud primordia on nearly every node of the stem started as early as 6 weeks after the experiment was begun.

C. Creeping-rhizome like basal portion—The effect of red light on the cuttings of this group seemed to show the same result as exemplified in group B—that is, the formation of new buds became obvious but the general appearance seemed to be less vigorous and the branches more or less slender and leaves thinner. Perhaps, the creeping rhizome can gain nutrition more easily from the substratum (medium) which favors its growth and development. This is one advantage over group A in which most part of the cuttings became contaminated and decayed shortly after the experiment started. A word must be mentioned here in regard to the plants receiving 9 hrs illumination with red light—The red light seemed to stimulate the production of bud primordia and give rise to bud formation on a rough-surfaced stem (Pl. II, 2, 3, 4, 6) especially near the tip, while those of the control and other cultures under different periods of exposure below 2 hrs all showed development on smooth-surfaced stem (Pl. II, 1). Moreover, sections of the tips of some erect branches showed that initiation of sex organs actually occurred in the present culture. Those under red light for 9 hr illumination have reached mature stages (Pl. III, 1, 2, 6–9) while those below 2 hr exposure have developed one or several cells in primary stages (Pl. III, 3–5), the latter were previously described by Campbell (2).

2. The effect of chemical treatments on the growth and development of *Haplomitrium rotundifolium* may be summarized as follows: (see Table 2)

DISCUSSION AND CONCLUSION

1. The effect of red light on growth and development of *Haplomitrium rotundifolium* indicates that exposure from 30 seconds to 15 minutes seemed to bring about a favorable influence on bud formation and vigorous tissue organization in the rhizome. The increase in the length of exposure seemed to produce a corresponding greater development of the gametophyte (Table 1, Group B, C). Exposures of red light longer than 15 minutes seemed to show a negative effect (Table 1, Group A) but plants under 9 hours of illumination maintained 48.5% of culture in good growth condition and this does not seem to agree with the former statement.

2. The 9 hours of illumination to red light causes several notable changes in

Table 2

Chemicals / effects		GA			IAA			NAA			Vitamins	Control I	GA + IAA				Control II
		10^{-4}M/1	10^{-3}M/1	10^{-2}M/1	10^{-4}M/1	10^{-3}M/1	10^{-2}M/1	1 mg/1	10 mg/1	100 mg/1	0.25 mg/1		GA100mg/1+ IAA100mg/1	GA 10mg/1+ IAA100mg/1	GA100mg/1+ IAA 10mg/1	GA 10mg/1+ IAA 10mg/1	
Gen. Characteristic	Branch	abundent	little	numerous	nothing special	none	numerous	not numerous	not numerous		no special	little branch	no branch.	no branch.	no branch.	have branch	no branch
	Leaves		larger thicker	increase in size		larger thicker	larger thicker			2-3 discolor	sign of change	av. ht. 2-2.5cm	av. ht. 3-3.5cm	av. ht. 5cm	av. ht. 4cm	av. ht. 3.5cm	av. ht. 3.5cm
	Growing Point					elongate	not elongate	slightly elongate	not elongate	not elongate							
	Gen. App.	not tall	tall		slender	not tall											
Chloroplast	Shape	ellipsoidal	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical	spherical
	Size	7.5μ	6.25μ	6.25μ	6.25μ	6.0μ	7.5μ	5μ	5μ	6.25μ	6.25μ	6.25μ	5μ	5μ	5μ	5μ	5μ
	Shape	constriction						ellipsoidal	constriction	constriction	constriction	constriction	ellipsoidal	constriction		ellipsoidal	ellipsoidal
	Size	10μ						11.25μ	8.75μ	8.75μ			7.5μ	7.5μ		8.75μ	8.75μ
Oil bodies	Shape	spindle	spindle	spindle	spindle	spindle	spindle	spindle	spindle	spindle	spindle		spindle	spindle	spindle	spindle	
	Size	5μ	6.35-7.0μ	12.5μ	3.75μ	8.75μ	3.75μ	7.25μ	8.75μ	8.75μ	10μ		5μ	10μ	7.5μ	8.75μ	
Initiation of Sex organ		Primary stage ♀ Pl. VII, 6	Primary stage ♀ Pl. VII, 6	Primary stage ♀ Pl. VII, 6	Primary stage ♀ Pl. VII, 5	Primary stage ♀ Pl. VII, 5	Primary stage ♀ Pl. VII, 5	none	none	none	none	none	Primary stage ♀	Primary stage ♀	Primary stage ♀	Primary stage ♀	none

the growth and development of *Haplomitrium rotundifolium* namely: a) conspicuous protuberances occurred on the surface of erect branches (Pl. II, 2, 3, 4, 6), while in the control or other cuttings below 2 hrs of exposure the stem had a smooth surface (Pl. II, 1), b) increase in the bud formation (Pl. I, 1, 3) as exemplified in erect stems with leaves and the creeping rhizome, and c) the most important of all the changes that occurred is the initiation of sex organs. A cluster of archegonia in mature stage were found scattered or in clusters on the surface of the tip of the shoot (Pl. III, 1, 2, 6, 7, 8, 9). There were no mature sex organs found on the control or other cultures under any of the different exposures other than 9 hours. However, one or two-celled structures projecting out from the surface of the shoot tip are numerous (Pl. III, 3–5). These may be considered as slimy papillae (Proskauer 1962) or sex organs in the primary stage of development (Campbell 1929) (Pl. III, 3, 4, 5). In spite of the fact that the sporophyte has not yet been artificially developed in this laboratory or elsewhere, yet it may be conceived that the development of the sporophyte of *Haplomitrium* will not be difficult to accomplish. Since *Haplomitrium rotundifolium* is unisexual, if both the male and the female gametophytes are brought together under the right conditions, the production of sex organs and the completion of sporophytic phase in culture seems very possible.

3. Among the chemicals, GA 10^{-6}M/1 seems to bring a more marked effect on the general appearance of the plant as a whole, giving a firm stem and larger, thicker leaves in most of the cultures. Vitamins made no special effect on the growth and development of *Haplomitrium rotundifolium*, nor does NAA, in fact, the latter treatment caused slightly retarded growth and development of the culture, and some leaves even became discolored. However, all GA, IAA and GA+IAA solutions in different concentrations showed that the surface cells of the shoot tip are highly potential for sexual initiation—but only in the primary stages. This agrees with what Campbell reported (1920) in his study on *Haplomitrium blumii*.

4. The occurrence of a cluster of archegonia in different stages of development (Pl. IX, 1–6), was found in some gametophytes propagated from cultures that have been growing in this laboratory since 1968 and 1969. They had not been treated by chemicals or red light, but were growing under 20°C, about 1200 lux of light intensity for 10 hours of daily illumination. This may indicate that these gametophytes possess the potential of sexuality, although they took one or two years to reveal the character. However, no shoot calyptra (Fulford 1956) (Yang 1966) have been found among the cultures.

5. The fact that no special changes occurred under chemical treatments may be due to two reasons: First, perhaps, the lower plants like bryophytes may not need an extra supply of chemicals such as auxins, vitamins, etc. Second, the time

for treatments may not be long enough to allow any specific results. At the time of making this report the cultures are still in vigorous growing condition. So close observation will be continued for further developments.

6. The cluster of archegonia found growing on untreated gametophyte (Pl. IX, 1, 4), the primary archegonia on plants treated with chemicals, (GA, IAA) (Pl. VIII, 5, 6) and the mature archegonia found on plants treated under 9 hour illumination of red light (Pl. III, 1, 6, 7, 9) are all first time discoveries for plants grown in artificial culture of *Haplomitrium rotundifolium.*

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Plate I

1, 2, 3. (Group 3) 30 seconds to one minute, numerous new buds and general appearance of cuttings. ×2.
4, 5. New buds and branching produced from creeping rhizome. ×6.3.
6. Elongating bud and shoot, 9 hour exposure on right; the same below 2 hour exposure on left, showing shorter shoot. ×2.5.
7. developing slender shoots from elongating tips. (9 hour exposure).

Plate II

1. Showing new shoot, smooth surfaced. Exposures below 2 hours.
2, 3, 4. Showing rough surfaced shoot, with protuberances. ×16.
5. Young branch developed within 6 weeks. Showing numerous buds and short branches. ×2.
6. Short branches developed from buds shown 2-4. ×6.3.

Plate III

1, 2. Mature archegonia found on the shoot tip of gametophytes. (9 hour exposure). ×40.
3, 4, 5. Series in initiations of sex organ—primary stages under 1 minute exposure. ×100.
6, 7. Enlargement of 1. ×100.
8, 9. Showing egg cells in respective venters. ×100.

Plate IV

1-4. Cultures after treatment with different chemicals.

Plate V

External views after treatment with GA and IAA.
1. GA 10^{-4}M/1. ×6.3 2. GA 10^{-5}M/1. ×1.5. 3. GA 10^{-6}M/1. ×1.5.
4. IAA 10^{-4}M/1. ×1.5. 5. IAA10^{-5}M/1. ×1.5. 6. IAA 10^{-6}M/1. ×1.5.

Plate VI

External views after treatment with NAA and Vitamins.
1. NAA 100mg/1. ×6.3. 2. NAA 10mg/1. ×6.3.
3. NAA 1mg/1. ×6.3. 4. Vit. 0.25mg/1. ×1.5.

Plate VII

Vigorous branches after treatment with GA combined with IAA. ×6.3.
1. GA 100mg/1+IAA 100mg/1. 2. GA 100mg/1+IAA 10mg/1.
3. GA 10mg/1+IAA 100mg/1. 4. GA 10mg/1×IAA 10mg/1.

Plate VIII

1-4. Showing chloroplasts and oil bodies in leaf cells taken from 3rd leaf of respective branches. ×400.
5, 6. Initiation of sex organ GA 10^{-4}M/1, IAA 10^{-4}M/1.

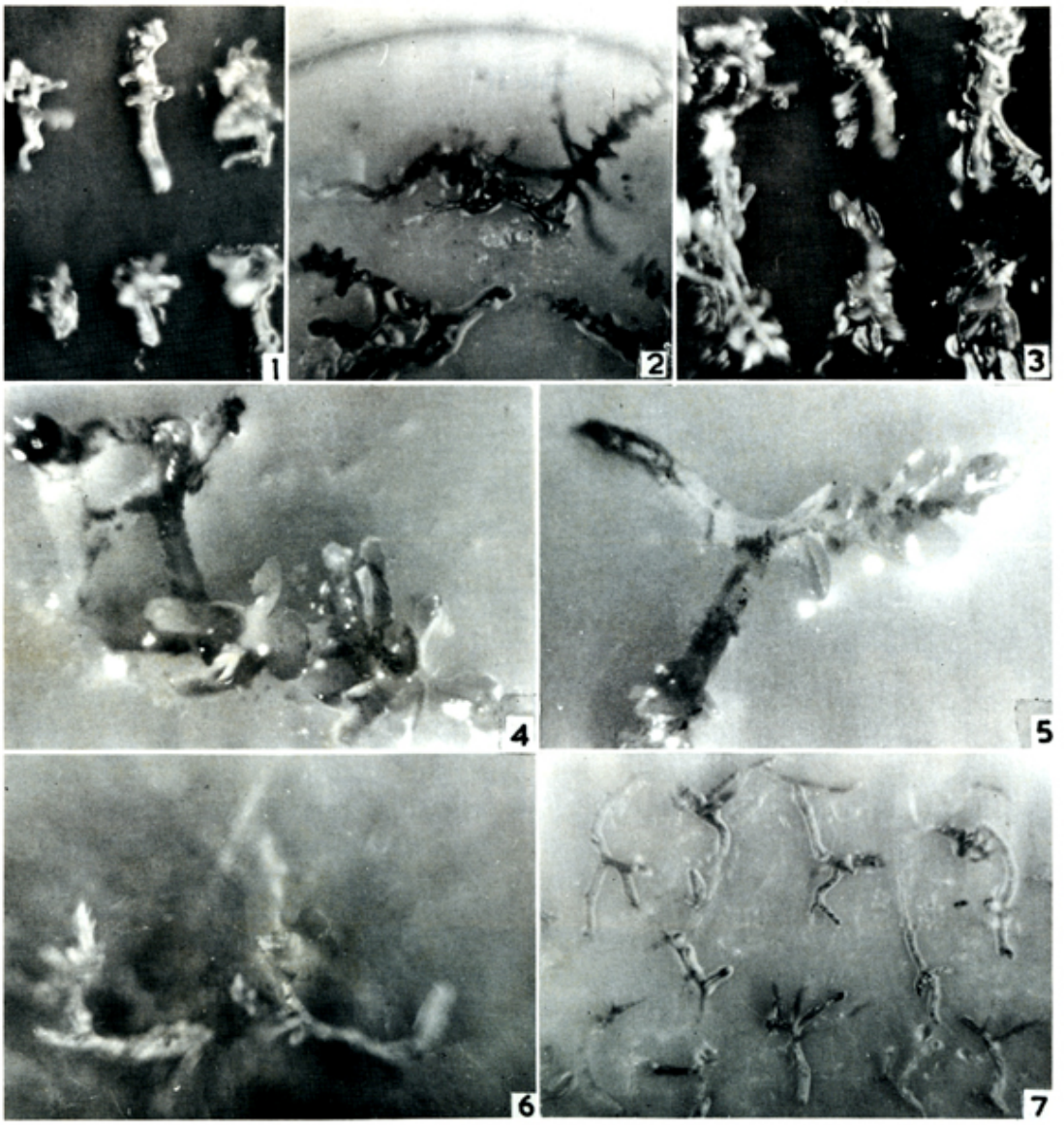
Plate IX

Initiation of sex organ occurring on gametophytes grown in the laboratory.
1. Young archegonia before differentiation of the neck cell, venter etc. ×100.
4. A cluster of mature archegonia with egg cells in the venter. ×100.
2-5. Ditto. ×400.
3. Archegonia dissected from another plant showing a male nucleus approaching the egg in the venter. ×400.
6. The male nucleus advanced into the venter. ×400.

Plate X

1, 2. Mature archegonia developed on the tip of shoot after 9 hours of exposure under red light.
3. Portion of cross section of a growing tip showing meristematic cells inside.
4. Portion of stem, c.s. ×100.
5. Elongating bud, 2 hours of exposure under red light. ×5.
6. Elongating shoot with leaves 9 hour exposure under red light. ×5.

Plate I



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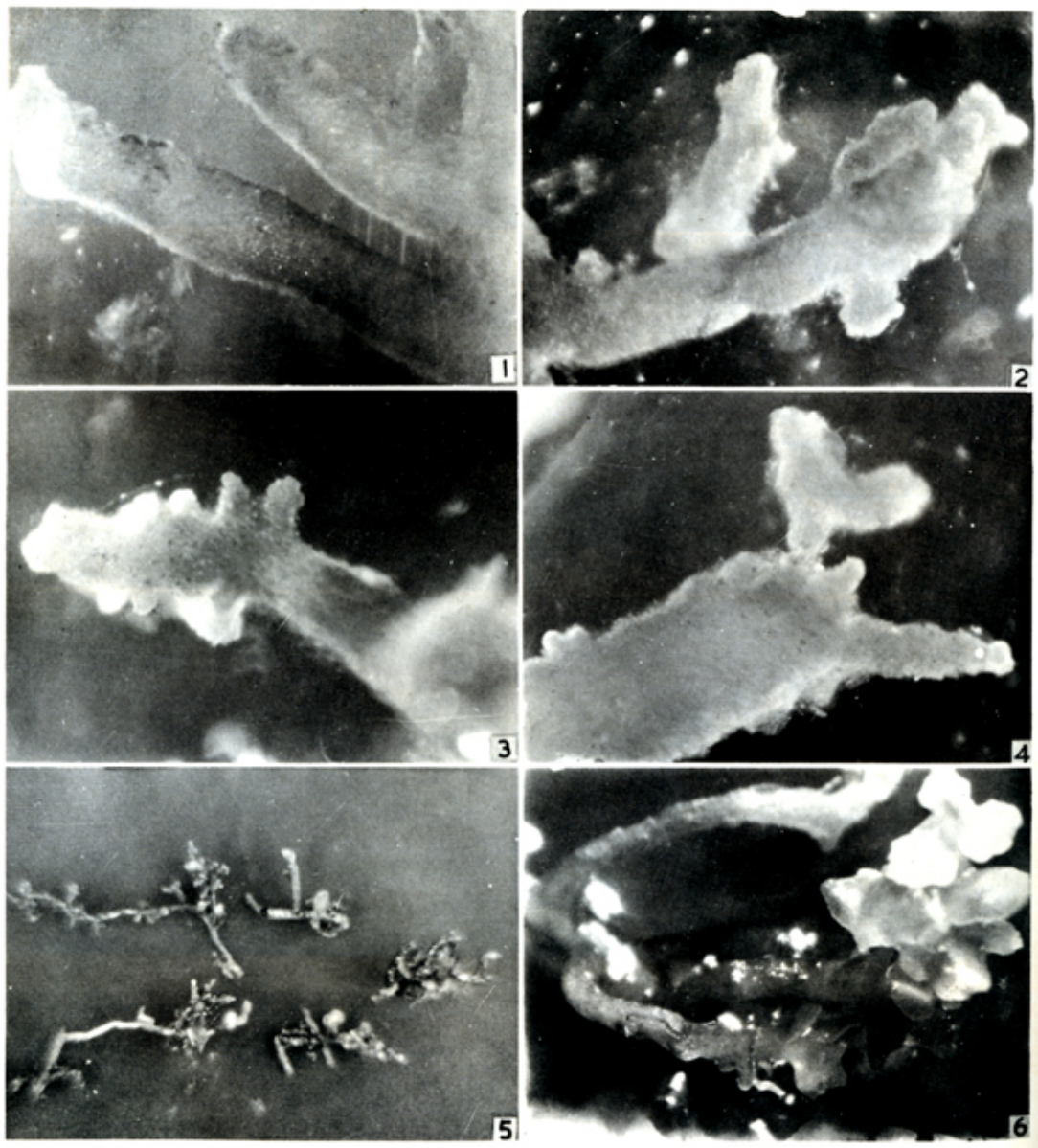

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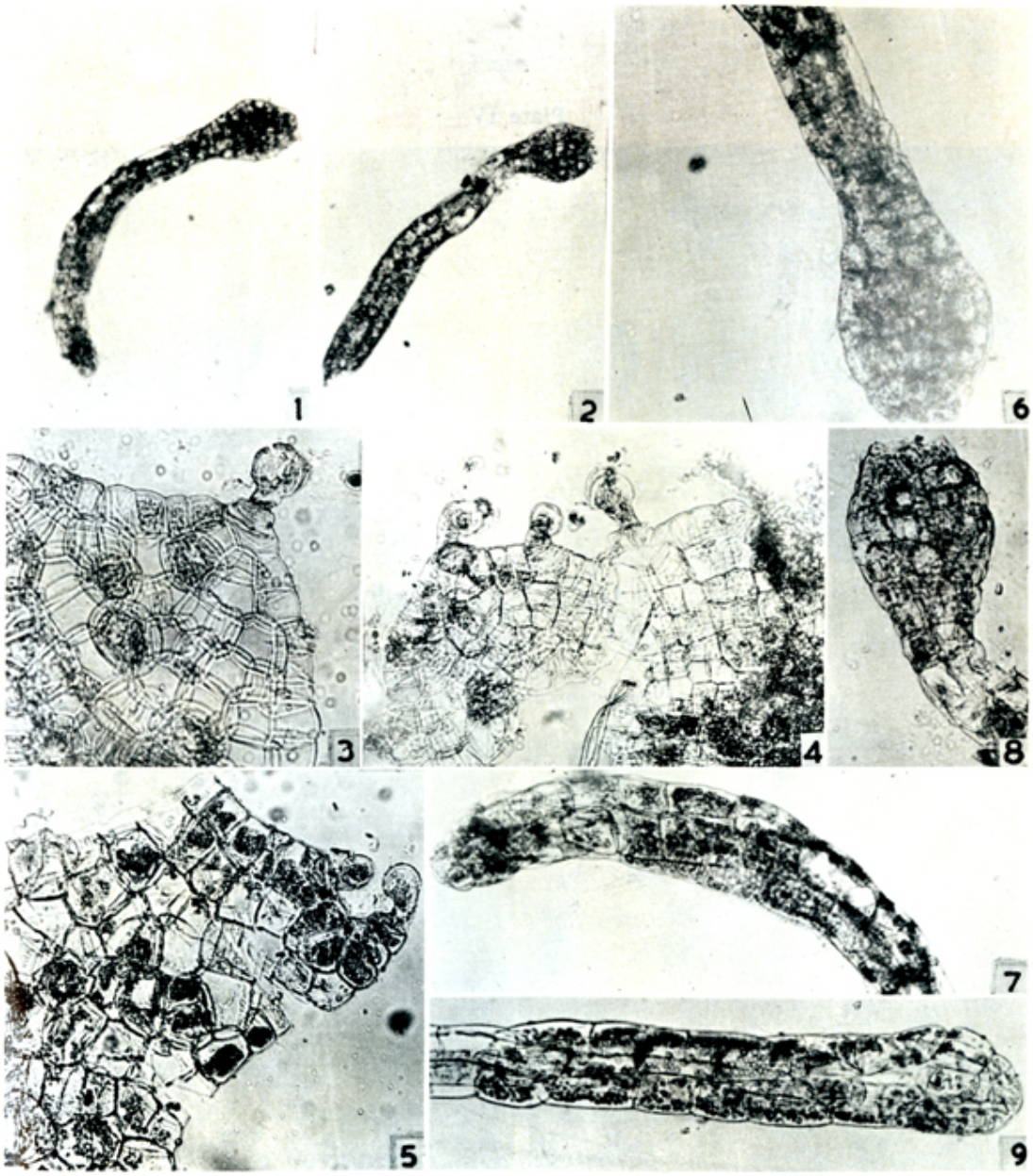

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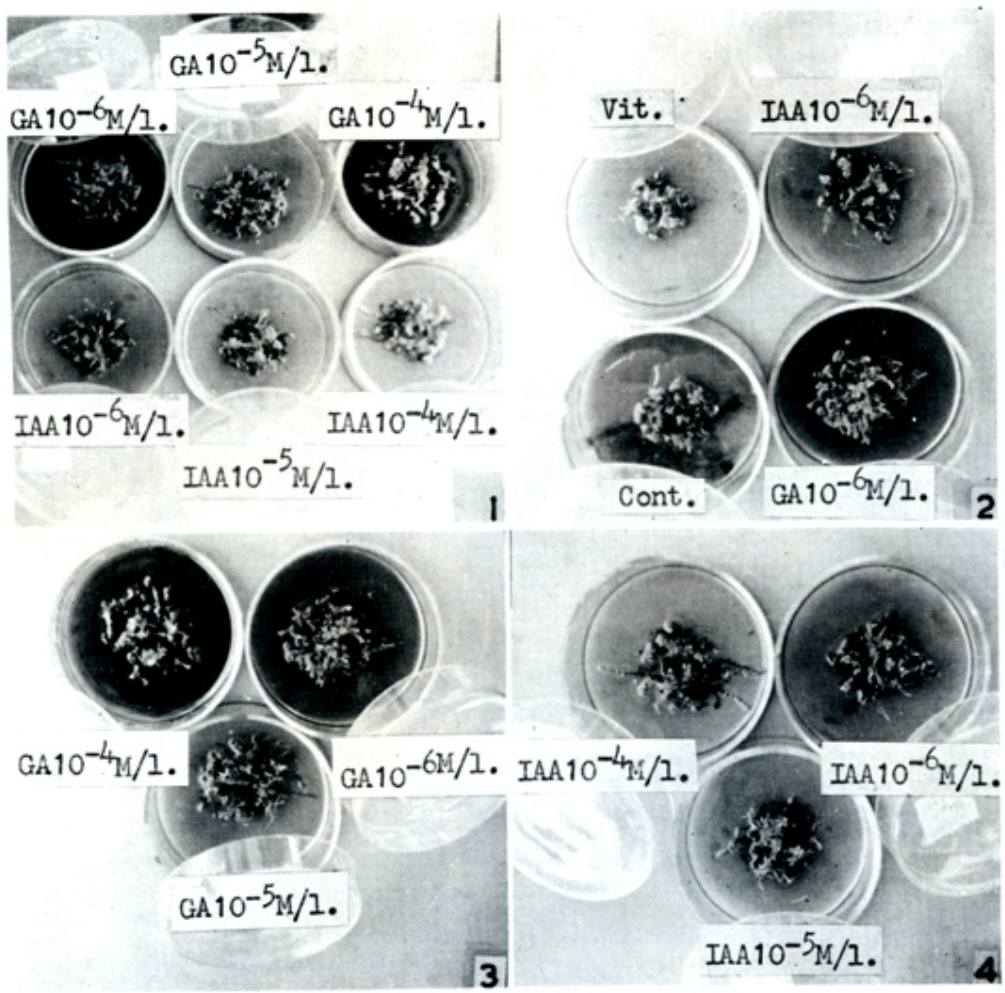

GA10^{-5}M/l.
GA10^{-6}M/l.
GA10^{-4}M/l.
IAA10^{-6}M/l.
IAA10^{-4}M/l.
IAA10^{-5}M/l.
1
Vit.
IAA10^{-6}M/l.
Cont.
GA10^{-6}M/l.
2
GA10^{-4}M/l.
GA10^{-6}M/l.
GA10^{-5}M/l.
3
IAA10^{-4}M/l.
IAA10^{-6}M/l.
IAA10^{-5}M/l.
4

Plate V

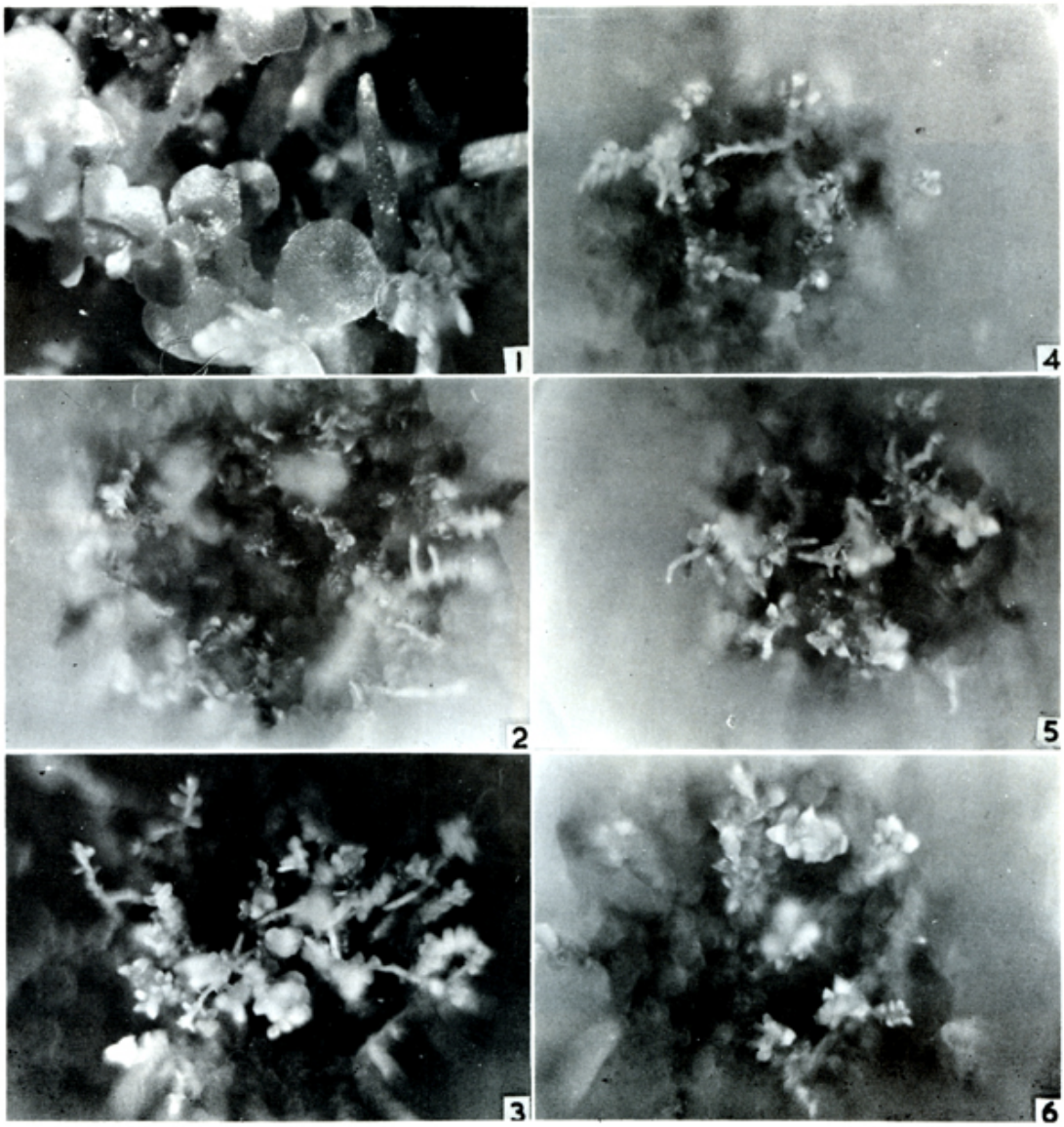


Plate VI

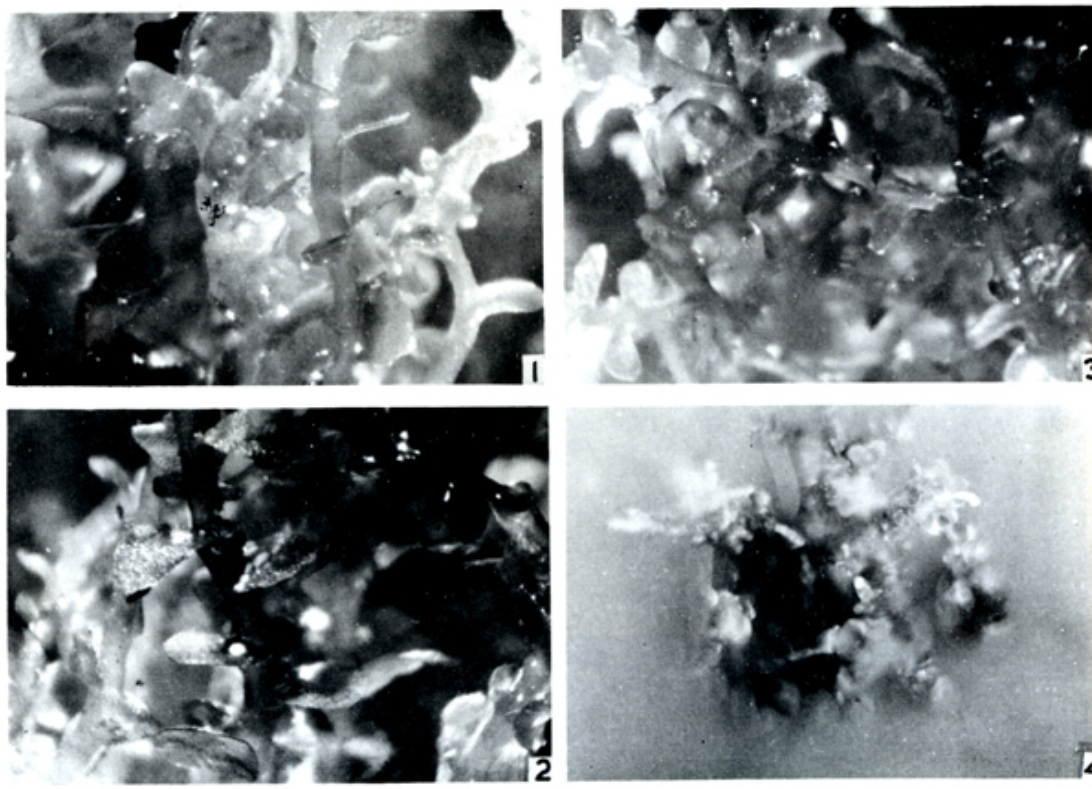


Plate VII

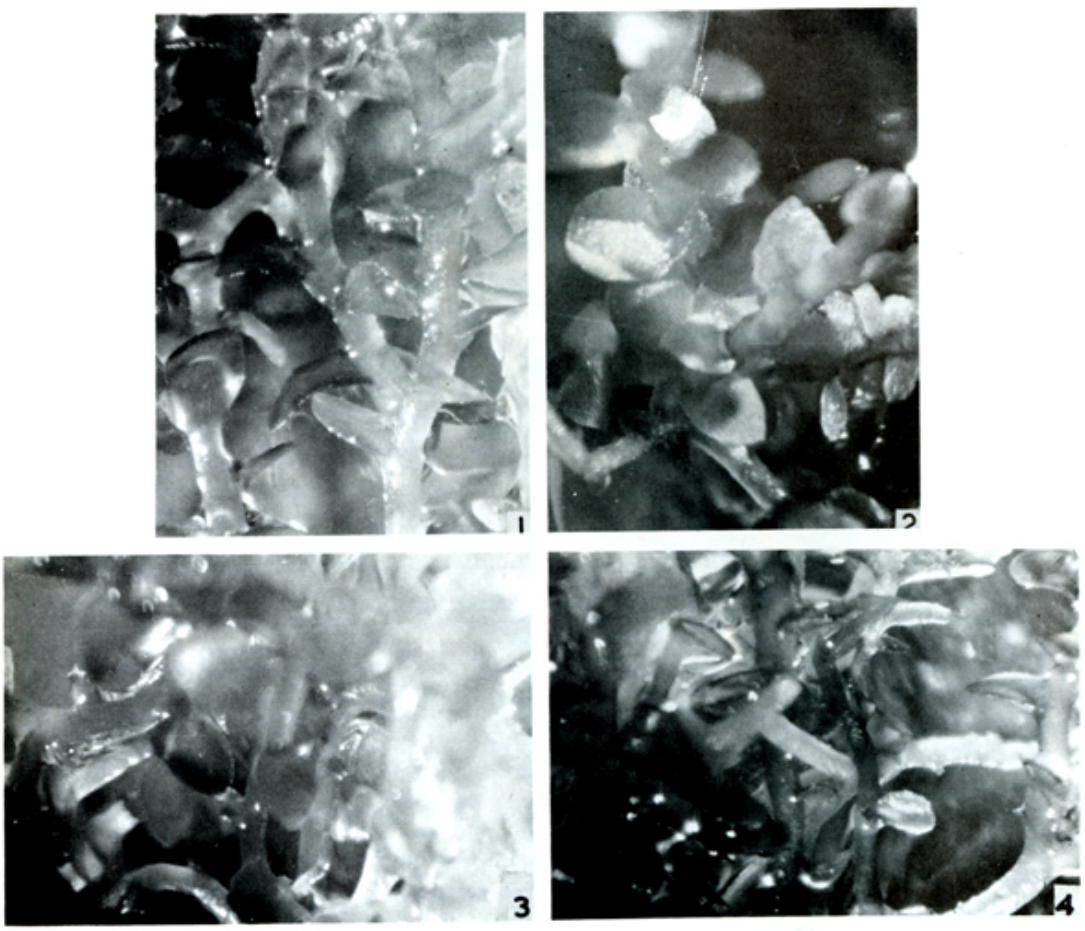


Plate VIII

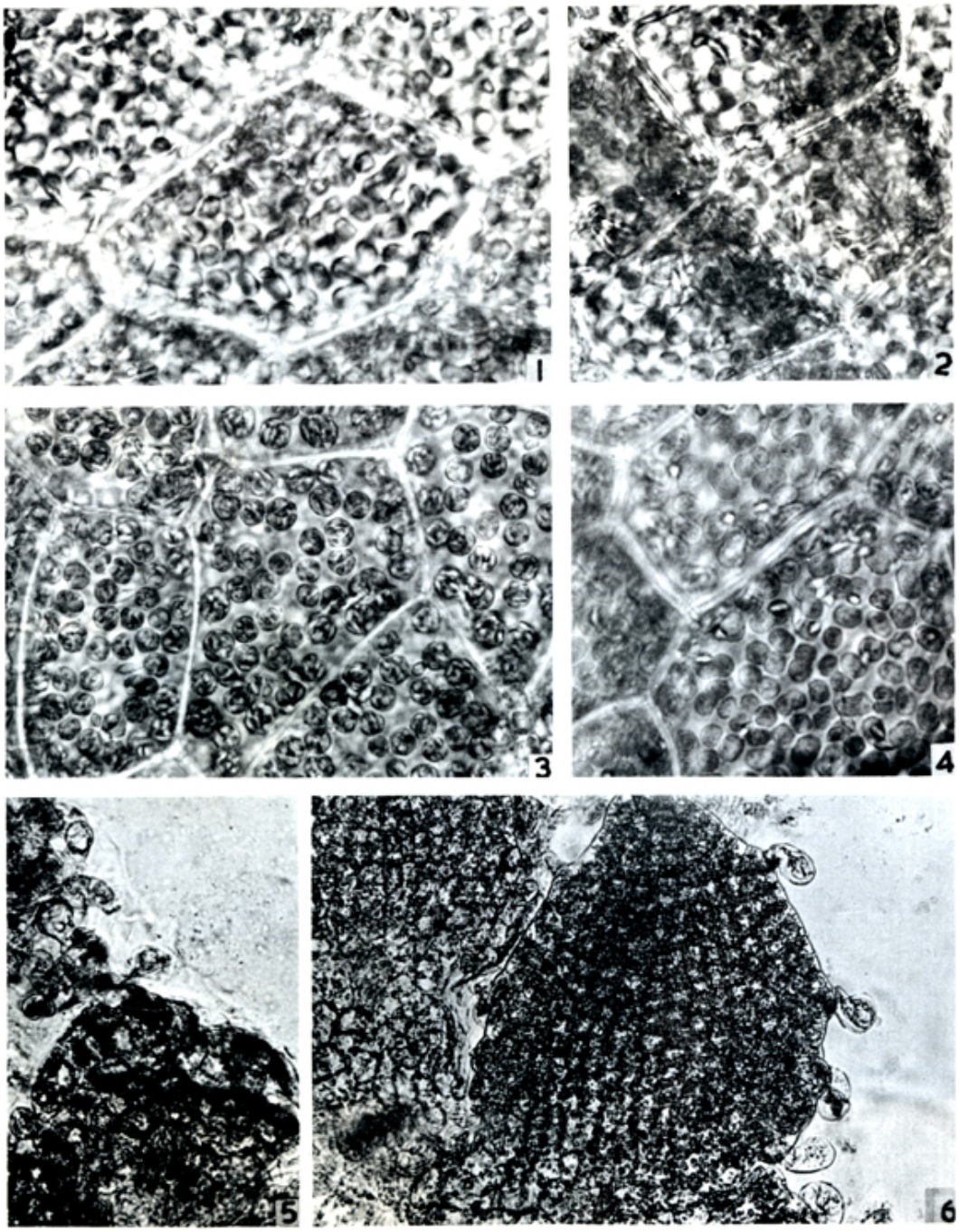
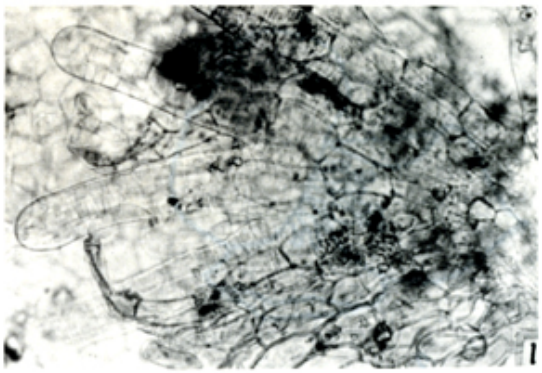
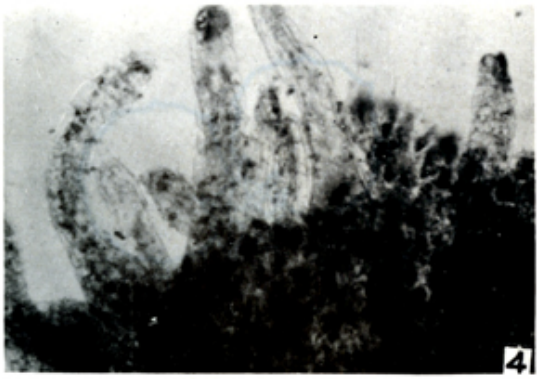
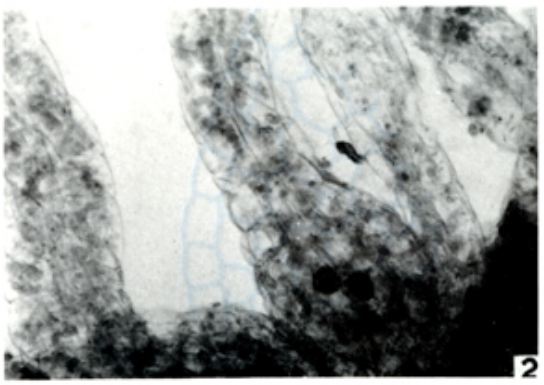
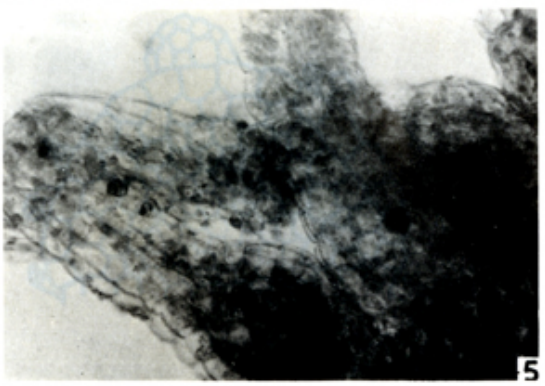


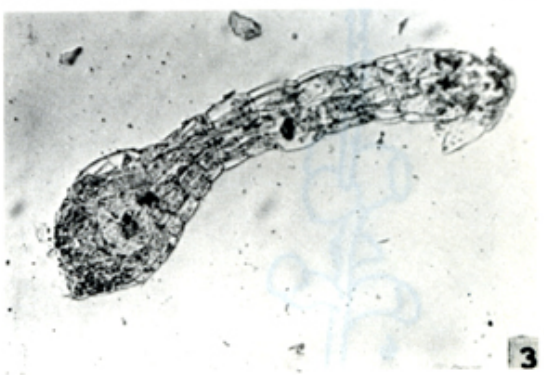
Plate IX











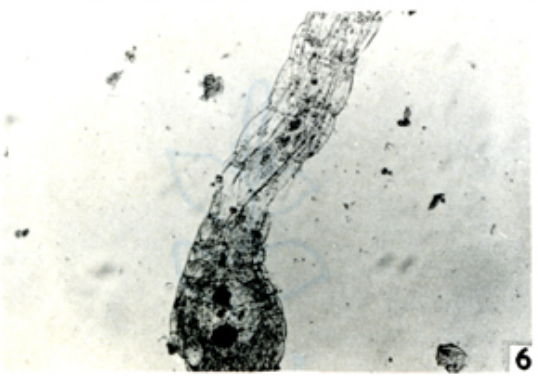


Plate X