THE GROWTH OF THE SPOROPHYTE OF *PORELLA NAVICULARIS* LINDB., *IN VITRO*

by

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THE GROWTH OF THE SPOROPHYTE OF *PORELLA NAVICULARIS* LINDB., *IN VITRO*

INTRODUCTION

In this study an attempt has been made to determine some of the physiological requirements of the sporophyte of *Porella navicularis* Lindb.[1] The interest in such a study lies in the problem of the nutritional inter-relationship of the hepatic gametophyte and sporophyte. The morphologist is concerned with this in his effort to establish a line of ascent leading to the independent sporophyte of the vascular plants. Presumably the form ancestral to the vascular sporophyte must have possessed most, if not all, the attributes of nutritional independence although it may have been physically attached to the gametophyte throughout its growth. The validity of the classical concept of the nutritional inter-relationship of the sporophyte and gametophyte in the hepatics is in doubt at present. This relationship was formerly considered to be one of true parasitism exerted by the sporophyte. Recent examinations of the sporophytes of a number of species have shown that chlorophyll is developed in the early ontogeny and is retained until the most mature stages are reached. Culture work has also helped to alter the concept by finding that some sporophytes are able to continue to mature stages when excised from the gametophyte thallus.

[1] Nomenclature in this paper follows that of Frye and Clark (4).

The classical morphologist has long asserted that the Anthocerotalean line best fulfills the requirements for the ancestral form of the vascular plants. In general, other hepatic sporophytes were cited as being completely dependent upon the gametophyte. The reason for the selection of the Anthocerotales as the ancestral form was that the sporophyte contains chlorophyll, has a basal meristem which could conceivably allow unlimited growth, has stomata, and often persists after the gametophyte thallus has disintegrated.

The sporophytes of the genera representing the Anthecerotales, however, are examples of the most extreme specialization that occurs within the hepatics. It is quite probable that they represent a higher form of the hepatics than do any of the other forms and are a diverging line from the other thalloid or foliose types.

The Psilophytales offer the most probable line of ascent from the bryophyte type of plant to the gymnosperm type. It is not so important that a foliose plant or sporophyte be derived but rather that vascularization and independency be established within the sporophyte generation. Therefore the sporophyte of Porella with its unspecialized structure and early presence of chlorophyll was selected to be studied as a possible independent structure. If independency could not be established it was thought that a study could be made to

determine what nutritional inter-relationship between the gametophyte and sporophyte was involved.

Campbell (2, pp.494–496), using Anthoceros pearsoni Howe, was the first worker to attack the problem of sporophyte independence through actual culture work. Since the Anthoceros sporophyte has a highly developed photosynthetic tissue, a long period of growth, and an active basal meristem, he suggested that if the sporophyte were able to absorb water directly rather than by the mediation of gametophyte tissue it might be possible for it to live and develop when separated from the gametophyte. Thus the behavior of the young fern sporophyte would be simulated. In Campbell's experiments it was found impossible to completely dissect the sporophyte from the gametophyte as the foot penetrates down into the gametophyte tissue. The foot was exposed by cutting away the surrounding tissue as much as possible and the foot was then inserted into a Knop solution. This was found to be unsatisfactory since bacteria soon enveloped the submerged portion. More satisfactory results were obtained by planting the sporophytes in sterilized earth. For a short period of time a slight increase in length was observed. The greatest amount of elongation was about 3 mm., but in most cases it was less. The most striking effect was that of hastening of spore formation. Most of the specimens selected for planting had not yet begun to show spores, but after a month in culture there appeared a darkening of the apical

region indicating the presence of mature spores. This darkening soon spread downward embracing the whole sporophyte. The spores thus formed germinated freely and the resulting gametophytes were apparently normal. The sporophytes with the foot missing lived for 2 months or more and behaved like the others.

After a lapse of approximately 20 years, the next report found in the literature on the culturing of hepatic sporophytes was by Studhalter (12, pp.153-154). He removed the sporophytes of Riella americana Howe and Underwood from the enclosing calyptra with spear pointed needles and cultured them in separate jars containing water. Usually the sporophytes remained alive for 61 days at which time they reached normal maturity. Similar cultures were made of the sporophyte of Sphaerocarpus texanus Aust. Although the genus is not aquatic as is Riella, the cultures were grown in water. Under these conditions, the sporophytes continued their internal development. During the culture of sporophytes of both genera all but the youngest continued their internal development. The nurse cells went through normal stages, spore mother cells developed into mature spores, and the capsule wall broke down normally at maturity. In all cases it was found that when the sporophyte grew to maturity it was somewhat smaller than normal. Studhalter concluded from these experiments that the sporophytes of both genera are nutritionally independent structures, at least from the time they have reached

a size one-half the mature diameter, and that they metabolize a considerable amount of food even at earlier stages of their development.

Similar success in the culturing of hepatic sporophytes was recently reported by Proskauer (9, pp.165-172). He isolated and planted in moist soil the sporophytes of the genus Pellia. Kept under moderately warm conditions and under light they soon matured fully, elongated rapidly, and dehisced. The sporophytes at the time of dissecting were, according to the author, well differentiated.

In spite of reports on the presence of chlorophyll in the sporophytes of various genera, it is usually accepted that the sporophytes of Marchantiales, Jungermanniales, and Sphaerocarpales are lacking in chlorophyll and/or in the capacity of self nutrition. Recent textbooks by authors such as Fuller and Tippo (5, p.712), McLean and Ivimey-Cook (8, p.389), Hill, Overholts, and Popp (6, p.488), Robbins and Weier (10, p.389), Smith (11, p.9), and Lutz (7, p.612) contain the information that the sporophytes of the above mentioned orders are dependent.

Bold (1, pp.551-552) lists the following Bryophytes as having sporophytes containing chlorophyll: Marchantia polymorpha L., Mannia rupestris (Nees) Frye and Clark, Reboulia hemisphaerica (L.) G.L. & N., Dumortiera hirsuta (Sw) Rienw. Bl. & Nees, Asterella

tenella (L.) Beauv., Ricciocarpus natans (L.) Corda, Pellia epiphylla (L.) Corda, Frullania sp., Cephalozia sp., and Blepharostoma sp.

Water conduction in the hepatics has been studied and is thought to offer possible evidence of independence in nutrition of the sporophytes. In a study by Clee (3, pp.105-111) of Pellia epiphylla (L.) Corda, it was suggested that the main source of water and minerals for the growth of the sporophyte is external. Using staining tests, he found that water passed over the surface of the gametophyte thallus, between the flaps of the involucre, and down the neck canal cells of the archegonia to the egg. He also found that the stained water was absorbed by the foot of the sporophyte, traveled up the seta into the elaterophore and out to the elaters. Ultimately it went out to the spores. He therefore concluded that the sporophyte was less dependent upon the gametophyte for supplies of water and minerals than was formerly thought.

Accepted as strong evidence for the parasitic nature of the sporophyte has been the specialized cells of the sporophyte foot. These cells (whether they are absorbing organs is yet to be determined) lie in the periphery of the foot. Studhalter (13, pp.638-650) on examining the foot found that the shape of the peripheral cells varied with the species being observed. They ranged from a papillate condition in Frullania to longer protruding processes as in the foot of Anthoceros. The presence of abundant

cytoplasm and large nuclei has been interpreted as an indication that the foot is actively engaged in the nutrition of the sporophyte. Studhalter in a previous experiment (see page 4 above) found the immature as well as the mature excised sporophytes could absorb all the necessary water and minerals directly from the surrounding medium through the cell walls of the capsule. He stated that it is only a step further to the absorption of these materials through an added thin calyptra which is in direct contact with the water of the habitat. With the sporophyte capable of manufacturing its own food then the foot, at least during the latter half of its development, does not function as an absorbing organ. If absorption does take place it is purely incidental. The early disintegration of the foot cells is often complete before maturity and lends corroborative evidence. The elongate peripheral cells of the foot may suggest vestigal structures from a former condition during which the sporophyte was less independent from a nutritional point of view. Now the main function of the foot seems to be that of anchorage.

MATERIALS USED

Collections of *Porella navicularis* Lindb. were made weekly in the McDonald Forest area, Benton County, Oregon. After each collection the material was dampened if necessary and stored until used in the cold room at 3 degrees centigrade. The dissecting procedure required the use of a binocular dissecting scope, a pair of fine pointed jewelers' tweezers, and micro-dissecting scapels. The sporophytes were measured under a compound microscope with an ocular micrometer whose readings were converted to millimeters with the use of a stage micrometer. One ounce glass specimen jars with screw top lids were used as culturing containers. Spun pyrex, filter paper, and agar were used as solid substrates.

Voth's Full Nutrient Solution and White's Basic Medium (Modified) were used for the culturing of the sporophytes. The composition of Voth's Medium was secured through a personal communication from Dr. Paul Voth.

Voth's Full Nutrient Solution has the following composition:

KNO_3	80.0 mg.
$Ca(NO_3)_2$	11.5 mg.
$Mg(NO_3)_2$	89.0 mg.
KH_2PO_4	54.5 mg.

$MgSO_4$ . . . . . 96.0 mg.

Distilled water to make 1 liter of culture solution.

pH adjusted to 5.94.

Trace Elements

(1 ml. of a mixture of the following per liter of culture solution.)

$MnSO_4$ . . . . . 2000 mg.

$Na_2B_4O_7$ . . . . . 2000 mg.

$ZnCl_2$ . . . . . 2000 mg.

Ferric citrate. . . . 150 mg.

Citric acid . . . . . 150 mg.

Distilled water to make 1 liter of solution.

White's basic medium contains the following concentrations of chemicals:

$Ca(NO_3)_2$ . . . . . .100 mg./liter distilled water

KNO_3 . . . . . 80 mg./ " " "

$MgSO_4$ . . . . . 35 mg./ " " "

KCl. . . . . 65 mg./ " " "

KH_2PO_4 . . . . . 25 mg./ " " "

KI . . . . . 0.75 mg./ " " "

$MnSO_4$	4.4	mg./liter	distilled	water
$Fe_2(SO_4)_3$	2.5	mg./ "	"	"
$ZnSO_4$	1.5	mg./ "	"	"
H_3BO_3	1.6	mg./ "	"	"
Sucrose	20,000	mg./ "	"	"
Glycine	2	mg./ "	"	"
Thiamin chloride. .	0.1	mg./ "	"	"
Nicotinic acid. . .	0.5	mg./ "	"	"
Pyridoxin	0.8	mg./ "	"	"

pH adjusted to 5.4 with 0.01 N NaOH

Indole -3- acetic acid and alpha naphthalene acetic acid were used in studying their action as the possible auxin in causing seta elongation in the mature sporophyte. In other experiments Biotin and yeast extract were used as supplements to determine their effect on the sporophyte cultures.

PROCEDURES IN ESTABLISHING CULTURES OF SPOROPHYTES

A. DISSECTION OF THE SPOROPHYTE FROM THE GAMETOPHYTE TISSUE

The dissecting procedure used was the same throughout each series of cultures. The gametophyte, well moistened, was placed under the dissecting scope. The perianth was removed with the jewelers' tweezers, thus exposing the sporophyte within the calyptra. The foot was exposed through the use of both the tweezers and a micro-scalpel. After loosening the gametophyte tissue surrounding the foot, a slight pressure usually forced the sporophyte foot clear of the gametophyte tissue. The sporophyte was then placed in either a beaker of water or on moist filter paper in a petri dish.

B. PROCEDURE FOR WASHING THE SPOROPHYTES

Washing of the sporophytes in three changes of sterile distilled water was the usual method employed. Other methods for sterilization were employed but in most cases they were unsuccessful due to the harsh oxidative or toxic action they had on the tender sporophyte tissue. Transfer utensils were kept in 100% alcohol for purposes of sterilization. There was no attempt to completely sterilize the sporophyte in the later experiments as no successful technique could be devised that did not harm the tissue. Rather, as much contamination

as possible was washed off so as not to interfere with the growth of the sporophyte. Usually this method was quite successful although in some cases contamination became so serious that the cultures had to be discarded.

C. MEASURING PROCEDURES

An ocular micrometer was used in a 10x occular for all measurements. This scale was calibrated against a stage micrometer under a 32 mm. objective and a 16 mm. objective. Measurements were taken and recorded of the greatest diameter of the capsule prior to washing the sporophytes. At the conclusion of the growth period the diameter was again taken and recorded.

D. PREPARATION OF CULTURE CONTAINERS

Spun pyrex, which was most commonly utilized as the substrate for growing the sporophytes, was inserted into the culture jars which had previously been washed and rinsed in distilled water. The loosely capped bottles were then sterilized in the autoclave for 15 to 20 minutes at 20 pounds pressure. When any other substrate was used, the sterilizing procedure was the same as that described above.

E. PREPARATION OF STOCK SOLUTIONS

Stock solutions of the nutrient media were made up in advance. Generally a two liter quantity was made up at a time and stored at 3°C. Because contaminants appeared in the Voth's nutrient solution, a layer of toluene was placed in the stock solution and thoroughly shaken throughout the solution. This was found to be quite effective in preventing contamination. Toluene, being very volatile, was entirely dissipated by autoclaving the solution when it was needed. Autoclaving of the White's nutrient solution was found to be all that was necessary for satisfactory storage at 3°C. In all cases, the stock bottles were stoppered with a cork wrapped in aluminum foil.

CULTURE SERIES

SERIES I. TABLE I

The sporophytes were dissected out according to the procedure indicated on page 11. The sporophytes were then measured with an ocular micrometer and the greatest diameter of the capsule was recorded. After having been washed three times in sterile distilled water, the sporophytes were planted on a spun pyrex substrate in autoclaved culture jars containing Voth's full nutrient solution. The culture containers were placed in diffused light and kept under moderately cool conditions. The cultures were checked periodically and at the time of death the increase in diameter of the capsule was recorded.

SERIES II. TABLE II

The procedure used in this experiment was the same as that used in Series I except for a 10% solution of Clorox which was used to surface sterilize the sporophyte. The sporophytes were then washed in sterile distilled water to remove the Clorox before planting.

SERIES III. TABLE III

The procedure was the same as in Series I except that sterile distilled water was used in place of the Voth's full nutrient solution.

SERIES IV. TABLE IV.

The procedure followed was the same as in Series I except that tap water was used as the nutrient solution for the sporophytes.

SERIES V. TABLE V.

The procedure utilized was the same as in Series I except for the source of nutrient. In this series the gametophyte thallus of Porella was mascerated in a Waring Blender. This solution was then autoclaved in the culture containers with the spun pyrex substrate.

SERIES VI. TABLE VI.

The procedure used was the same as in Series I except that White's Basic Nutrient Solution was used as a source of nutrition for the sporophyte cultures.

SERIES VII. TABLE VII.

The procedure used was the same as in Series I except that White's Nutrient Solution with the supplements omitted was used.

SERIES VIII. TABLE VIII.

The procedure used was the same as in Series I except that White's Nutrient Solution with the sucrose omitted was used for the nutrition of the sporophytes.

SERIES IX. TABLE IX.

The procedure used was the same as in Series I except that White's Nutrient Solution with added yeast extract was used. (1%).

SERIES X. TABLE X.

The procedure used was the same as in Series I except that White's Nutrient Solution with various concentrations of Biotin was used as a medium.

Part A. 0.5 gamma Biotin/ cc. White's nutrient solution.

Part B. 1.0 gamma Biotin/ " " " "

Part C. 1.5 gamma Biotin/ " " " "

SERIES XI. TABLE XI.

The procedure used was the same as in Series I except that Voth's full nutrient solution with various concentrations of Biotin was used as a medium.

Part A. 0.8 gamma Biotin/ cc. Voth's full nutrient solution.

Part B. 0.4 gamma Biotin/ " " " " "

Part C. 0.2 gamma Biotin/ " " " " "

SERIES XII. TABLE XII.

The dissecting and measuring procedure was the same as used in Series I. The sporophytes were planted in autoclaved petri dishes containing a filter paper substrate moistened with White's nutrient solution. The cultures were then placed in a room maintained at approximately 3°C. A 60 watt light bulb provided illumination for eight hours a day.

SERIES XIII. TABLE XIII.

The procedure used was the same as in Series XII except the supplements were omitted from the White's Nutrient Solution.

SERIES XIV. TABLE XIV.

The surrounding perianth was removed from the sporophyte but the foot was left intact with the gametophyte branch on which it was borne. The sporophyte capsule diameter was measured with an ocular micrometer, and the sporophyte was then planted in autoclaved culture jars containing:

Part A. White's Basic Nutrient.

Part B. White's Basic Nutrient without supplements.

SERIES XV.

Sporophytes in which the capsule was of mature size but in which the seta had not begun elongation were dissected out according to the procedure on page 11. Culture jars containing spun pyrex substrate and the following concentrations of alpha naphthalene acetic acid were autoclaved and the sporophytes planted therein.

a. 10 ppm.

b. 1 ppm.

c. 0.01 ppm.

d. 0.0001 ppm.

SERIES XVI.

The procedure followed was the same as in Series XV except that indole acetic acid was used. The concentrations of the indole acetic acid used were the same as those of the alpha naphthalene acetic acid used in Series XV.

SERIES XVIII.

The procedure followed was the same as in Series XV except that tap water was used instead of auxins.

RESULTS

A. DISCUSSION OF FIGURES

The sporophyte of Porella navicularis Lindb. consists of three major structures: (1) a foot embedded in and attaching the sporophyte to the gametophyte, (2) an elongated stalk or seta, and (3) the capsule, or spore case, surmounting the whole structure. The foot consists of two main cellular regions: (1) a periphery of elongated, thin walled cells having a dense cytoplasm, and (2) large, somewhat spherical, thin walled cells comprising the interior. Beneath the maturing sporophyte, in the gametophyte tissue, is a region of small thin walled cells that appear to have the same density as the young maturing peripheral cells of the sporophyte foot. This dense zone of gametophyte cells has been termed the subcalyptral pad by Studhalter in his description of the developing sporophyte of Riella americana Howe and Underw. (12, p.638). This term will be used to describe the zone as it occurs in Porella navicularis Lindb. Surrounding the subcalyptral pad are gametophyte cells which are highly vacuolated and have thick cell walls. These cells mark what will be the outer limit of penetration of the foot.

Before the sporophyte shows evidence of sporogenous tissue, the foot has penetrated a considerable distance into the subcalyptral pad. In Figure I the subcalyptral pad appears as the dark zone continuous with the calyptra and surrounding the sporophyte foot. The

foot cells at this stage of sporophyte development are not well differentiated, nor is the sporophyte divisible into three major regions as when more mature. The peripheral cells of the foot appear densely stained but are not too different from the remainder of the sporophyte cells. The zone between the subcalyptral pad and the foot of the sporophyte shows residual cell wall material remaining from disintegrating cells of the subcalyptral pad. No sign of compression of living cells is evident in the gametophyte cells surrounding the foot.

In Figure II sporogenous tissue is differentiating within the capsule of the sporophyte and stains a darker color than the surrounding sporophyte capsule walls. The subcalyptral pad at this stage is still relatively thick. The foot cells of the sporophyte have greatly increased in number and the peripheral cells of the foot are well differentiated. Cells of the subcalyptral pad lying immediately adjacent to the base of the foot have lost most or all of their cytoplasm and are slightly crushed or compressed.

Figure III shows the foot and surrounding gametophyte tissue, the seta, and a portion of tne capsule of the sporophyte. Relatively undifferentiated sporogenous tissue may be seen in the lower right hand corner of the figure. The foot has increased considerably in size and consequently the subcalyptral pad has been greatly reduced. The peripheral cells of the foot in this figure are in

intimate contact with the cells comprising the subcalyptral pad. No large zone of disintegrating gametophyte cells may be seen, as in Figure II, but rather the disintegrated cells are compressed against the intact cells of the subcalyptral pad. Very thick walls now appear in the gametophyte cells surrounding the subcalyptral pad.

Figure IV is a more highly magnified photograph of the foot of a sporophyte whose sporogenous tissue is at approximately the same stage of differentiation as that shown in Figure III. Although this is not a true median section of the foot it is used to show the papillate cells comprising the periphery of the foot. The number of cells in the subcalyptral pad has been reduced and these do not appear to have as dense a protoplasm as those shown in Figure I. The dark area at the base of the foot and in the subcalyptral pad appears to be residual cellular material from disintegrating gametophyte cells. The foot as shown in median section actually penetrated to the depth of the disintegrating gametophyte cells and was in contact with them.

The condition of the foot of the sporophyte remains relatively constant from the preceding stage until the sporophyte is mature (spores and elaters formed and seta elongated). As seen in Figure V, the foot of a mature sporophyte undergoes considerable change. Here the cells of the subcalyptral pad have been completely disintegrated and only the heavy walled material of the gametophyte tissue remains. This outer layer of gametophyte cells (those which surround the subcalyptral pad) show signs of being compressed by the

foot. The cells of the foot have enlarged considerably and have become highly vacuolated. The cytoplasm in the cells of the seta and the capsule wall has also become greatly vacuolated.

Figure VI is an example of a sporophyte grown for sixty-two days in Voth's Full Nutrient Solution. At the time of killing and fixing in preparation for staining, the sporophyte was a healthy green color. All the cells of the sporophyte appear to be becoming highly vacuolated with the cells of the foot much more so than those of the seta and capsule. The sporogenous tissue is apparently disorganized and most of the tetrads appear to be disintegrating.

Figure VII is a more highly magnified portion of the foot of the same section that appears in Figure VI. The peripheral cells are large protruding processes and may be compared to those shown in Figure IV. These peripheral cells as well as the interior cells are highly vacuolated structures. It may be noted that some of the interior cells are beginning to disintegrate. The tissue that appears at the base of the foot is residual gametophyte cells that were incompletely dissected off at the time of removal of the sporophytes for planting. The cells of this tissue are highly vacuolated. The tissue at the top of the foot and partly surrounding it is a portion of the calyptra. The cells of the calyptra, like those of the other tissues, are becoming highly vacuolated.

B. RESULTS OF CULTURE WORK

The results of the culture work were negative in the sense that the sporophytes in all cases failed to mature. The culture work does show that the nutritional requirements of the sporophyte are complex and that it is not simply a matter of minerals or sugar that is required to maintain the growth of the sporophyte.

C. ANALYSIS OF DATA SHEETS

An analysis of the data sheets shows little correlation between growth and the nutrients supplied or their modification. The overall picture derived from the data is one of complete randomness of distribution of growth in size classes. There appears to be no correlation between length of time and growth. One series of cultures using White's basic medium seems to indicate that it is of possible nutritional benefit to the sporophytes. Less active growth seems to be shown when the sporophytes are grown in simpler media such as tap water, distilled water, and Voth's Full Nutrient Solution. The data sheets are, however, primarily a record of the results of the experiments and are not designed to determine rates of growth or the relative effectiveness of age or size. Rather the aim was to determine whether a particular culture solution would affect growth in any way and at any stage.

Since the sporophytes were measured only twice, once at the time of planting and again at the time of death (in most cases), it is not known whether the growth period occurred shortly after planting or whether it was spread out over the entire length of life of the sporophyte. It was deemed desirable to measure the sporophytes only at the time of planting and at the time of death to avoid contaminating the cultures.

CONCLUSIONS

The early morphologists regarded the foot as a haustorial organ in the nutrition of the entire sporophyte. It was not until recently that the supposed parasitic nature of the foot was questioned, mainly on the basis of the presence of chlorophyll in the sporophyte of a great number of the hepatics. Some culture work, done in not too exact a fashion, also helped to formulate this new concept of nutritional independency of the sporophyte. The development of the foot both *in vivo* and *in vitro* will be discussed in its possible role in the nutrition of the sporophyte of *Porella navicularis* Lindb.

In the early ontogeny of the sporophyte the foot penetrates down to the subcalyptral pad, which stains a dark color and is apparently rich in cytoplasm. A portion of the gametophyte cells disintegrate well in advance of the peripheral cells of the foot in the early stages of development of the sporophyte. It is not known whether the peripheral cells of the foot break down the gametophyte cells through dissolution of the cell walls or whether the protoplasm is released due to compression rupturing the cells. The presence of compression is doubted, however, as sectioned material does not indicate compression of living cells.

As the sporogenous tissue of the sporophyte continues to mature, the cells of the foot continue their deeper penetration into

the subcalyptral pad. It is not until the sporophyte has matured and the seta has elongated that the cells of the foot mature. At maturity these peripheral cells of the foot become highly vacuolated and enlarge to about the same diameter as the interior cells of the foot.

As indicated in the data tables none of the sporophytes were successfully cultured to maturity although they grew for considerable lengths of time. It is most probable that the nutritional demands are not inorganic salts but rather an organic substance (or substances) that was not supplied in the cultures. Mascerated tissue of the gametophyte thallus failed to stimulate growth to maturity although it is possible that in this case the needed organic substance was destroyed either in the mascerating process or during autoclaving. Yeast extract which provides many amino acid and vitamins failed to promote growth. Biotin, an essential metabolite for many micro-organisms, also failed to promote growth to maturity. Similar failure of growth resulted when the sporophyte was left intact with the gametophyte branch on which it was borne. It was therefore concluded that this unknown organic factor or factors must be supplied to the sporophyte by the intact and actively growing gametophyte.

Correlated with the failure of the sporophytes to mature was the failure of the seta of the sporophyte to undergo normal elongation. In cultured sporophytes which were relatively mature when excised the seta failed to elongate. When the sporophyte was left intact

with the gametophyte tissue this same failure occurred. Evidently the auxin causing cellular enlargement is produced by the intact gametophyte thallus as is the organic factors for cellular nutrition. Indole acetic acid and alpha naphthalene acetic acid in the concentrations used in the culture work by this author failed to supplement the normal auxin supply.

When the sporophyte is cultured the cells of the foot become highly vacuolate. It is possible that the high degree of vacuolation is caused by a difference in osmotic concentrations between the nutrient medium and the cells of the foot. The subcalyptral pad is thought to prevent this until these gametophyte cells are completely disintegrated and the foot is in contact with the heavier walled cells that surround the subcalyptral pad. Chlorophyll found to be present in the capsule and seta may produce sufficient photosynthetic products to partially nourish the cells. This may explain why the cells of the capsule wall and the seta are not as highly vacuolated as the foot which apparently does not contain chlorophyll.

The mechanism by which the foot of the sporophyte penetrates into the gametophyte tissue is thought to be one of enzymatic action. As seen in Figures I and II the cells of the subcalyptral pad disintegrate well in advance of the peripheral cells of the foot. The lack of compression that occurs in these gametophyte cells corroborates this conclusion. This enzyme that is secreted by the foot appears to

attack the gametophyte cell walls and possibly the protoplasm they contain. The enzymatic action, however, seems to be limited to the cells of the subcalyptral pad and does not attack the heavier walled cells comprising the rest of the gametophyte thallus.

The following theory is proposed for the nutrition of the sporophyte of Porella navicularis Lindb. The sporophyte is a nutritionally dependent generation. A necessary part of the nutrition of the sporophyte is derived from the actively growing gametophyte generation to which it is attached. The necessary metabolites are secured through the penetration by the foot through the subcalyptral pad which in turn is nourished by the intact gametophyte thallus. The auxin causing the seta elongation is generated also by the gametophyte thallus and is probably present at all stages of the maturing sporophyte but shows its greatest activity when the cells of the sporophyte have reached a certain point in their maturation. The chlorophyll present in the seta, the capsule, and the sporogenous tissue probably accounts for part of the nutrition of the sporophyte but is not sufficient to maintain active growth.

SUMMARY

Due to some doubt concerning the nutritional interrelationships between the sporophyte and the gametophyte generation of the Hepaticae, a series of culture experiments were carried out using the sporophyte of Porella navicularis Lindb. In conjunction with this, sections were made of the sporophyte growing in vivo, showing the penetration of the foot down through a region of thinly walled cells called the subcalyptral pad. From an analysis of the cultures and of the sections made of the sporophytes, it was concluded that this sporophyte is a parasitic generation during its entire growth period.

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APPENDIX A

DATA TABLES

Table I

	Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1.	11.20	13.10	17.0	54
2.	8.26	12.60	52.4	47
3.	6.33	7.53	19.0	14
4.	5.25	6.90	31.4	96
5.	4.08	3.66	-	83
6.	3.86	4.75	23.0	83
7.	3.66	4.28	16.9	65
8.	3.46	3.46	0.0	17
9.	2.78	3.08	10.8	65
10.	2.02	2.30	13.9	76
11.	1.63	1.63	0.0	32

Table II

	Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1.	6.90	8.26	19.7	64
2.	4.75	6.33	33.3	91
3.	4.28	6.33	48.0	57
4.	2.78	3.86	38.9	64
5.	2.14	2.78	29.9	91
6.	1.76	1.88	6.82	79
7.	1.63	3.86	137.00	27
8.	1.13	1.41	24.8	79

Table III

Initial Volume in cu.mm. x 10^{-2}	Final Volume in Cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 20.70	26.20	26.60	36
2. 16.10	20.70	28.60	63
3. 2.15	2.28	6.04	25
4. 1.63	1.63	0.00	27
5. 1.21	1.41	16.50	14

Table IV

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 19.50	24.80	27.2	59
2. 18.30	24.80	35.5	27
3. 11.30	12.10	7.1	25
4. 2.77	2.77	0.0	14
5. 2.44	3.09	26.6	14
6. 1.21	1.41	16.5	27

Table V

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 156.0	183.0	17.3	62
2. 120.0	144.0	20.0	80
3. 105.0	110.0	4.76	80
4. 85.6	120.0	40.2	80
5. 40.8	46.1	13.0	80
6. 38.2	46.1	20.7	80
7. 16.5	28.8	74.7	80
8. 16.5	23.0	39.3	80
9. 10.2	11.2	9.8	80

Table VI

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 95.2	120.0	26.1	58
2. 23.0	58.6	155.0	58
3. 23.0	55.5	108.0	62
4. 18.8	*[2]	*	*
5. 18.8	*	*	*
6. 2.06	9.2	346.0	48

Table VII

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 132.0	170.0	28.8	29
2. 46.3	69.5	50.2	50
3. 10.2	13.7	34.3	29
4. 1.15	*	*	*

Table VIII

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 110.0	126.0	14.5	50
2. 52.4	69.5	32.7	48
3. 26.7	35.6	33.3	47
4. 0.905	1.44	58.6	29

[2] An asterisk has been used to denote the withdrawal of the culture because of contamination.

Table IX

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 184.0	214.0	16.3	35
2. 62.3	73.4	17.8	35
3. 19.5	26.7	36.9	23
4. 1.41	*	*	*

Table X

Part A

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 99.8	143.0	43.2	62
2. 46.2	94.7	105.0	36
3. 28.8	52.4	82.9	62
4. 24.8	*	*	*
5. 5.76	11.3	96.2	51

Part B

Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1. 104.7	143.0	36.6	58
2. 49.2	69.5	41.3	62
3. 23.0	46.3	101.0	62
4. 13.7	*	*	*
5. 13.7	28.8	110.0	36

Part C

	Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1.	131.0	143.0	91.6	62
2.	35.6	*	*	*
3.	30.9	55.5	79.9	62
4.	30.9	46.6	49.6	44
5.	7.33	10.2	39.2	12

Table XI

Part A

	Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1.	32.70	42.00	28.4	61
2.	8.26	11.20	35.6	59
3.	7.54	7.54	0.0	39
4.	1.20	1.20	0.0	14
5.	0.87	1.03	18.4	25

Part B

1.	31.00	42.00	35.4	61
2.	13.10	17.20	31.3	59
3.	4.28	5.00	16.9	14
4.	1.21	1.41	16.6	32
5.	1.21	1.11	*	*

Part C

1.	9.70	15.10	55.6	61
2.	4.76	5.75	20.8	59
3.	2.77	3.46	24.9	39
4.	1.03	1.21	17.5	39

Table XII

	Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1.	144.0	157	9.0	17
2.	144.0	183	27.1	17
3.	132.0	183	38.6	17
4.	132.0	183	38.6	17
5.	115.0	150	30.4	17
6.	110.0	144	30.9	17
7.	105.0	120	14.3	17
8.	95.4	150	57.3	17

Table XIII

	Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1.	132.0	144.0	91.0	17
2.	115.0	162.0	41.0	17
3.	105.0	115.0	9.5	17
4.	90.5	90.5	0.0	17
5.	81.6	132.0	61.7	17
6.	73.8	110.0	49.0	17
7.	69.5	90.5	30.2	17

Table XIV

Part A

	Initial Volume in cu.mm. x 10^{-2}	Final Volume in cu.mm. x 10^{-2}	% Increase	Days of Growth
1.	107.0	126.0	17.8	41
2.	99.5	126.0	26.6	15
3.	90.5	115.0	27.0	15
4.	86.0	110.0	28.0	15
5.	7.38	19.5	163.0	15

Part B

1.	110.0	132.0	20.0	65
2.	105.0	120.0	14.3	65
3.	90.5	99.5	10.0	41
4.	10.2	16.4	60.0	61
5.	2.05	2.88	40.5	61

APPENDIX B

Figure I. Undifferentiated sporophyte of Porella starting penetration into the subcalyptral pad.

Figure II. Well differentiated foot of the sporophyte of Porella showing disintegration of the subcalyptral pad well in advance of the foot.

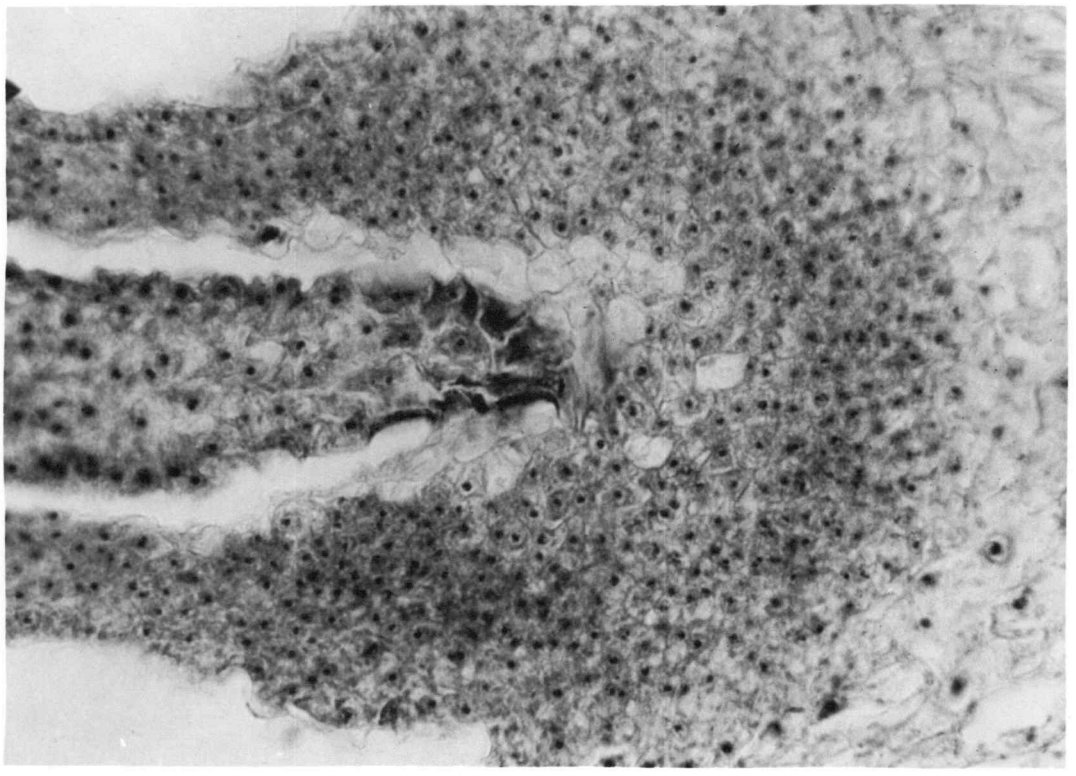

Figure I. 475x

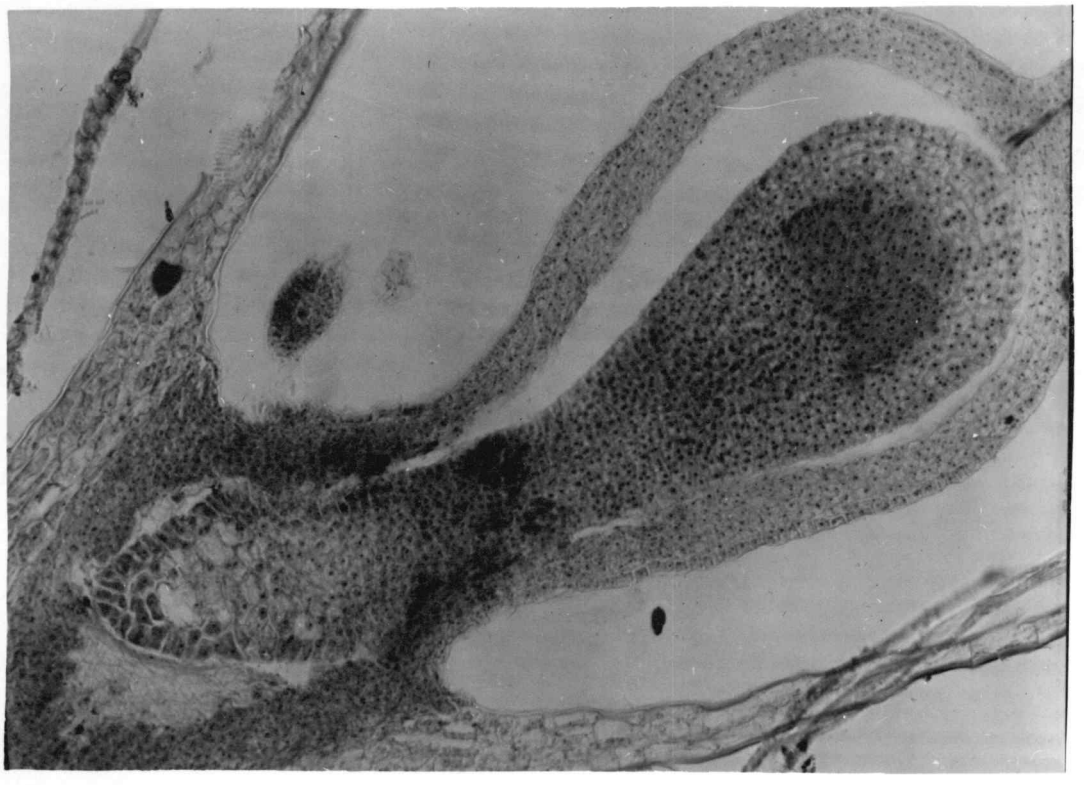

Figure II. 150x

Figure III. Foot of the sporophyte of Porella which has penetrated through most of the subcalyptral pad.

Figure IV. Highly magnified portion of the foot showing the papillate peripheral cells of the foot and the disintegrating gametophyte tissue.

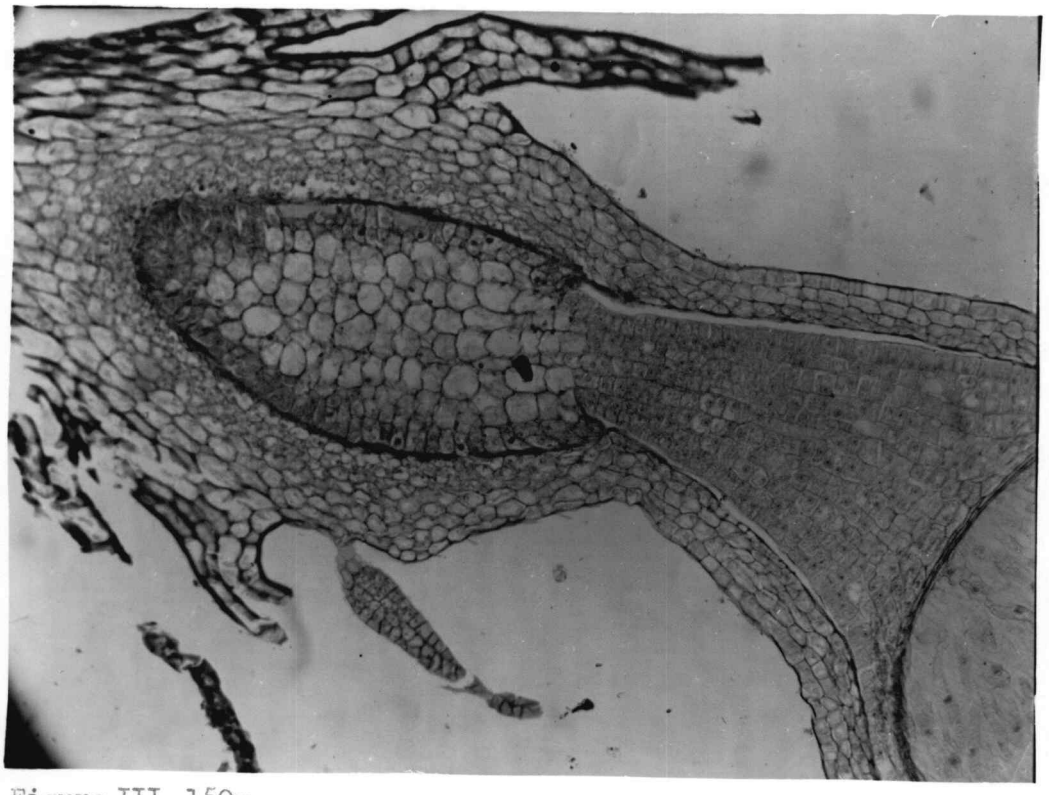

Figure III. 150x

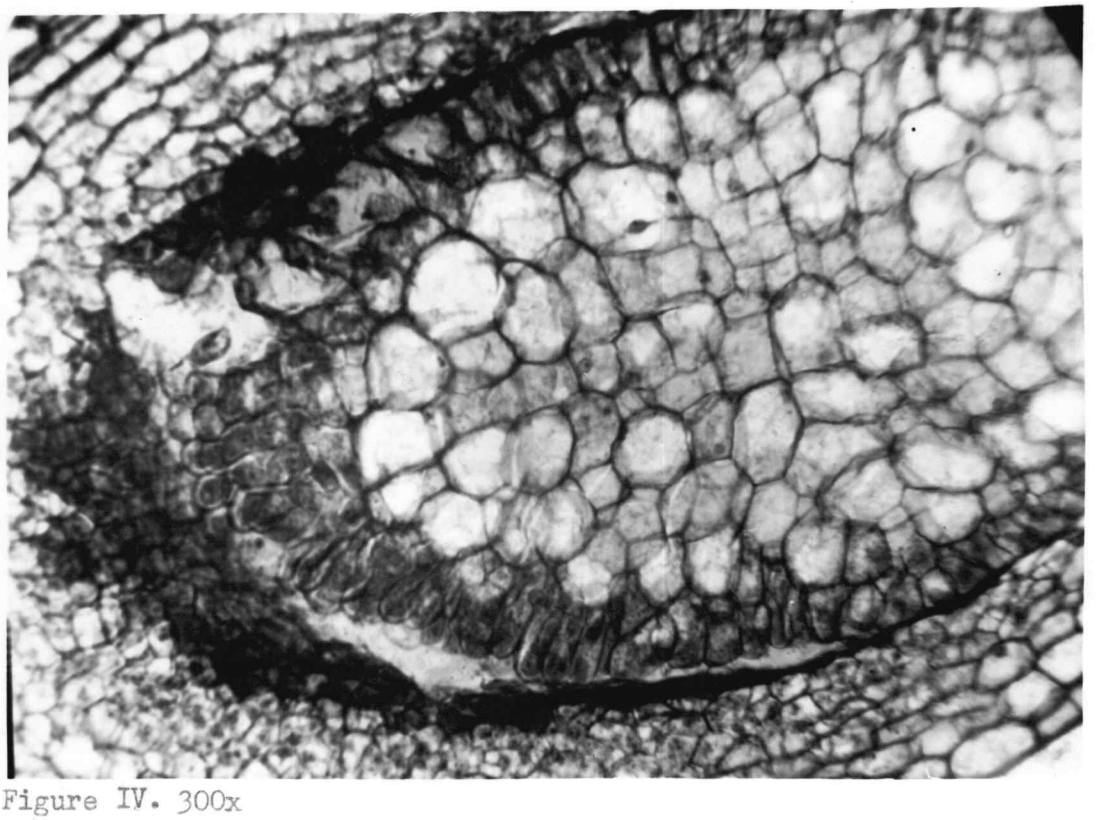

Figure IV. 300x

Figure V. Condition of the foot cells of a fully matured sporophyte.

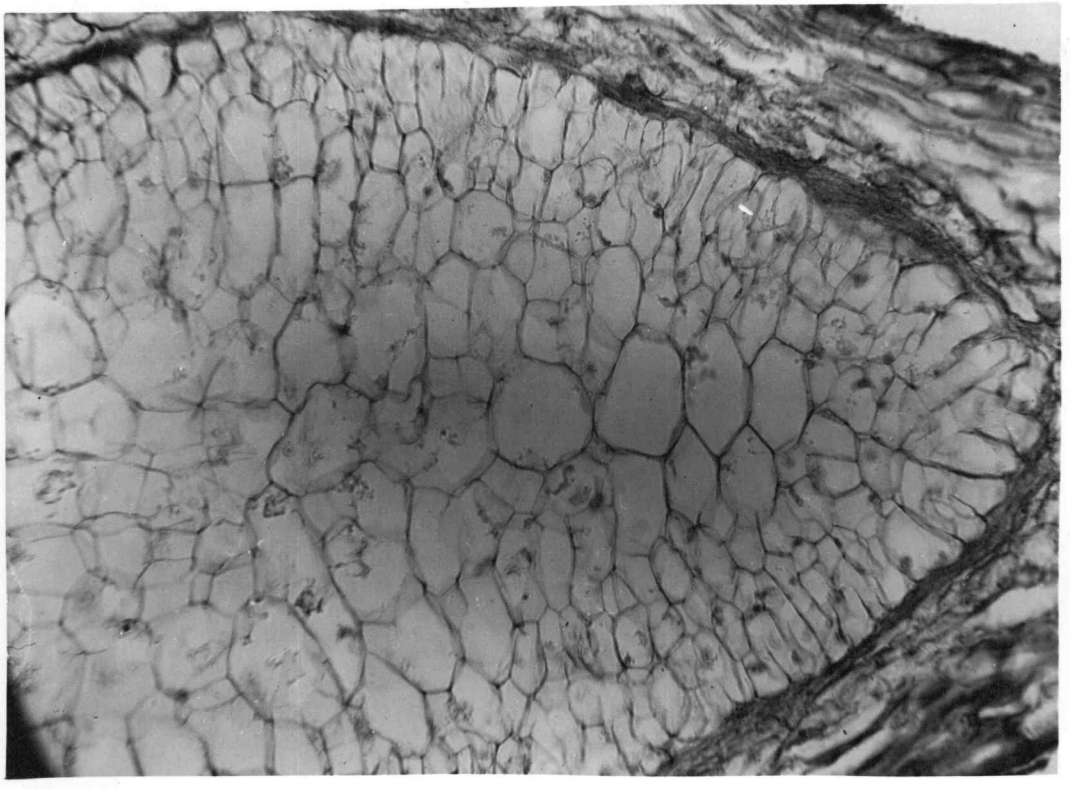

Figure V. 400x

Figure VI. Condition of the cells of the sporophyte when grown in Voth's Full Nutrient Solution for 62 days.

Figure VII. The foot of the same sporophyte enlarged showing the much elongated peripheral cells and the very considerable vacuolation.

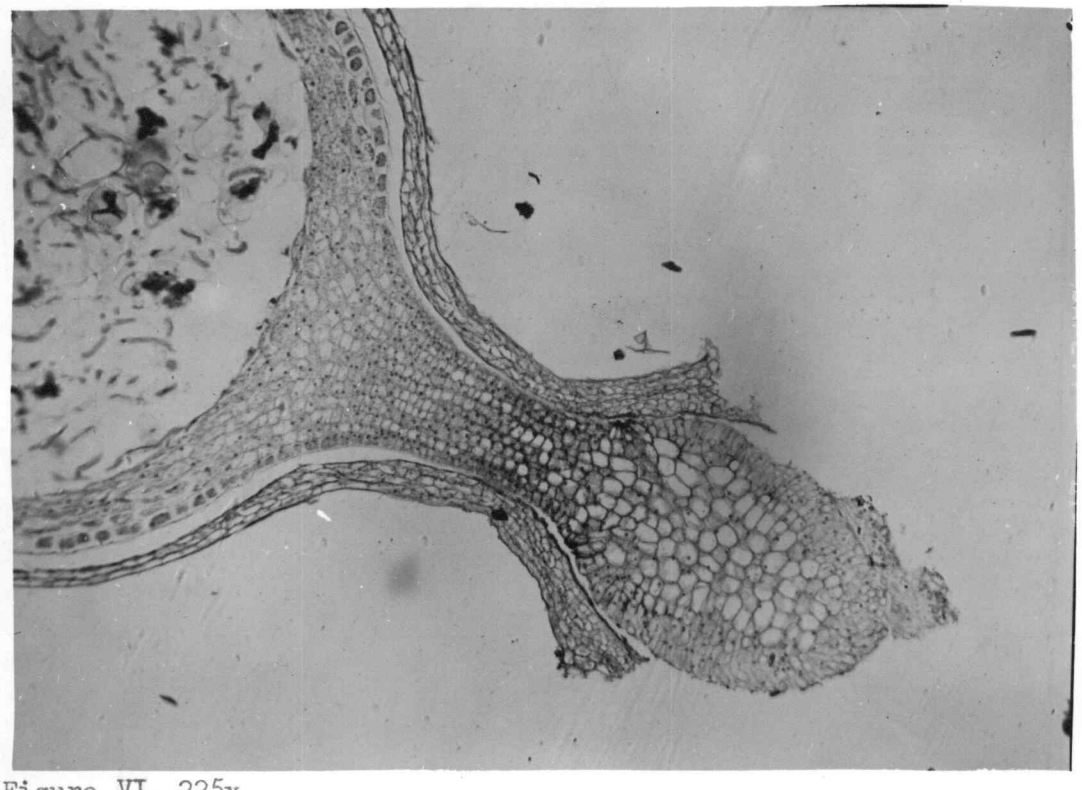

Figure VI. 225x

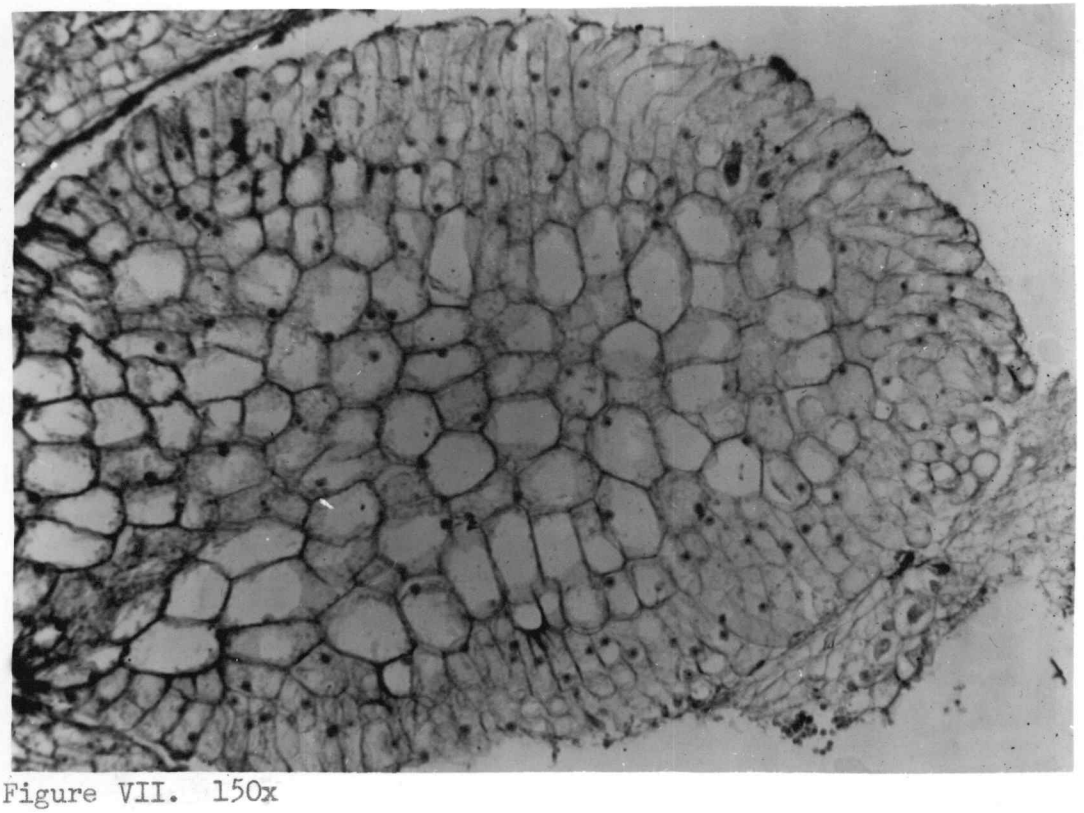

Figure VII. 150x