

INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.
2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.
3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again -- beginning below the first row and continuing on until complete.
4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.
5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

75-10,462

BELTZ, Carolyn Kay, 1946-
THE INFLUENCE OF NATURAL ENVIRONMENTAL
CONDITIONS ON THE GROWTH, DISTRIBUTION, AND
MORPHOLOGICAL EXPRESSION OF THE CARNIVOROUS,
AQUATIC PLANT UTRICULARIA MACRORHIZA LE CONTE,
WITH LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS
OF BLADDER ONTOGENY. (VOLUMES I AND II)

Iowa State University, Ph.D., 1974
Botany

Xerox University Microfilms, Ann Arbor, Michigan 48106

© 1975

CAROLYN KAY BELTZ

ALL RIGHTS RESERVED

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED.

The influence of natural environmental conditions on the
growth, distribution, and morphological expression of
the carnivorous, aquatic plant Utricularia macrorhiza
Le Conte, with light and electron microscopic
observations of bladder ontogeny

by

Carolyn Kay Beltz

Volume 1 of 2

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Botany and Plant Pathology
Major: Botany (Morphology)

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1974

Copyright © Carolyn Kay Beltz, 1975. All rights reserved.

TABLE OF CONTENTS

	Page
DEDICATION	iv
INTRODUCTION	1
SURVEY OF THE LITERATURE	4
Introduction	4
Taxonomy	5
General Morphology	8
Habitat Studies	11
Trapping Mechanisms of Bladders	12
Bladder Contents	19
Digestion and Need for Animal Nutriment	21
Studies of the Bladder Ontogeny and Anatomy	23
Bladder ontogeny	23
Antennae	26
Wall cells	26
Vascular tissue	27
Outer surface trichomes	28
Door	29
Trigger trichomes	30
Door surface associated trichomes	31
Pavement epithelial trichomes	33
Velum	34
Quadrifid and bifid trichomes	35
MATERIALS AND METHODS	40
Field, Taxonomic, and Statistical Methods	40
Plant collection	40
Taxonomy	40
Transplants	45
Statistical analysis	45
Laboratory Methods	52
Anthocyanin determination	52
Light microscopy (LM)	54

	Page
Transmission electron microscopy (TEM)	55
Scanning electron microscopy (SEM)	55
OBSERVATIONS	57
Field Observations	57
Silver Lake Fen	57
Jemmerson Slough	59
Three Corners Pond	60
Spring Lake	61
Transplant experiments	61
Growth Chamber Results	62
Statistical Results	63
Anthocyanin Determination	65
Microscopy	65
Winter-buds	65
Bladder ontogeny	67
Door	73
Trichomes and antennae surrounding the aperture	76
Pavement epithelium	79
Bifid and quadrifid trichomes	82
Bladder outer surface trichomes	88
DISCUSSION	93
SUMMARY	141
LITERATURE CITED	144
ACKNOWLEDGEMENTS	158
APPENDIX A: TABLES FOR THE LITERATURE CITED	160
APPENDIX B: FIELD METHODS	175
APPENDIX C: LABORATORY TECHNIQUES	179
APPENDIX D: KEY TO FIGURES	188
APPENDIX E: FIGURES	191

DEDICATION

This dissertation is dedicated to DWIGHT CALVIN BELTZ, who never lived to see its completion, but through his earlier and continuing influence instilled in his children and grandchildren the necessary discipline to approach and complete all tasks in life with pride and determination, with the hope of self-improvement and a worthy contribution to future generations.

INTRODUCTION

In general, the only safe conclusion to be drawn from a study of the available evidence regarding the nature of the organs in the Bladderworts, seems to be that--in the present state of our ignorance--the attempt to fit so elusive a genus into the Procrustean bed of rigid morphology, is doomed to failure.

Agnes Arber (1920)

Biologists have accepted easily the need for plant nutriment in animal survival; however, the need by some plants for animal nutriment remains, even to date, difficult to comprehend. For this reason, carnivorous plants have for centuries been intriguing to mankind. Those genera of the family Lentibulariaceae which are of small size and have complex trapping mechanisms remain the least understood of the carnivorous plants.

Taxonomists generally agree that the Lentibulariaceae consists of five genera and approximately 290 species: Utricularia (200 sp.), Genlisea (32 sp.), Pinguicula (35 sp.), and the two smaller genera of Polypompholyx and Biovularia. Because of the large number of species comprising the genus Utricularia, there have naturally been a number of taxonomic modifications. With the advent of more morphological investigations some order has been brought to the genus; however, the number of species which have been investigated morphologically remains small and those studies which have been done are incomplete in many respects. Consequently, there is a need for additional morphological information before the taxonomy can be

greatly improved.

In an attempt to better understand the morphology of the genus a readily available species was selected for study. Utricularia macrorhiza occurs in several Iowa locations and is also fairly universal in distribution throughout the world. Plants which appeared morphologically different were collected from four aquatic habitats. In examining the gross morphology of the plants from these different habitats, morphological plasticity was observed among various populations. This raised the question as to whether or not the plants were actually the same species or different species. Therefore, specimens from each location were sent to Dr. Peter Taylor, Kew Botanical Gardens, England, who has worked on the taxonomy of the genus for the past 15 years. Accordingly, all plants were verified to be the same species using currently available taxonomic information for the genus. This suggested that the differences observed were possibly due to environmental conditions. In order to substantiate this possibility, environmental features were monitored, statistically analyzed, and transplants of each population were made from one habitat to another.

The investigation also included light and electron microscopic observations of the various stages of bladder ontogeny. The bladders are the most complex and more intriguing structures of the plant. Their development has been studied only partially by previous workers. Other than three electron microscopic studies concerned with the winter-bud stage of development, nothing has been

done ultrastructurally to provide any information on the cellular events which occur in the bladders.

The intention of this investigation was to provide additional information concerning environmental conditions which influence growth and survival of Utricularia in various habitats, the morphological and anatomical features of one species (U. macrorhiza), with emphasis on development of the complex bladder trap, and bladder function. The morphological information obtained concerning a single species perhaps will stimulate similar studies of other species and provide information for taxonomists attempting to provide order to this large and complex genus. In addition, this study presents a more complete picture because material from natural habitats was used so that naturally occurring variations were observed rather than the more uniform development under artificial growth conditions.

SURVEY OF THE LITERATURE

Introduction

Utricularia (Lentibulariaceae) is interesting from both the layman's and scientist's viewpoints. The layman hears of meat-eating plants and imagines the monstrous plant-like beings which are so common in science fiction and superstition. Prior (1939) discussed the facts and myths surrounding carnivorous plants and attributed the greatest economic value to their sale as novelty plants. The only other known economic value for Utricularia appears to be the use of it as manure and fodder for cattle and pigs in tropical Africa, India, and Southeast Asia (Sculthorpe 1967). Although unsuccessful attempts were made to use it as a control for mosquito larvae (Matheson 1930), it reportedly caused concern in New York as a "fish-eating" plant (Dean 1890). In the latter case this is rare and hardly of economic importance.

To the scientist, Utricularia and other carnivorous plants represent a taxonomically heterogeneous group, of which according to Lloyd (1942) the Lentibulariaceae are the most numerous and widespread. They show few consistent common features except their carnivorous habitat, along with the adaptation of a highly evolved, extremely plastic dicotyledonous angiosperm to an aquatic or moist terrestrial habitat, make Utricularia a genus which presents challenging problems in the fields of development, phylogeny, morphology, anatomy, ecology, physiology, biochemistry, and taxonomy.

Taxonomy

Members of the Lentibulariaceae, including Utricularia, are taxonomically defined by Benson (1957). He describes the family as consisting of annual or perennial, predominantly insectivorous, herbaceous plants inhabiting wet terrestrial or aquatic habitats. Leaf polymorphism is frequent in both terrestrial and aquatic species, with one to several species, or groups of species, having distinct leaf and stem structures and other associated appendages (to be explained later). Flowers are bracteate and pedicels of some species bear a pair of bracteoles. The inflorescence may be represented by a single flower or a raceme (which consists of flowers borne on a pedicel attached to a rachis). The flowers are bisexual, zygomorphic, and are composed of a two or five lobed calyx or a divided calyx whose segments are open or imbricate, spurred, frequently possessing a palate. The palate is variable in form. Two stamens are always present; in addition, two staminoides are present in some species. The anthers are unilocular, longitudinally dehiscent and the ovary is superior, unilocular with two carpels and free central placentation. Numerous ovules and seeds are generally produced. The embryo is poorly differentiated or undifferentiated and has no endosperm associated with it. Kamienski (1877), Merz (1897), Khan (1954) and Kumazawa (1967) have best described anatomically embryo and seedling development.

As mentioned previously, the taxonomy of the genus Utricularia is complex and constantly in revision. As it is only superficially

related to the problem discussed in this dissertation I shall not belabor the issue but note that Taylor (1964) has undoubtedly done the best single synopsis of the genus. Although this work strictly monographed species found in Africa and Madagascar, its introduction provides the most current analysis of the taxonomy and the biology of the genus to date. Table A1, Appendix A, also outlines a number of the species investigated and those investigating them. Although it is by no means complete it will give the reader an idea of the vastness of the genus and refers to many of the earlier taxonomic descriptions. Other references not included in Table A1, Appendix A, which may be of taxonomic value include: Smith (1819), Meyer (1837), De Candolle (1844), Gardner (1846), Benjamin (1847), Edgeworth (1848), Bentham (1869), Burrell and Clarke (1911), Nieuwland (1914), Barnhart (1915), Fries (1916, 1924), Phillips (1917), Hoehne and Kuhlmann (1918), Mildbraed (1922), Stephens (1923), Pellegrin (1930), Hutchinson and Dalziel (1931), Gleason (1931, 1952, 1968), Rossbach (1939), Carr (1940), Fernald (1941, 1946, 1950), Cain (1944), Eyles and Robertson (1944), Muenscher (1944), Suessenguth (1951), Czech (1952), Taylor (1954, 1961, 1963), Core (1955), Perrier de la Bathie (1955a, 1955b), Bosser (1956, 1958), Benson (1957), Mason (1957), Exell and Roziera (1958), Dawson (1960), Lewis, Satripling, and Ross (1962), Gleason and Cronquist (1963), Bell (1967), Fassett (1969), and Correll and Correll (1972).

As would be expected when dealing with such a large genus,

various attempts have been made to sub-divide the genus into smaller related groups. Only two such attempts are significantly related to this dissertation. Lloyd (1929a, 1929b, 1930, 1931a, 1931b, 1932a, 1932b, 1933, 1935, 1936, 1942) worked with the mechanisms and structures of the bladders of numerous species of Utricularia (See Appendix A, Table A1). It is therefore not surprising that his artificial subdivisions of the genus are based on bladder structure. He distinguishes four general groups on the basis of the traps: the U. vulgaris type (of which U. macrorhiza belongs), the "Biovularia" type, and U. purpurea type, and the U. dichotoma--U. monanthos type. These large groups were then divided according to type of habitat if it varied (it does not vary in the Biovularia type or in the U. purpurea type) and these subgroups were then further divided into groups on the basis of morphological characters other than bladder structure; most commonly stem or petiole features.

Taylor (1964) grouped the species by habitat and habit into five groups: submersed aquatics, free-floating aquatics, affixed aquatics (anchored in the substrate), terrestrial, and epiphytic. The characteristics of species in each of these groups were categorized by: stolon, leaf, corolla, etc..

General Morphology

Perhaps one of the more spectacular features of the morphology of Utricularia is its plasticity. Arber (1920), Taylor (1964), and Sculthorpe (1967) emphasized that any portion of the vegetative plant thallus may become meristematic and produce any number of a variety of other plant parts by adventitious budding or continued apical growth. Therefore, a leaf may give rise to a stolon, bladder, or another leaf by adventitious budding from its margin, its lamina, or its petiole, or by prolongation at its apex. There is no real distinction between stems, leaves, stolons, etc., so one is plagued with the question of how the structures relate to those more orthodox organs of other plants. The peculiar organs of Utricularia display similarities in function to the traditional stem and leaf; however, their structure and their derivation are curious. Unfortunately, at present there is no fully acceptable answer.

Arber (1920) and Sculthorpe (1967) indicated that the vegetative plant body has been variously interpreted as a modified stem, a much dissected leaf, a modified root system, and a combination of stem and leaf elements. "There has probably been more controversy about the morphological nature of the different organs of these plants, than about such problems in the case of any other Angiosperm" (Arber 1920). The best historical review of this issue is by Goebel (1891). Sculthorpe (1967) points out the concept of a modified root system was highly unlikely. Far too much modification would have had to occur to be readily explained, and the infrequency of true roots in

the Lentibulariaceae argues strongly against such a theory. Meierhofer (1902) interpreted the bladder as a modified leaf and although the idea has merit it is still disputable because no attempt has been successful in defining a leaf specifically for Utricularia. The possibility that the entire plant is a much dissected leaf creates a number of problems which Goebel (1891) has defined as: this "leaf" would have to possess characteristics we normally attribute to stems alone such as continued apical growth, as described by Hovelacque (1888) for Utricularia, and the ability of producing leaves from a leaf, axillary branches, and development in more than one plane. However, Goebel (1904) argues that the theory cannot be completely refuted as adventitious shoots are produced on the leaves of other members of the Lentibulariaceae. As with many aquatic plants, Utricularia possesses a regenerative ability, producing new plants from fragments of the plant. Glück (1906) was even able to demonstrate that plants could arise solely from pieces of the bladder stalk, an observation not so surprising today with modern tissue culture techniques, but certainly of considerable merit for the time.

In addition to dealing with the bladder-bearing laterals (typically called "leaves" or "stems" for convenience) there are a number of other unusual structures which must be considered. The first of these was defined as a "tuber" by Darwin (1875). This structure measured 11.4 mm to 25.4 mm in length and was either buried in the substrate or floated near the surface of the water.

Its surfaces were covered with glands. It contained vascular tissue but had no starch reserves. Darwin suggested that the tuber served as a reservoir for water during dry seasons. Ridley (1888) mentioned the presence of "tubers" in epiphytic forms of Utricularia but suggested no function. Cohn (1875) detected large amounts of storage material in the tubers which he believed was starch. At germination the starch was lost. Reinert and Godfrey (1962) observed that tubers form only on plants that become impacted in "muck" after the water has almost completely receded. They did not observe tubers germinating in the field but when immersed in water in the laboratory they did so readily.

Pringsheim (1869), Goebel (1889, 1891), and Glück (1906) noted the presence of "Ranken" (tendrils) in Utricularia. Arber (1920) called these structures "air-shoots" and described them as reduced inflorescences bearing small individual "leaves," thread-like, white, and covered with stomata. She suggested that they provide a source of oxygen for the plants when they are growing under tangled, crowded conditions.

Arber (1920) identified a third structure called "earth-shoots" and described them as penetrating the mud and producing bladders, which trap animals, and serving for anchorage and absorption of nutrients. Glück (1906) briefly mentioned them, and Benjamin (1848) described U. intermedia as rooted, evidently observing the so-called "earth shoots."

Two other structures have been described that reportedly aid

in keeping the inflorescence erect and afloat. The first was termed a rhizoid by Goebel (1889), who described them as bearing no bladders but having gland-covered leaves bent in a claw-like fashion. The second specialized shoots surround the base of the inflorescence of U. stellaris, U. inflexa, and U. inflata, and consist of elongated aerenchymatous floats. Goebel (1891) illustrated these for U. inflata. Spruce (1908) described them in detail and noted a similar structure in U. quinqueradiata. For additional discussion see Reinert and Godfrey (1962).

Habitat Studies

Utricularia is widely distributed in the tropics, and a few species, all free-floating aquatics, occur in the north temperate regions (Taylor, 1964). According to Kondo (1972a) about twenty species occur in North America as free-floating or anchored aquatics, epiphytes in wet mosses, or terrestrial plants of wet to moist, sandy soils.

Moyle (1945) investigated the chemical factors influencing aquatic plant distribution in Minnesota. Utricularia vulgaris var. americana (now considered U. macrorhiza) was placed in his "hard-water flora" group and described as growing in quiet waters over fertile soil. Ranges were determined for the following chemical factors: total alkalinity 16.5-297.5 ppm, sulphate ion 0.0-317.6 ppm, and pH 6.8-8.8.

Glück (1913) did an extensive investigation of the habitats

in which Utricularia grew in Great Britain. He omitted water chemistry, but water depth, sulphate, shoot, leaf, and bladder measurements, and presence or absence of flowering were observed for eight different habitats.

According to Sculthorpe (1967), Utricularia is exceptional among most free-floating plants in that some species frequently colonize fen and bog waters in which pH is low, and calcium, magnesium, and nitrate concentrations are very low.

Trapping Mechanism of Bladders

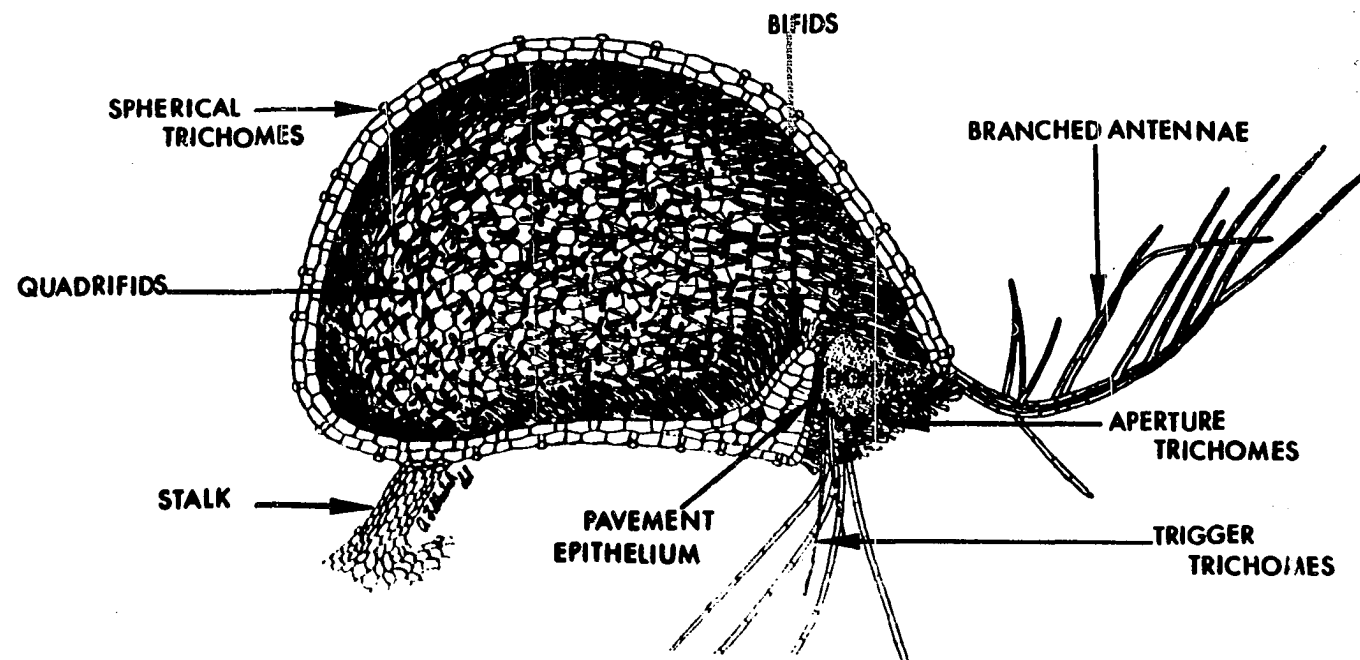
The literature of the most complex and intriguing structures of Utricularia, the bladders, remains to be discussed. It has now been a century since the classical works of Darwin (1875) in England and Cohn (1875) in Germany were published on Utricularia. Both made impressive contributions to bladder anatomy, and, although they were not the first to publish on Utricularia, their observations were the most extensive to that time. Crouan (1858), a pharmacist in France, Holland (1868) in England, and Treat (1875) of America had observed animals in the bladders of Utricularia. Darwin (1875) knew of the works of Crouan (1858) and Holland (1868) and published his own similar observations concluding that Utricularia was truly a carnivorous genus. None of these workers had any reasonable understanding of the mechanism by which the animals entered the bladders. I will add that despite numerous reports over the years regarding the problem of how the trapping mechanism works, no satisfactory explanations

exist to date.

Skutch (1928; Illustration 1) described the bladders of U. vulgaris (closely allied to U. macrorhiza) as "roughly lenticular in shape" and reaching 2.5 to 3.0 mm in length. Each bladder is attached by a short, slender stalk inserted on its ventral or adaxial surface near the inner axil of a fork of the filiform leaf. Kamienski (1877) described the bladders of the same species as bilaterally symmetrical and as equivalent to the "leaf" or a segment of the "leaf." Skutch (1928) stated that the dorsal region of the bladder is elongated and arched, with the ventral portion shorter and straighter. A semicircular aperture occurs dorsally and on the ventral side. The aperture is closed by the door which rests on the pavement epithelium, originally termed a "collar" by Darwin (1875). Continuous with the dorsal surface, and branching on either side of the aperture, are two multicellular trichomes. Darwin (1875) called these "antennae" because the bladder's appearance reminded him of a crustacean.

Located on the outer surface of the door are four trigger trichomes which Darwin (1875) illustrated quite nicely and called "bristles." He was unaware of their function. In addition, he illustrated the various trichomes associated with the inside of the door, as well as those on the inner surface of the bladders. He called these latter trichomes "quadrifids" and "bifids."

The earliest reports regarding bladder function implicated it in supplying air to the plants or in helping keep the plants afloat (De Candolle, 1844; Treviranus, 1848; Benjamin, 1848; Van-Tieghem,



ILLUST. 1. UTRICULARIA BLADDER DRAWN BY SKUTCH 1928 LABELS ADDED

1868). Benjamin (1848) was the first to demonstrate that the door actually opened inwardly and implied that this was only to let air in. Darwin (1875) and Goebel (1889) discredited these theories, however, both mistakenly believed that they observed animals entering the bladders merely by forcing their way through the slit-like orifice with their head serving as a wedge. This idea was also popular with Goebel (1891), Meierhofer (1902), and Luetzelburg (1910).

Cohn (1875), Treat (1875, 1876), Büsgen (1888) and Brocher (1911) all agreed that the door was held shut by water pressure and that the animals must press against the door to enter the bladder. Brocher (1911), an entomologist, first noticed actual springing of the traps. He observed that as animals disappeared the bladders "jerked" and broadened in profile, thus he concluded that prey was sucked in. He heard a "crepidulation" as the plants were removed from the water and noted that bladders which had been triggered had convex sides, whereas set bladders had concave sides. By "titillating" the door bristles (trigger trichomes) he could get the set traps to trigger and become convex.

Brocher (1911) hypothesized that the concave form of the bladder was explained by the principle that the rate of development of the tissues was greater than the rate of increased lumen volume. Because the walls were concave, the tensions of their tissues were greater and when triggered the wall tension was released so that the bladders became convex.

The concave wall condition was further augmented by the fact

that the interior of the bladder possessed a "negative pressure." To prove this view, Brocher punctured concave bladders and they returned to their normal convex position. The door itself was observed to be curved outward which caused it to be held tightly against the threshold, where it was made water-tight by mucilage secretion. The position of the door could be changed because of its "sensibility" and "contractility," shrinking when touched and thus allowing the water pressure to exert its force. He believed the trap action occurred so rapidly that it closed before the walls could be fully expanded, and this might possibly explain why the bladders could trap repeatedly. However, he felt this was doubtful and remarked that a more reasonable explanation was that the inner trichomes removed water from the interior of the bladder. Not being a botanist, he implied that he had no desire to consider proving this. Brocher (1911) unfortunately published his work in a journal not generally read by botanists. Therefore, Ekambaram (1916), Withycombe (1916), using only a hand-lens at the age of eighteen, and Hegner (1926), all without any knowledge of the other's work, made similar observations to that of Brocher (1911). Since their works, numerous theories have appeared and a great deal of experimentation has been done on the trapping mechanism. Skutch (1928) and Lloyd (1942), who did a comparative study of the trapping mechanisms of many species, published two excellent and exhaustive reviews of the literature concerning trapping mechanisms. Therefore, only the two most recent accounts of this aspect will be presented. What I describe is a

rather brief interpretation of Lloyd's (1942) description, which he somewhat facetiously compared to a "complicated mousetrap" at the end of his book. The door can be thought to have two "hinges," one at its upper origin and the other in the central region just below the trigger trichomes. That portion of the door below the lower "hinge" swings independently of the upper "hinge." If pressure is applied to the upper "hinge" the door does not open, but if pressure is applied to the lower "hinge" by a thrust on the trigger trichomes so that it swings on the upper hinge, it will clear the "stop" on which it rests on the pavement epithelium, allowing an inward swing of the door. As the bladder is triggered, the lateral walls spring outward and water rushes in, bringing with it any associated small animals or plants. The door then closes and is sealed by a second valve, the velum, a thin but firm transparent membrane which lies against the lower edge of the door and is attached to the pavement epithelium from which it is secreted. This seals the bladder door tightly and no leakage has been detected via the small slit between the door and its stop, because of the effectiveness of this back-up seal.

For the bladder to trap again, water must be removed from the interior of the bladder. Czaja (1923) and Nold (1934) demonstrated that the entire inner and outer surfaces of the bladder were covered by a cuticle, except for the terminal cells of the trichomes. Therefore, the most reasonable path for water movement out of the bladder lumen was through the quadrifids and bifids. Nold (1934) further

suggested that it would probably have to pass out via the "spherical cells" on the bladder surface. Lloyd (1942) concluded that there is little or no experimental evidence to prove the involvement of the outer spherical cells. Whatever the mechanism is, within 15 to 30 minutes, depending on the species, the bladder is back in the concave position to be triggered again.

The most recent paper on bladder movement is by Sydenham and Findlay (1973). They describe the changes which occur in the internal pressure, volume, membrane potential difference, and membrane resistance during the bladder triggering process. There has been a long controversy over whether the trap action is mechanical or excitatory. Lloyd (1942) was convinced that the process was strictly mechanical and provided a rather lengthy defense for his view. However, with Sydenham and Findlay's (1973) paper there is more evidence that the mechanism is excitatory. Triggering a bladder causes an increase in internal hydrostatic pressure from about -17 kPa (= -17,000 Pascal units or 0.025 psi) to about -5 kPa (-5000 Pascal units or 0.007 psi) and an increase in luminal fluid of more than 40% by volume. The opening and closing action of the door takes 10-15 milliseconds after triggering. When a bladder is triggered, changes occur in the potential difference between the lumen of the bladder and the outside solution, in the potential difference between the lumen of the bladder and the interior of a wall cell, and in the resistance between the lumen and the outside solution. The potential difference changes are not due to short-circuiting but result from actual changes in the

respective membrane potential differences. They have shown that in a concave (set) bladder, sodium and potassium ions are actively transported into the lumen from the outside solution, and chloride ions are actively transported out of the lumen.

Diannelidis and Umrath (1953) were able to trigger bladders by electrical stimulation, suggesting an excitatory step. Sydenham and Findlay (1973) were unable to demonstrate this. They presented the following evidence for an excitatory step: when a bladder is triggered repeatedly the door partially collapses inwardly before opening. This is due to a loss of turgor in the cells of the door, resulting from stimulation. It was difficult for them to understand how a small displacement of the tip of one of the trigger trichomes could cause a loss in door turgidity. They eventually concluded that the first stimulation of the trigger trichomes causes a loss of solute, and consequently loss of turgor. This causes the door to become flaccid and open, triggering the trap. If the trigger trichomes are repeatedly triggered after the first stimulation, loss of turgor in a greater number of cells is necessary before turgidity is decreased enough to open the door.

Bladder Contents

Various kinds of organisms have been reported trapped in the bladders of Utricularia. There appears to be little specificity in the organisms trapped. Treviranus (1848) reported the presence of snails and beetles, Crouan (1858) found various kinds of crustaceans,

and Darwin (1875) and Cohn (1875) described crustaceans and insect larvae in the bladders.

Goebel (1891) observed that some differences occurred in the organisms trapped by U. intermedia and U. vulgaris growing in the same pond. He explained that this was because U. intermedia was anchored to the substrate and, therefore, more prone to trap the bottom dwelling Cypris. Utricularia vulgaris was free-floating and caught the free-swimming copepods. Withycombe (1924) saw a tadpole held with its head in one bladder and its tail in another. He also noted the presence of small molluscs and a colonial polyzoan.

Gibbs (1929) and Darwin (1875) both noticed that lower organisms remained alive longer. Gibbs was especially curious as to why Euglena remained alive and reproduced itself in older bladders, asking "what are the digestive enzymes of the bladders and is secretion stimulated when animals are trapped?" He thought Euglena was either immune to the bladder enzymes or that the traps were too old to be functional.

Hada (1930) stated that the animals captured by Utricularia are killed chiefly by the mechanical power of compression of the bladder. Smaller organisms remained alive longer, and following the death of any animals there is an increase in number of bacteria. Schumacher (1960) observed 308 desmids in 50 randomly sampled bladders representing 25 taxa.

These examples demonstrate that a wide variety of organisms are trapped indiscriminately. However, the perplexing question still

remains as to why some organisms die while others remain alive and reproduce within the bladders.

Digestion and Need for Animal Nutriment

Darwin (1875) was the first to investigate the value of digested products of Utricularia. He pushed minute fragments of roasted meat, small cubes of albumen, and pieces of cartilage through the bladder orifice and left them there for 1 to 3½ days. The bladders were then cut open and their contents examined. He saw no signs of digestion or dissolution and concluded Utricularia could not digest the animals it captured. Cohn (1875) agreed with Darwin because he observed that some animals remained alive for days and, therefore, no "poisonous" substance could be present in the bladders.

Treat (1875) came to the opposite conclusion and stated that in less than two days after a large animal was trapped the "fluid contents of the bladders began to assume a cloudy or muddy appearance, often became so dense that the outline of the animal was lost to view." She suggested that this indicated the possible presence of a "ferment hastening" secretion which would cause rapid decay.

Arber (1920) and Sculthorpe (1967) both stated that no digestive enzyme has been demonstrated and that the animals probably undergo bacterial decay. In contrast, Luetzelburg (1910) was able to identify benzoic acid in the bladders. He believed that it inhibited bacterial decay and consequently digestion of the trapped animals. Therefore, digestion must be accomplished by a secretory

product in the bladders. This supported Gibbs' (1929) earlier observation that the death of a "worm" takes place in a couple of hours or less than a day. He considered this far too rapid for bacterial action to be responsible. No solution to the actual process of animal "digestion" has been reached to date.

With the fact established that animals were trapped by Utricularia and that at least some of them were digested or decayed, other questions were raised. Of what benefit was animal nutriment to the plant and what specific elements of the animals were used by the plants? Pringsheim and Pringsheim (1962) demonstrated that U. exoleta would grow vegetatively in an inorganic medium, but flowers were only produced when meat extract and peptone were added to their culture media. Pringsheim and Pringsheim (1967) tried the same experiments with U. minor and U. ochroleuca but found they could only get good vegetative growth by adding peptone and beef extract. They could never get flowering.

Sorenson and Jackson (1968) fed paramecia to U. gibba growing on a near optimal inorganic medium and no changes in growth were observed compared to controls not fed paramecia. However, when they fed paramecia to plants growing on complete but poorly balanced inorganic media, or plants deficient in either magnesium (Mg) or potassium (K), they got an increase in both total number and length of internodes. They also noted an increase in the number of bladders in fed plants. The results of their investigation suggested that

animals captured by Utricularia under natural conditions provide significant quantities of inorganic elements, such as Mg and K, as well as unknown organic compounds required by the plant.

Lollar, Coleman, and Boyd (1971) fed labelled ostracods, using ³²phosphorous, to U. inflata to determine if phosphorous contained in trapped animals was absorbed by the bladders and translocated to the leaves and stems. They found that radiophosphorous uptake was greater in plants with bladders than in plants without bladders, indicating that phosphorous in the animals was absorbed by the plants. Although only trace amounts were involved, they considered the mechanism important in nutrient-poor habitats such as oligotrophic lakes and ponds.

Studies of the Bladder Ontogeny and Anatomy

Bladder ontogeny

Pringsheim (1869) was the first to illustrate and discuss the apical development of Utricularia. He showed some of the earlier stages of bladder ontogeny for U. vulgaris, noting the presence of trichomes on the surfaces of the shoots and bladders, the coiled appearance of the shoot tip, and the formation of the bladder inner wall by division and invagination of cells which divided off from the shoot.

Darwin (1875) also investigated the development of U. vulgaris. He observed that in early autumn the floating stems formed large buds which sank to the bottom and remained dormant throughout the

winter. This was also observed by Benjamin (1848) and Glück (1906). The young leaves which formed the bud bore various stages of early bladder development. At the earliest stage Darwin observed, the young "leaves" had broad flattened segments, which bore young bladders that appeared to be formed by an oblique folding over of the apex and margin of the "leaf." The bladders were circular in outline with a narrow, almost closed, transverse orifice which lead to a lumen filled with water. He noted that the orifices faced inward toward the main plant axis when the bladders were young and then turned at a right angle to that position as they matured. The young exterior bladder surface was covered with "papillae" of different sizes, many of which were elliptical in shape. Darwin considered the "valve" (door) and "collar" (pavement epithelium) to be extensions of the bladder wall. Trichomes were detected on the door quite early, along with minute cellular projections he called the "antennae." Quadrifids and bifids were not observed until the bladders reached mid-development.

Meister (1900) stated that the turions (winter-buds) were immune to depredation by aquatic herbivores such as snails. He assumed this was because of their surface coat of spines on the "leaves" and the coat of mucilage on the entire plant surface.

Meierhofer (1902) carried out the most extensive ontogenic study to date. Unfortunately, his work on U. vulgaris had received little attention in previous reviews of the literature. He noted the presence of two types of trichomes on the surfaces of the shoots

and bladders, the coiled appearance of the shoot tip, and demonstrated that the inner walls of the bladders were formed by a division and invagination of the shoot epidermal cells. Meierhofer illustrated a series of young bladder developmental stages showing the continued invagination of cells to form the door and pavement epithelium, the early development of the antennae, and a developmental sequence for the quadrifids and bifids. He further observed that young bladder surfaces were covered with mucilage glands but more mature bladders had smaller "knopfartigen" (button-shaped) glands.

Arber (1920) described winter-bud formation in U. vulgaris, but not bladder development. She generally agreed with the earlier works discussed previously but made a few additional observations. Winter-buds were formed under normal conditions between the first of August and mid-November. However, they could be induced to form at any time under poor nutritive conditions. The parent plant became "water-logged" and sank to the bottom, carrying with it the winter-buds. Because the winter-buds were lighter than water they would float if not pulled down by the parent plant. The winter-buds remained at the bottom of the pools throughout the winter with their apices pointed upward. In the spring, the winter-buds were able to rise to the surface because the parent plant had rotted away during the winter to nothing but the vascular cylinder. The young shoot tip could be found coiled up deep within the winter-bud surrounded by the protective leaves. The protective leaves of the winter-bud were

firmer than the other vegetative leaves of the plant and had a more conspicuous development of glandular trichomes.

Antennae

Darwin (1875) described the antennae as "prolongations" of the bladder. As mentioned earlier, he believed the bladder had the appearance of a crustacean and hence gave these structures the name "antennae." The term is one of the few to remain unaltered over the years in the literature. Meierhofer (1902) illustrated a young developing antenna. He described it as composed of many short cells rich in protoplasm. Withycombe (1924) stated that the antennae are long, branched processes which arise from the dorsal "corners" of the aperture. They bent down and out and made a funnel-like region to lead prey toward the bladder aperture. Lloyd (1942) noted that the branched antennae varied considerably among different species. They were long and slender in some species, and much reduced, or absent, in others.

Wall cells

Darwin (1875) described the walls as green and two cell layers thick. The exterior layer was made up of larger, polygonal cells with outer surface "papillae" (trichomes) arising at the angles between the cells. Compton (1909) agreed with Darwin except that he said the wall was composed of "about three cell layers" with the middle layer being somewhat disorganized.

Merl (1921), Czaja (1922), Nold (1934), and Lloyd (1936)

believed the wall cells acted as glandular tissue, and pumped water from the interior of the bladder to the outside.

Gibbs (1929) stated that the wall cells varied from being almost colorless to delicately green to dark blue with age. The blue color was attributed to anthocyanin. He agreed with Darwin (1875) that the bladder was two cell layers thick except for the prolongation of the vascular tissue of the stalk along the ventral and dorsal region of the bladder and the massive pavement epithelial layer.

Slinger (1954) also observed the bladder walls to be two cell layers thick except in the pavement epithelial layer. He further noted that the entire plant surface, including the bladders, was highly cuticularized.

Vascular tissue

Darwin (1875) described the vascular tissue as a bundle of vessels, formed of simple elongated cells, which progress through the short footstalk and divide at the base of the bladder. One of the branches of the vascular strand extends up the middle of the dorsal surface, and the other strand runs through the middle of the ventral surface. The latter divides again beneath the pavement epithelium and its branches traverse on each side to where the door unites with the pavement epithelium. Compton's (1909) description of the vascular tissue was similar to Darwin's except that he noted that the vascular strand consists of phloem but no xylem.

Chandler (1910) and Arber (1920) both observed spiral elements

in the center of the vascular strand but considered the vascular tissue weakly developed. Arber called the tracheary elements "imperfect vessels." The conducting elements were incomplete and she felt this was consistent with their relative unimportance for transpiration in a rootless submerged aquatic. The central vascular cylinder was observed as being surrounded by a lacunar cortex.

Slinger (1954) did not observe the branching described by earlier investigators and merely stated that a single vascular strand passed into the bladder via the stalk. It ran dorsally in the wall layers without branching and terminated in the upper door region.

Outer surface trichomes

Darwin (1875) reported that the surface of young bladders was covered by trichomes of different sizes, many of which were elliptical in shape. These trichomes arose at the angles between the exterior wall cells and consisted of short conical projections surmounted by two hemispherical cells. These cells appeared in close apposition and united. However, when he immersed them in various solutions they often separated. Darwin, along with Compton (1909), considered these trichomes to be identical with those on the shoots and leaves.

Withycombe (1924) and Gibbs (1929) observed that all of the trichomes were similarly constructed. Gibbs (1929) said that the fundamental three-celled unit consisted of "basal, stalk, and

head cells."

Slinger (1954) and Sculthorpe (1967) described the bladder surface as covered with scattered spherical mucilage glands. Lloyd (1942) indicated that these spherical trichomes were devoid of a cuticle.

Door

Cohn (1875) described the door as composed of two layers of small cells which were continuous with the wall cells of the bladder. Darwin (1875) observed that the door sloped into the cavity of the bladder and was attached to the bladder on all sides except at its posterior margin. This edge was free and formed one side of the slit-like orifice leading into the bladder. The free edge rested on the edge of the pavement epithelium. The door could only open inwardly, was colorless, transparent, elastic, and convex in the transverse direction. Compton (1909) agreed that the door was two cell layers thick and outwardly convex. Additionally, he observed that the cell walls of the door were thicker than those of the remainder of the bladder.

Withycombe (1924) described the door as continuous with the dorsal wall of the bladder, consisting of two cell layers thick, and having three ill-defined regions. The upper dorsal region of the door was composed of similar cells, was slightly concave when viewed from the outside, and had numerous club-shaped trichomes which arose from the sides. The middle region bulged slightly outward.

The inner wall regions of these latter cells have ingrowths which project into each cell's cytoplasm. The inner layer of the door was composed of larger, thin-walled cells which structurally would allow an inward movement of the door. In the region surrounding the trigger trichomes, and radiating out from it to the free edge of the door, was a region Withycombe termed the "principal motor tissue." The cells of this region had thicker walls than other cells of the door and had many ridges projecting into the cells. Although the other cells of the middle region possessed these projections, they were not as extensive.

Gibbs (1929) reported that the intact door had a much different shape than after it was removed or damaged. His other observations were much the same as those of previous investigators.

Lloyd (1936) described the door as longer than the pavement epithelium on which it rested, which caused the door to buckle and appear convex. He further observed that the door rested in a "groove" in the pavement epithelium. Slinger (1954) agreed with this and divided the door outer surface into two regions, an "upper glandular" one and a "lower nonglandular" one.

Trigger trichomes

Darwin (1875) described four bristles that projected from the door as transparent and about as long as the door itself. These bristles arose from the posterior free margin of the door and pointed obliquely outwards in the direction of the antennae.

Merl (1921) sectioned the door longitudinally and illustrated the trigger trichomes as extensions of four of the outer door surface epidermal cells. They were initially filled with protoplasm but later appeared empty.

Withycombe (1924) reported that the trigger trichomes were four in number, bicellular, and tapered to a point. They developed from outgrowths of the epidermal cells of the door and were sensory, causing the bladder to respond.

Hada (1930) described the trigger trichomes as a double pair of bristles which projected out of the door at near right angles. He believed they were so transparent that animals never recognized them.

Door surface associated trichomes

Darwin (1875) observed three types of trichomes on the door surface. Those located around the anterior margin of the door were very numerous and crowded together. Each trichome had an "oblong head cell" on a "long pedicel" which consisted of an elongated cell and a shorter cell which adjoined the head cell. The trichomes near the free posterior margin of the door were much larger, fewer in number, and almost spherical. These trichomes had short pedicels and two head cells each. The third type of trichome had transversely elongated heads, and short pedicels, so that they were parallel and close to the door surface. He called these latter trichomes "two-armed" glands and they were different from his two-armed "bifids."

Darwin believed that all trichomes on the door were absorptive glands. He doubted that they ever secreted anything.

Goebel (1891) briefly noted the presence of trichomes on the door and around the aperture. He stated that these secreted mucilage which attracted animals to the bladders. Meierhofer (1902) illustrated the door surface trichomes in face view, side view, in longitudinal section, and transverse section. He described the surface trichomes as "Köpfchendrusen" (capitate trichomes) which consisted of three cells the "Basalzelle" (basal cell), "Gelenkzelle" (hinge cell), and "End-oder Köpfchenzelle" (end or head cell). He believed that these trichomes secreted something. Luetzelburg (1910), Hada (1930), and Sculthorpe (1967) all agreed that they secreted mucilage and sugar to attract animals.

Withycombe (1924) observed that numerous "club-shaped" trichomes arose from the side of the concave region of the door. Again he noted these consist of three cells; "an elongated stalk cell, short square middle cell, swollen distal club-shaped cell." Similar but less numerous trichomes were located around the free edge of the door. Three or four peculiar club-shaped trichomes were described near the trigger-trichomes. The terminal cell of these latter trichomes was larger and almost spherical. The other club-shaped trichomes were more exterior, smaller, and not spherical.

Gibbs (1929) merely described the door surface trichomes as long and slender with three cells, basal, stalk, and head cells. Slinger (1954) indicated that the numerous door surface trichomes

consisted of four cells. The lowermost or "wall cell" was an elongation of an epidermal cell. This was followed by a "basal cell, a narrow cylindrical middle cell, and a capital glandular cell."

Pavement epithelial trichomes

Darwin (1875) called the pavement epithelium a "rim or collar." It dipped deeply into the bladder and was covered by numerous trichomes. Those trichomes along the sides were identical to the trichomes on the adjoining door. He stated that the pavement layer was formed by an inward projection of the bladder walls. The brownish outer cells facing the door had rather thick walls, and were minute, very numerous, and elongated. Beneath these trichomes the cells were continuous with those of the whole inner bladder surface. The space between the inner and outer surface consisted of "coarse cellular tissue." The inner side of the pavement epithelial layer was covered with delicate bifid processes.

Compton (1909) indicated that the pavement epithelium was quite massive in comparison to the remainder of the bladder tissue and provided firm support for the door. Withycombe (1924) described the pavement epithelium as a semicircular pad of large parenchymatous cells. The entire pavement layer consisted of three cell layers with thick walled elongate cells. The cells were not fused to one another but were merely in close proximity. He defined the three layers as an "upper columnar layer, middle layer of smaller cells, and basement layer." The middle region of the pavement

epithelium was specialized for mucilage secretion, which he demonstrated with hematoxylin stain. He observed a groove just in front of these cells, in which the door rests. This groove was as wide as the middle layer of the pavement epithelium.

Gibbs (1929) referred to the pavement epithelium as a "doormat" composed of larger cells than those of the rest of the bladder. It was rigid, immediately under the door, and merged gradually with the lateral walls in an upward sweep.

Lloyd (1942) described a "threshold" covered by the pavement epithelium which was composed of sessile cells that secreted mucilage. The velum was attached to its outer margin.

Velum

Lloyd (1929a) and Gibbs (1929) are the only investigators to observe the velum intact. Lloyd first observed its presence and Gibbs, later the same year, agreed that he had seen a "veil." Slinger (1954) observed what he thought was part of the velum. Lloyd (1929a) stated that the upper surface of the pavement epithelium was composed of closely packed cells which "exfoliated" their cuticles to form a "veil" which was pressed against the door edge. Lloyd (1936) indicated that water pressure pressed the velum more firmly against the door edge to create an effective seal. When he cut the velum the trap could not reduce the volume of water in the bladder. In Lloyd's (1942) later work he described the velum in greater detail as a thin transparent membrane formed by the cuticles

shed from the "capital cells" of the pavement epithelium. The cuticles remained attached to one another and to the "capital cells" of the outer zone of the pavement epithelium. Only the cuticles of the outer and middle regions of the pavement layer form the velum, while those of the inner zone acted separately. These enlarged and burst, forming a cushion of "balloon-like" tissue which blocked the small gap between the door edge and pavement epithelium.

Quadrifid and bifid trichomes

The quadrifid trichomes were first observed by Crouan (1858), who thought they were root hairs. He suggested that the bladders protected these fragile organs from damage by direct light and crustaceans.

Darwin (1875) first named them "quadrifids" and "bifids." In very young bladders these processes consist of a conical protuberance, which narrows into a very short stalk topped by minute cells. They resemble the outer surface trichomes but are more prominent. The two terminal cells first became much elongated in a line parallel to the inner bladder surface. Then each is divided by a longitudinal partition. Soon after this the two "half-cells" separate from one another and form four cells. He stated that since there was no space for the two new cells to increase in breadth in their original plane, one slides partially under the other. Their manner of growth then changes and their outer sides, rather than the apices, continue to grow. The two lower cells which slide partly beneath the upper

two cells form the longer, more upright arms of the quadrifid, whereas the two upper cells form the shorter, more horizontal arms. The bifid processes develop in the same manner except that the two terminal cells never divide and only increase in length.

Darwin (1875) observed that the longer cells of the quadrifids are always directed toward the posterior end of the bladder and the shorter cells toward the anterior end of the bladder. The walls of these cells are composed of a thin transparent membrane and can be bent in any direction without being broken. They are lined with a thin layer of protoplasm and each arm contains a minute, faintly brown particle that is either round or elongated and constantly exhibits Brownian movement. These were observed in young quadrifids when they were only about one third their mature size. Although Darwin admitted these particles did not resemble ordinary nuclei, he believed they were nuclei.

Darwin (1875) investigated the absorptive nature of the quadrifid and bifid processes and found they absorbed decayed matter. He observed clean bladders and saw nothing in the processes except the "modified nucleus." In those bladders that contained decayed matter, the quadrifids contained transparent or yellowish spherical or irregularly shaped masses of matter. These masses become confluent and then separate again. He suggested that this matter was protoplasm generated by the absorption of nitrogenous matter from the decayed animals. He further used ammonium nitrate to demonstrate that the quadrifids were absorptive rather than secretory.

Cohn (1875) suggested that the quadrifids withdrew water from the bladder lumen at a faster rate than it could pass in through the wall cells. If this were not the case, he felt, the bladders could not have responded so quickly.

Goebel (1891) observed that the inner epidermis of the bladder had a cuticle, except for the four cells atop the quadrifid trichomes. He noticed oil droplets in the quadrifids of bladders containing decayed animals but no oil droplets in those which were free of digested material. Therefore, he assumed that the droplets were derived from the absorption of decayed matter.

Meierhofer (1902) illustrated the developmental sequence for the "vierstrahlige Drüsen" (four-radiating trichomes; i.e., quadrifids). Some of the inner epidermal cells elongated and protruded beyond the normal limits of the epidermis. These cells then divided again, producing cells directly above the elongated epidermal cells. The upper cells then underwent two further divisions to form four terminal cells which became much elongated. He was only able to observe the basal cells and the four-armed upper cells.

Brocher (1911) observed the bifids which he called "rhizoids." He believed they were responsible for absorption of the inner contents of the bladder but was never able to prove his theory. Merl (1921), Czaja (1923), and Benjamin (1924) accepted this theory but were also unable to provide any proof for it.

Withycombe (1924) was the first to actually present any experimental evidence that the quadrifids removed water from the bladder

lumen. He noted that the quadrifids constantly absorbed fluid from the bladder interior, whether it was pure water or an infusion of decayed animals. Methylene blue placed in the bladder lumen was absorbed rapidly by the quadrifids, whereas the other regions of the bladder took several days to absorb it.

Gibbs (1929) described the quadrifids as digestive glands or water-absorbing hairs composed of four radiating head cells, a stalk cell, and basal cell. The bifids were similar but located only on the back of the pavement epithelium and had just two head cells.

Lloyd (1942) agreed with Gibbs' (1929) description but added that the "capital" (terminal) cells were devoid of cuticle whereas the rest of the quadrifid had a cuticle. He also felt that they removed water from the bladder lumen.

Slinger's (1954) report of these trichomes for U. transrugosa is much the same as that reported by earlier workers except that the four arms of the quadrifids were all of equal length. They were also closely appressed to one another rather than spread apart as reported in other species.

Kurz (1959) observed the developmental sequence of the quadrifids under normal conditions and after colchicine and kinetin treatment. She was able to produce various freakishly shaped "quadrifids" with only two or three arms of unusual length and width dimensions.

Abel and Denffer (1961) investigated the relationship of organ polarity to quadrifid orientation and development. They

found that the long arms of the quadrifids were always oriented toward the point where the stalk attached to the bladder. They compared the bladder to a peltate leaf, and believed that the cell polarity and organ polarity of the bladder inner wall were always related.

MATERIALS AND METHODS

Field, Taxonomic, and Statistical Methods

Plant collection

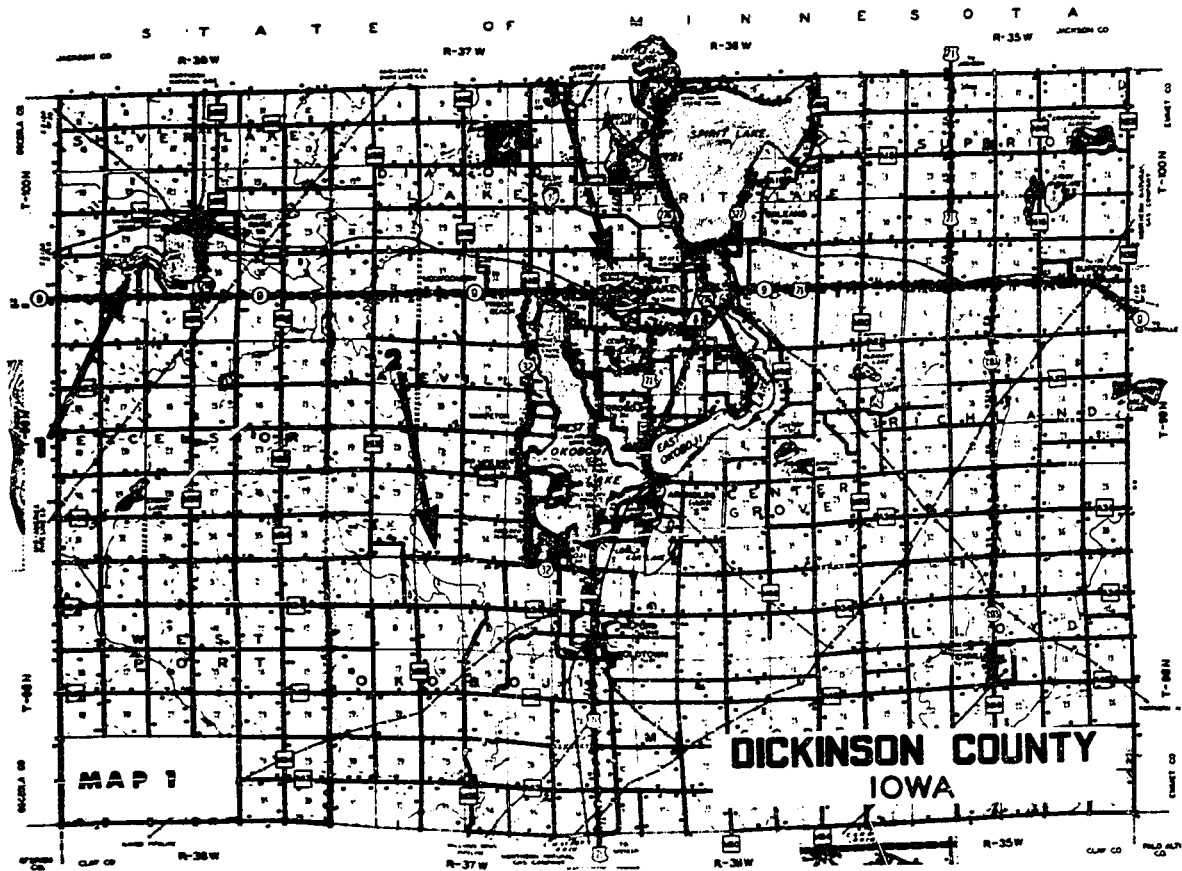
Utricularia macrorhiza Le Conte was collected from four Iowa aquatic habitats at specific intervals throughout the summer months of 1972 and 1973. The collection sites were: (1) Silver Lake Fen on the west edge of Silver Lake, Dickinson County (Map 2, D; Map 1, arrow 1, T-100N, R-38W); (2) Three-Corners Pond, a small farm pond, Dickinson County (Map 2, D; Map 1, arrow 2, between T-98N and T-99N); (3) the boat launching area on the shore of Jemerson Slough, Dickinson County (Map 2, D; Map 1, arrow 3, between T-99 and T-100N, R-36W and R-37W); (4) a small ditch at the north end of the south camping area road at Spring Lake, Green County (Map 2, G; Map 3, arrow 4, T-84, R-30W). The plants were collected (Appendix B, Table B1) and the water monitored for temperature, depth, total hardness, iron, pH, ortho-phosphate, turbidity, and color using a Hach DR-EL Direct Reading Engineer's Laboratory (Appendix B, Table B2).

Taxonomy

Pressed and formalin-alcohol-acetic acid (FAA) preserved specimens were sent to Dr. Peter Taylor, Royal Botanic Gardens, Kew, England for identification. All specimens were identified as U. macrorhiza in agreement with Le Conte's (1823) description. Voucher specimens from each collection site have been deposited

Map 1. Dickinson County, arrow 1 points to location of Silver Lake Fen, arrow 2 to location of Three Corners Pond, and arrow 3 to location of Jemmer-son Slough

Map 2. State of Iowa map with Dickinson County located at D and Green County located at G



Map 3. Green County, arrow 4 points to location of
Spring Lake

in the Iowa State University Herbarium (ISC: CKB-U26, U28, U29, U33, U34, U35, U36).

Transplants

Transplants were made from each location to the other locations. Three types of containers were used to isolate and maintain the plants. The first type of container, used in 1972, consisted of a screen rectangle anchored in the substrate by wooden stakes attached to each side. Small screen cylinders with closed bottoms and tops and anchored by stakes were also used in the same year to contain transplanted winter-buds. Flooding in the early Spring of 1973 made the rectangular containers completely ineffective and only the winter-buds in the small cylinders were salvaged. A third type of container was therefore constructed, consisting of a screen cylinder open at the top end and surrounded by a flat rectangle of green styrofoam (Fig. 58). Each floating container was anchored to a brick by a long nylon rope so that the container could fluctuate with the water level.

Statistical analysis

From June, 1972 to August, 1973, field data for water chemistry and plant characters were tabulated (Table 1, 2) and sent to Dr. Richard Pimentel, a biometrician, at California Polytechnic State University, San Luis Obispo, for statistical analysis. The method used was the principal component analysis. There were 21 variables and 28 subjects used in the correlation matrix analysis. The program

Table 1. Coding used for statistical and field data

3-CORN	3-Corners Pond
SILFEN	Silver Lake Fen
SPRING	Spring Lake
SLOUGH	Jemmerson Slough
TIME	Time from Spring to Fall
HARDNESS	Total hardness in the water ppm
TEMP	Temperature in degrees C
PHOSPH	Orthophosphate in the water ppm
IRON	Iron in water ppm
SULFAT	Sulphate in water ppm
PH	pH of water
TURBID	Water turbidity in Jackson Turbidity Units
COLOR	Water color
BLADCL	Bladder color on a scale of red to blue ^a
BLADE	Maximum number of bladders per branch
BLADSZ	Maximum bladder size
BRANCH	Maximum branch length
FNBLAD	Functional bladders
FLOWER	Flowering present or absent
WINBUD	Winter-bud present or absent
DEATH	Dead plants present or absent

^aRed = 4; brown = 3; green = 2; blue = 1; missing = 0.

Table 2. Field data

Jemmerson Slough (SLOUGH)

TIME	HARDNESS	TEMP	PHOSPH	IRON	SULFAT	PH	TURBD	COLOR
6/23/72	220	25	0.40	0.03	5.0	7.1	24	150
7/7/72	200	27	0.08	0.01	9.0	8.4	20	175
7/21/72	200	27	0.10	0.15	2.0	8.2	30	120
8/15/72	100	20	0.15	0.20	3.0	7.4	30	90
6/15/73	230	28	0.30	0.05	8.0	8.0	30	110
7/18/73	110	25	0.55	0.05	10.0	7.5	25	100
8/9/73	160	28	0.22	0.08	5.0	8.6	120	400

TIME	BLADCL	BLADE	BLADSZ	BRANCH	FNBLAD	FLOWER	WINBUD	DEATH
6/23/72	1	16	2mm	2cm	+	+	-	-
7/7/72	1	16	5mm	2½cm	+	+	-	-
7/21/72	1	6	2½mm	2½cm	+	+	-	-
8/15/72	1	12	3mm	3cm	+	-	-	-
6/15/73	2	16	2mm	2cm	-	-	-	-
7/18/73	1	12	2½mm	3cm	+	+	-	-
8/9/73	0	0	0	0	-	-	-	+

Table 2. (continued)

Spring Lake (SPRING)

TIME	HARDNESS	TEMP	PHOSPH	IRON	SULFAT	PH	TURBD	COLOR
6/23/72	210	26	0.20	0.00	230.0	8.3	25	85
7/7/72	230	26	0.15	0.28	130.0	7.0	15	100
7/21/72	250	24	0.04	0.20	98.0	6.9	15	130
8/15/72	250	24	0.10	0.18	120.0	7.2	17	100
6/15/73	180	34	0.20	0.05	65.0	8.6	20	70
7/18/73	170	26	0.20	0.05	70.0	7.6	40	60
8/9/73	150	25	0.18	0.00	75.0	8.0	50	160

TIME	BLADCL	BLADE	BLADSZ	BRANCH	FNBLAD	FLOWER	WINBLAD	DEATH
6/23/72	1	8	2½mm	2cm	+	+	-	-
7/7/72	1	10	2mm	2cm	+	+	-	-
7/21/72	1	10	2mm	2cm	+	+	-	-
8/15/72	3	0	0	0	-	-	+	-
6/15/73	1	6	1½mm	1½cm	+	+	-	-
7/18/73	1	6	1½mm	1½cm	+	-	-	-
8/9/73	0	0	0	0	-	-	-	+

Table 2. (continued)

Three Corners Pond (3-CORN)

TIME	HARDNESS	TEMP	PHOSPH	IRON	SULFAT	PH	TURBD	COLOR
6/23/72	170	24	0.47	0.30	8.0	6.5	15	70
7/7/72	170	23	1.20	0.90	18.0	6.9	100	350
7/21/72	150	29	0.18	0.10	5.0	7.1	15	80
8/15/72	120	22	0.20	0.30	2.0	7.1	10	70
6/15/73	170	27	1.10	0.15	15.0	8.1	20	75
7/18/73	160	26	0.55	0.08	15.0	7.2	50	150
8/9/73	150	26	0.40	0.05	5.0	8.1	20	100

TIME	BLADCL	BLADE	BLADSZ	BRANCH	FNBLAD	FLOWER	WINBUD	DEATH
6/23/72	1	8	2mm	2cm	+	+	-	-
7/7/72	1	8	2mm	2cm	+	+	-	-
7/21/72	1	6	2 $\frac{1}{2}$ mm	2cm	+	-	-	-
8/15/72	1	10	2 $\frac{1}{2}$ mm	2 $\frac{1}{2}$ cm	+	-	-	-
6/15/73	2	8	2mm	2 $\frac{1}{2}$ cm	-	-	-	-
7/18/73	1	8	2 $\frac{1}{2}$ mm	2 $\frac{1}{2}$ cm	+	+	-	-
8/9/73	1	10	2 $\frac{1}{2}$ mm	3cm	+	-	-	-

Table 2. (continued)

Silver Lake Fen (SILFEN)

TIME	HARDNESS	TEMP	PHOSPH	IRON	SULFAT	PH	TURBD	COLOR
6/23/72	1320	20	0.30	0.18	1300.0	7.2	20	80
7/7/72	1220	25	0.25	0.09	1750.0	7.5	0	10
7/21/72	1800	33	0.18	0.03	1000.0	7.7	10	70
8/15/72	700	20	0.10	0.10	1075.0	7.4	0	30
6/15/73	1300	33	0.10	0.01	2400.0	8.3	25	70
7/18/73	1250	25	0.12	0.10	1820.0	8.1	10	50
8/9/73	1100	30	0.19	0.05	2800.0	8.0	10	30

TIME	BLADCL	BLADE	BLADSZ	BRANCH	FNBLAD	FLOWER	WINBUD	DEATH
6/23/72	2	12	2mm	1½cm	-	-	-	-
7/7/72	4	12	2mm	1½cm	-	-	-	-
7/21/72	4	12	2mm	1½cm	-	-	-	-
8/15/72	4	12	2½mm	2cm	-	-	-	-
6/15/73	4	12	2mm	1½cm	-	-	-	-
7/18/73	4	12	2½mm	2cm	-	- (+)	-	-
8/9/73	4	12	2½mm	2cm	-	-	-	-

Table 3. Component correlations--components are columns, variables are rows

Row	1	2	3	4	5
3-CORN	-0.411	0.318	-0.440	0.337	0.562
SILFEN	0.918	0.002	-0.153	0.231	-0.158
SPRING	-0.212	-0.397	-0.075	-0.757	0.061
SLOUGH	-0.294	0.077	0.667	0.190	-0.465
TIME	0.082	-0.501	0.276	0.260	0.534
HARDNESS	0.889	-0.027	-0.138	0.193	-0.161
TEMP	0.236	-0.245	0.410	0.019	0.411
PHOSPH	-0.409	0.209	-0.380	0.563	0.213
IRON	-0.433	0.223	-0.655	0.299	-0.209
SULFAT	0.885	-0.040	-0.082	0.210	-0.057
PH	0.219	-0.396	0.690	0.172	0.222
TURBID	-0.595	-0.478	0.028	0.533	-0.179
COLOR	-0.667	-0.353	0.049	0.471	-0.324
BLADCL	0.913	0.050	-0.227	0.085	-0.031
BLADE	0.375	0.754	0.265	0.157	-0.228
BLADSZ	0.118	0.844	0.338	0.089	-0.000
BRANCH	-0.084	0.893	0.219	0.133	0.229
FNBLAD	-0.670	0.538	0.154	-0.289	0.109
FLOWER	-0.538	0.404	0.159	-0.235	-0.204
WINBUD	0.010	-0.363	-0.404	-0.435	-0.111
DEATH	-0.254	-0.803	0.178	0.240	-0.106
VARIANCE	5.777	4.491	2.518	2.311	1.501
% VARIANCE	27.5	21.4	12.0	11.0	7.1
CUM %	27.5	48.9	60.9	71.9	79.0

also included eigen vectors and component correlations (Cooley and Lohnes, 1971; Table 1,3). Only the print-out results of the latter method is illustrated as this method provided the most error-free information.

Laboratory Methods

Anthocyanin determination

The bladders of Utricularia macrorhiza were pigmented blue at Spring Lake, Jemmerson Slough, and Three Corners Pond, and red at Silver Lake Fen. These pigments were microscopically localized in the vacuoles and assumed to be anthocyanin. However, in order to verify experimentally that the pigments were anthocyanin, the following procedure, modified from Harborne (1964), was carried out.

One hundred dark blue bladders were removed from plants collected at Three Corners Pond. These bladders were extracted with 0.5 ml hydrochloric acid (HCl) in absolute ethanol (ETOH), 97:3 volume/volume or 0.5 ml HCl and methanol (MeOH), 97:3 volume/volume. Both extractions were then centrifuged for use in cytophotometry. Cytophotometry was done with a Leitz Microspectrophotometer fitted with an in-line monochromator, xenon light source, and galvanometer calibrated for transmission/absorption.

The eluted pigment was placed in a depression slide and coverslipped to keep the solution from evaporating and becoming more concentrated. Because anthocyanins absorb between 500 and 550 m μ in methanolic HCl, the wavelength was set at 500 m μ .

The depression of the slide was moved out of the field, a blank reading taken, and the galvanometer zeroed. The depression was then moved into place and a reading taken for transmittance of the solution. Readings were recorded at the following increments: 510 m μ , 515 m μ , 520 m μ , 522 m μ , 524 m μ , 525 m μ , 530 m μ , 540 m μ , 550 m μ , 560 m μ , and 570 m μ . The instrument was re-zeroed after each reading.

To further substantiate the presence of anthocyanin, paper chromatographic techniques were used. Four hundred blue bladders were dissected from plants collected at Three Corners Pond. These were ground with a mortar and pestle, extracted with 1.0 ml MeOH-HCl (97:3 volume/volume), and centrifuged.

Six-tenths (0.6) ml of the extract was applied to Whatman no. 3 filter paper. The chromatogram was equilibrated in a chromatography tank for 24 hours with the lower phase of a mixture of butanol-acetic acid-water (4:1:5, by volume). It was developed descendingly with the upper phase of the same mixture and then turned and developed with water-acetic acid (85:15, volume/volume). Ten separate runs were done using the above procedure. The R_f values (= the distance traveled by a substance relative to the distance of the solvent traveled) were determined for butanol-acetic acid-water, and the percent error determined. An ultraviolet (UV) chromatography light source was used to determine the anthocyanin spots on the chromatography paper. These spots appeared dark purple-black under UV.

Light microscopy (LM)

The developmental anatomy of the bladders, winter-buds, and other specialized vegetative propagules was observed using 10 μ m thick paraffin sections (Appendix C, Table C1, C2) and 1 μ m thick plastic sections of material fixed and embedded for electron microscopy (Appendix C, Table C7). Paraffin sections were cut on a rotary microtome and stained with safranin-fast green (Jensen, 1962) or with certain histochemical stains. Plastic sections were cut with glass knives, stained with Paragon dye mixture (Paragon C. & Co. Inc., Bronx, N.Y.; Spurlock Skinner, and Kattine, 1966), and mounted in Permount (Fisher Scientific Company, Fairlawn, N.J.). Whole and cut unfixed fresh materials were also observed.

Histochemistry using paraffin-embedded or fresh material included: total water insoluble carbohydrates using the periodic acid-Schiff reaction (Appendix C, Table C3); nucleic acids--azure B with RNAase extraction (Appendix C, Table C4); acridine orange fluorescence technique (Appendix C, Table C5); and callose--aniline blue-fluorescence technique (Appendix C, Table C6).

Photographs were taken on a Leitz Ortholux microscope fitted with an Orthomat Camera and phase-contrast or bright-field optics. Kodak Panatomic-X film (bright field and phase-contrast) or Tri-X or High Speed Ektachrome film (polarized light, dark field, fluorescence) was used for the LM photography and developed in Kodak Microdol-X (1:3 dilution).

Transmission electron microscopy (TEM)

Bladders and winter-buds were fixed in 2-3% glutaraldehyde, buffered in 0.025 M phosphate buffer or filtered pond water, pH 7.0-8.0 at 4 C, for 12 hr. The plant material was then washed in buffer and post-fixed in buffered 1.0% osmium tetroxide (Stevens Metallurgical Corp., New York, N.Y.) for 1 hr at room temperature. This was followed by a buffer rinse, dehydration in a graded acetone series, and embedding in Araldite-Epon (Appendix C, Table C7). Best results were obtained when the water in which the plants were found growing in the field was used as a buffer solution, as hard water lakes have an excellent buffering capacity. All of the plants used were from hard water habitats (Table 2).

Plastic sections 50-70 nm thick were cut on a Reichert OM-U2 ultramicrotome, using a DuPont diamond knife. Sections were picked up on 100 mesh Formvar coated grids and stained with 2% methanolic uranyl acetate for 10 min and lead citrate for 10 min (Stempak and Ward, 1964). Grids were observed on a RCA EMU-3F or Hitachi HU-11C electron microscope operated at 50 KV. Micrographs were taken using DuPont COS-7 Cronar film and developed in Kodak D-19 developer (1:1 dilution).

Scanning electron microscopy (SEM)

Material was fixed and post-fixed in the same manner as for TEM. However, dehydration was in a graded ethanol series followed by a graded ethanol-Freon TF series (Appendix C, Table C8). Once the material was in pure Freon TF (Polysciences, Inc., Warrington,

Pa.) it was dried in a critical point apparatus using liquid carbon dioxide as the transitional fluid. Dried material was left whole or cut and mounted on brass stubs with silver tape and paint. The material was then coated with about 10 nm carbon and 30 nm of gold using a Varian VE30 vacuum evaporator.

The developmental stages of the bladder were viewed and photographed on the following SEM instruments: JEOL JSM-S-1, Science II or JEOL JSM-U-3, Engineering at Iowa State University, Ames, Iowa; JEOL JSM-35, Boston, Massachusetts; MR-1000A, Boston, Mass.; Kent Cambridge S4-10, Cleveland, Ohio and Morton Grove, Illinois; ETEC Autoscan, Sunnyvale, California; Coates and Welter Cwikscan-104, Sunnyvale, California; and Hitachi HHS-2R, Palo Alto, California. These microscopes were operated at 4, 10, and 25 KV. Micrographs were taken on Kodak Ektapan, Professional Copy 4125 film, or 52 or P/N55 Polaroid film.

OBSERVATIONS

Field Observations

Utricularia macrorhiza was found growing in several Iowa aquatic habitats. All of these habitats had the common feature of providing protection for the plants from wind and wave action. The observations of this investigation were limited to four geographical habitats that appeared different in general morphometry and in plant associations.

Silver Lake Fen

Silver Lake Fen (Map 1, arrow 1; Fig. 5), consists of small, shallow pools located in mounds above and around the actual Silver Lake water level on the southwest side of the lake. Water is supplied to the pools by an underground source. The water is always very cold as it rises to the surface but warms rapidly because of the shallowness of the pools, which are rarely more than four inches deep. The bottom of each pool contains flocculent sludge (Fig. 3); however, the water above the sludge is very clear (Table 2). Silver Lake Fen water is extremely high in amounts of total hardness and sulphate (Table 2), when compared with the other three habitats, and is always low in phosphate and iron (Table 2). The only significant plants associated with U. macrorhiza in the pools are Chara, Utricularia minor, various diatoms, green and blue-green algae. The pools are somewhat protected from wind by a Typha-grass stand at the north end of the fen (Fig. 5) and hills to the east

and south. There is little wave action because of the shallowness of the pools and the presence of grass and Typha stands around them.

Utricularia at the fen rarely flowered and reproduction was predominately vegetative. Only on two occasions were flowers observed. The first flower seen was aborted and the second was observed when the water level of the pools was unusually high. Generally, the plants were floating in such shallow water that they were in contact with the flocculent substrate (Fig. 3), as opposed to, the more usual condition, floating free above the substrate (Fig. 2). Therefore, most bladders observed were filled with this flocculent material which was "sucked in" when the bladders were triggered. This rendered them incapable of trapping again because the bladders could not return to the concave position. As a result of their inability to trap repeatedly, the number of zooplankters trapped, and the amount of animal nutriment available to the plants were greatly reduced. The pools were also limited in variety and number of zooplankters on which to feed. These restrictive factors are apparently significant in the suppression of flowering at the fen because animal nutriment has been shown to be essential in flowering by Pringsheim and Pringsheim (1962).

The plants were typically smaller with shorter internodes and branches, many bladders were present per branch, and the bladders were comparatively smaller (Table 2; Fig. 57). The mature bladders were pigmented with red anthocyanin in the bladder wall cell vacuoles (Fig. 11, 12).

Germination of the winter-buds began in June and new ones were formed in mid-August. In addition, other vegetative propagules were observed throughout the summer months. These were fine white filaments ending in a starch-filled bud protected by "leaf-like" scales with stomates (Fig. 3--arrow, 18, 61).

Jemmerson Slough

Jemmerson Slough (Map 1, arrow 3) had dense stands of Typha around its periphery which provided protection for Utricularia from wind and wave action. The most dense growth of Utricularia macrorhiza occurred in a narrow area that had apparently been cleared for boat launching (Fig. 6). In addition to Typha, there was Myriophyllum, Utricularia minor, Ceratophyllum, Lemna, and various algae growing in association with Utricularia macrorhiza. The water was hard but not nearly as much as at Silver Lake Fen, and it had greater turbidity and color (Table 2). Jemmerson Slough had an abundant supply and variety of zooplankters on which the plants could feed. Flowering was common and began in mid-June and continued through late July (Fig. 1, 6).

Winter-bud formation occurred later than at Silver Lake Fen, in late September or early October of 1972. The filamentous vegetative propagules were also observed. In early August of 1973, no plants were observable in the area. The water surface was densely covered with Lemna and the Typha community had greatly restricted the previous open area.

Plants sampled at Jemmerson Slough prior to August of 1973 were more robust than those of any other habitat. The plant length, branch length, bladder size, and internode length were greater than comparable measurements on plants from the other three habitats (Fig. 54). The mature bladders were pigmented with blue anthocyanin (Fig. 13, 14, 15).

Three Corners Pond

Three Corners Pond (Map 1, arrow 2) is a large farm pond with a wide range of aquatic vegetation. Utricularia macrorhiza was growing in the marginal areas of the pond (Fig. 4, arrow) and associated with Utricularia minor, Sagittaria, Alisma, Sparganium, Myriophyllum, Typha, and several grasses, sedges, and algae. The water was fairly hard but less so than at Silver Lake Fen and was more turbid with greater color than the fen (Table 2).

There was an abundant supply of zooplankters. Flowering was common from early June to late July. Winter-buds were formed during September and October. Filamentous vegetative propagules were occasionally observed but not with the degree of frequency as at the other three habitats.

The plants were not as large as those at Jemmerson Slough but displayed a greater number of dissections (sub-branches) in the branches coming off the central plant axis (Fig. 56). The branches were intermediate in length, between those at Jemmerson Slough and at Silver Lake Fen (Table 2). The mature bladders were pigmented with blue anthocyanin.

Spring Lake

Spring Lake (Map 3, arrow 4) is a man-made lake formed from an old quarry. Utricularia macrorhiza occurred at the west end of the lake along the north shore, at the spring inlet into the lake, and in a ditch adjoining the east end of the lake until the summer of 1971. At that time the lake was poisoned with Diquat, an aquatic weed killer. The only population of Utricularia that survived in substantial numbers was in the small ditch adjoining the lake. The ditch was filled by rain water, rather than with water from the lake. The plants flowered from mid-June to late July in 1972 (Fig. 7, arrow). However, in 1973 the plants only flowered in June and were dead by July 13. The plants at Spring Lake were always comparatively more fragile, limp, and oily to the touch. During the period the plants began to die, the water was brown and stagnant.

Winter-buds were formed in 1972 (Fig. 55, arrow) but not in 1973. The bladders were pigmented blue with anthocyanin at maturity. Bladder size was comparatively small, especially during 1973, and branch length was shorter compared with Utricularia at Jemmerson Slough and Three Corners Pond.

Transplant experiments

The transplant experiments provided inconclusive results with regard to morphological variability. Time and nature became overwhelming obstacles. The first series of transplants failed because

of poor transplant container design. Flooding of the aquatic habitats was not considered as a possibility when the containers were designed and all of the plants floated out the top of the containers during a rainy spring.

The second year the styrofoam floating containers were used with only erratic results. Green styrofoam was used in an attempt to camouflage the containers from potential vandals. The transplants remained healthy in these containers and I was hopeful they could survive long enough for two years of observations. However, the green styrofoam was devoured by birds in two months time. As the collection sites were not within close driving range the maintenance of the containers became prohibitive. Almost daily surveillance of the transplant containers would be essential to get successful results. This is especially true for a free-floating aquatic plant, such as Utricularia macrorhiza, which is difficult to tag.

Growth Chamber Results

Plants from each location were grown in a growth chamber in plastic crispers under 12 hour daylength using fluorescent lighting (1,000 ft. c.) at 80 F and 12 hour nights at 60 F. Plants from Silver Lake Fen flowered when put into the growth chamber in their natural water free of the flocculent material common to their normal habitat (Fig. 59); however, until the plants became dormant, there was little change in their general morphology (Fig. 60), and

their bladder pigmentation remained red.

In general, the growth chamber results were poor because the plants became dormant in three to four weeks or were killed by large algal blooms. Buds that germinated after three months of dormancy, produced small plants reaching only 4 to 5 cm in length. Therefore, it was impossible to draw any conclusions as to whether the morphological differences in the plants were genotypic or phenotypic.

Statistical Results

The following interpretations are summarized from Table 3 using all values between 0.325 and 1.000 or -0.325 and -1.000. For example in column 1 the negative value of -0.411 would be considered significant but the -0.212 would not be considered a significant value for interpretation. The same is true for positive values. The 0.918 value would be significant but the 0.082 value would not be. Each variable is treated individually in the program and compared in relation to all other variables. Only five of the 21 component columns are used because 79% of the total variation occurs in this segment of the print-out. The remaining 21% variation is dispersed in columns 6-21 in a manner requiring methods of interpretation beyond the limits of this dissertation.

Columns 1, 3, 4, and 5 show the following significant correlations for Three Corners Pond: a positive correlation for phosphate, iron, water turbidity, water color, functional bladders, flowering,

winter-buds, and temperature; and a negative correlation for Silver Lake Fen, total hardness, sulphate, red bladder color, maximum number of bladders per branch, Jemmerson Slough, temperature, pH, bladder size, Spring Lake, and winter-buds. In some instances both negative and positive correlations are shown for the same variable in different columns. This is true for temperature, maximum bladders per branch (which tends to be more positive than negative), and winter-bud formation (more negative than positive). In these instances the data is insufficient to separate variables because of environmental or other influences which were not measured.

Columns 3 and 5 show correlations for Jemmerson Slough which are positive for temperature, pH, and bladder size; and negative for Three Corners Pond, phosphate, iron, winter-bud formation, time, and temperature.

Columns 2 and 4 show significant correlations for Spring Lake. The positive correlations are for time, pH, turbidity, water color, winter-bud formation, and death. Negative correlations are shown for maximum bladders per branch, bladder size, maximum branch length, functional bladders, flowering, phosphate, turbidity, and red bladder color.

In general the statistical results of component correlation support the field observations. Discrepancies occur in a few instances because unmeasured influences such as surface shading by Lemna, Diquat poisoning, climatic changes, and various other factors were not included in the statistical program. These influences

and a comparison of the biological, field, and statistical results can be found in the Discussion section.

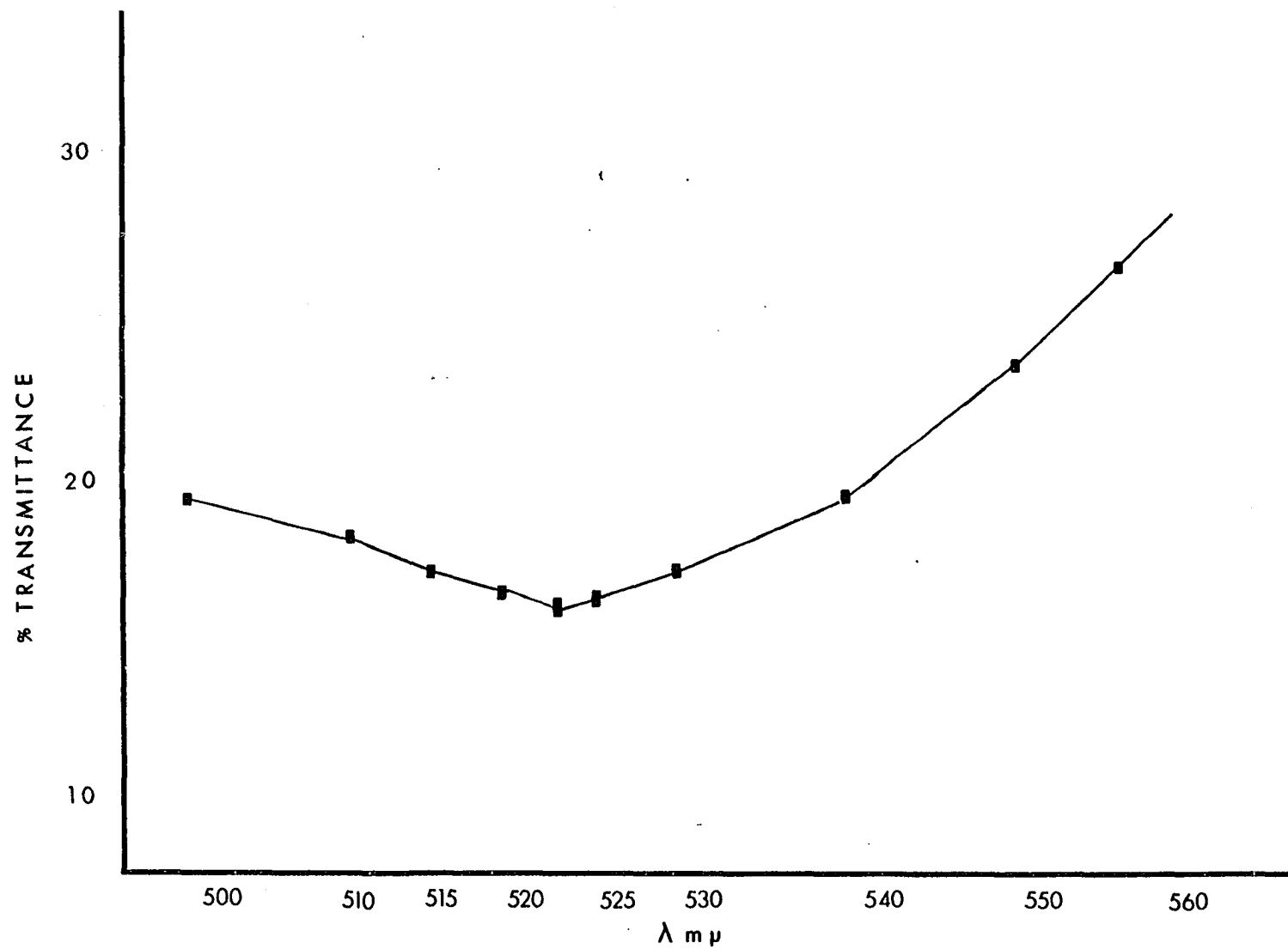
Anthocyanin Determination

With the Leitz Microspectrophotometer the lowest transmittance reading was 522 m μ (Illustration 2), within the 500-550 m μ range for anthocyanin. The range for R_f values of 10 runs, using two dimensional chromatography, was between 0.368 and 0.438 with an average of 0.394. The percent error was 2.1%. The average R_f value (0.394) is close to the R_f values for previously determined anthocyanins such as Peonidin and malvidin (Harborne, 1958). However, the specific anthocyanin for Utricularia macrorhiza was not determined, and may belong to a group of unidentified anthocyanins rather than to either of the above.

Microscopy

Winter-buds

The shoot tip is coiled and covered by numerous multicellular lenticular trichomes (Fig. 24--arrow, 25--arrow, 64). Branch and bladder primordia develop below the shoot apex (Fig. 9, 24, 25, 26). In the winter-buds the arenchymatous shoot (Fig. 20, 63) and branches contain large quantities of starch (Fig. 20, 24), whereas the "Summer-buds" contain little or no starch (Fig. 25) as shown with PAS and darkfield optics. The winter-buds are protected by a secretory product produced by the lenticular outer trichomes (Fig. 75, 79, 80).



ILLUST. 2. ANTHOCYANIN TRANSMITTANCE

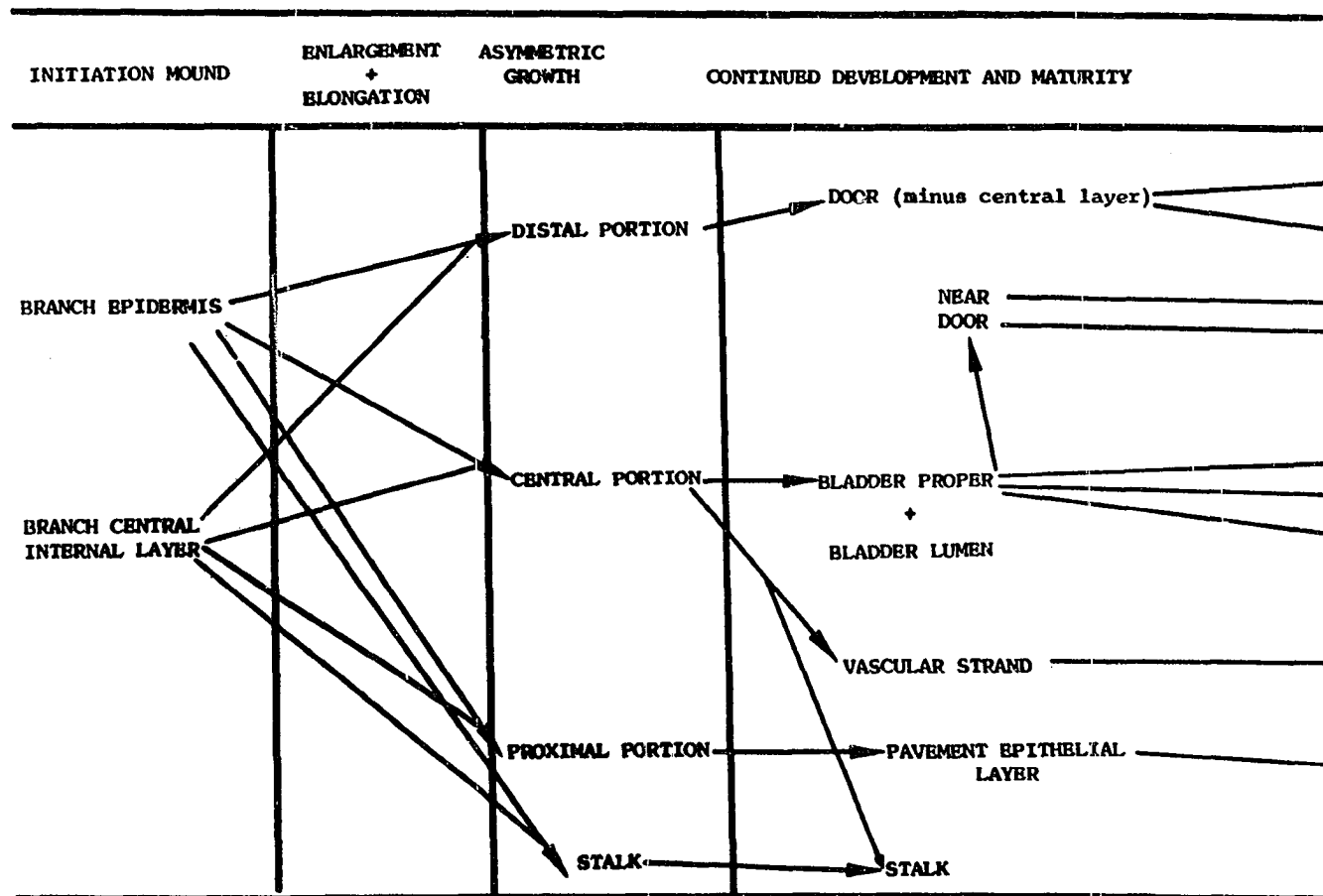
This secretory product stains positively for water insoluble carbohydrates using PAS and will be loosely defined as mucilage (Fig. 20). In addition, the shoot apex is protected throughout the year by small dissected branches (Fig. 65) which are alternately arranged and closely appressed to form the bud (Fig. 55, arrow).

Bladder ontogeny

Bladders from all four habitats were used in studying the development and fine structure. Although there were differences in gross morphology the basic anatomy of the bladders appeared to be the same. The individual photomicrographs are designated by the following abbreviations for each collection site: Spring Lake (SPRING), Three-Corners Pond (3-CORN), Silver Lake Fen (SILFEN), Jemmerson Slough (SLOUGH).

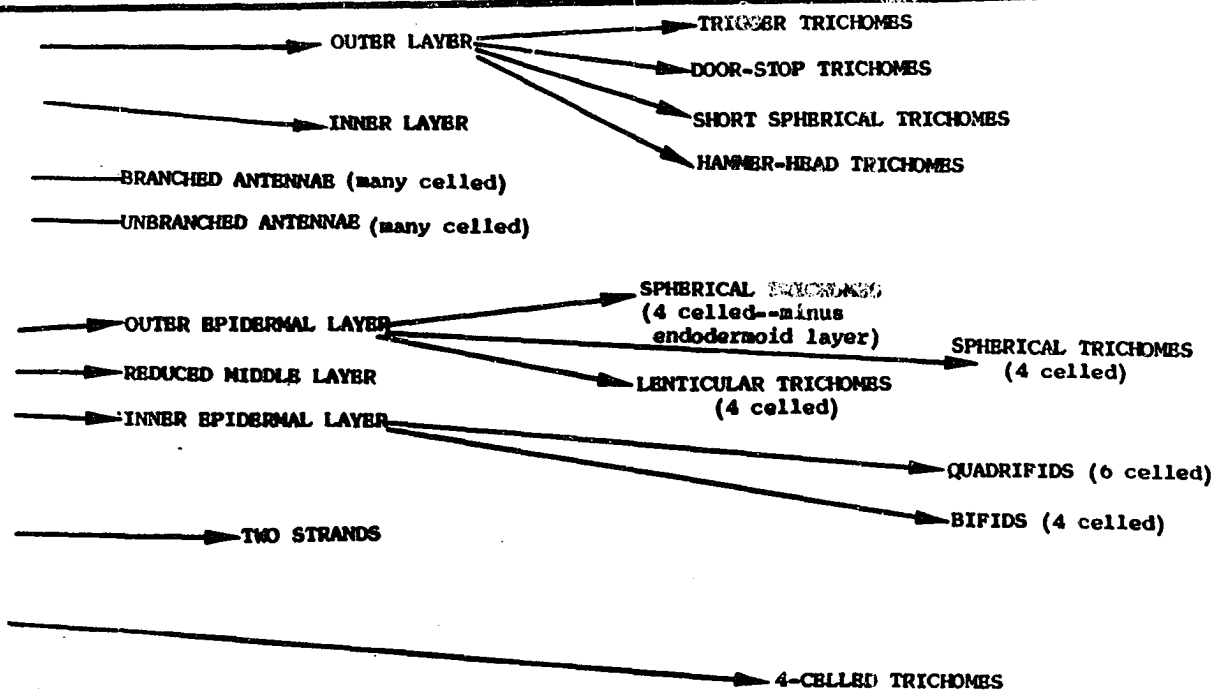
By sectioning the buds (Fig. 62) or removing bladders along the length of the shoot it was possible to observe various stages of bladder development. The developmental processes observed are summarized in Illustration 3. Bladder size was not a good indicator of the stage of development. Generally, mature bladders were pigmented red or blue, whereas younger bladders that were not yet capable of trapping organisms were green.

Epidermal and internal cells of the plant branch divide to form a mound of densely staining cells which I will define as bladder primordium (Fig. 16, 62, 73, 74, 75). When stained with azure-B for nucleic acids, these cells stain much more intensely than the



ILLUST. 3. SUMMARY OF BLADDER DEVELOPMENT

CONTINUED DEVELOPMENT AND MATURITY



ILLUST. 3. SUMMARY OF FLADDER DEVELOPMENT (CONTINUED)

cells of the branches from which they arise (Fig. 16). Ultrastructurally the cytoplasm is quite dense with small vacuoles, mitochondria, proplastids, and a large central nucleus (Fig. 248). Cell division and enlargement then take place more frequently on one side of the bladder primordium than on the other side (Fig. 76, arrow) so that the cells of the opposite side grow inwardly. As this unequal rate of division and enlargement continue, the bladder primordium becomes more strongly curved and develops into three regions: a distal epidermal layer, a middle layer, and a proximal epidermal layer. The distal and proximal epidermal layers are actually continuous with one another and are only separately defined here for convenience in presentation of later events. The proximal layer which has grown inwardly contains cells that are smaller and have dense cytoplasm, whereas the other two layers contain cells that are increasingly larger and more vacuolate (Fig. 77, 78).

Unequal cell division and enlargement continue, enhancing the invagination process, thus, forming the bladder lumen (Fig. 26, 80, 81). The middle layer of cells ceases to divide and enlarge. Spaces develop in the middle layer of cells as the outer (distal) and inner (proximal) cell layers continue to grow (Fig. 81-90). Bladders at this stage of development, stained with acridine orange, fluoresce intensely under ultraviolet light (UV) indicating the presence of RNA. The brightest fluorescence is from the innermost layer of cells adjacent to or lining the lumen (Fig. 32, 33).

The young immature bladders are oriented so that the invaginated region faces downward toward the branch from which it was derived (Fig. 66). A single vascular bundle, seen using polarized light (Fig. 8), traverses the center of the stalk and passes into the bladder. The vascular bundle branches at the base of the bladder just above the stalk attachment to the bladder base to form two strands. One branch runs through the mid-dorsal region of the bladder (Fig. 86, 93) and the other runs through the mid-ventral region (Fig. 28, 83, 93). Ultrastructurally the vascular bundle consists of sieve tube elements with p-protein, and adjacent companion cells and phloem parenchyma (Fig. 134, 135). The inner wall cells become more vacuolate except in the regions which will become the door and pavement epithelium (Fig. 81-88). The future door and pavement epithelial regions have dense cytoplasm and stain intensely for nucleic acids using azure B (Fig. 17). The door primordium is composed of two layers of cells which divide anticlinally to produce a flap-like structure (Fig. 27, 28, 85-95). The future pavement epithelial region consists of a single layer of cells very similar to those of the door primordium (Fig. 85-91). If the bladder is cross sectioned vertically across the lateral margins of both regions the lower cell layer of the door can be seen to be continuous with the pavement epithelial cell layer (Fig. 89, 91, 93, 94).

The primordial cells of the pavement epithelium undergo periclinical divisions in the anterior region first (Fig. 92, 95, 96).

Each division produces 2 cells, a basal cell and an outer cell. The basal cell becomes more vacuolate while the outer cell remains small and spherical and has a dense cytoplasm. A second series of periclinal divisions occurs in the outer cell producing a middle and a terminal cell; these two cells along with the basal cell elongate to become the secretory trichomes surrounding the aperture of the bladder (Fig. 102).

Following the initiation of cell division in the region anterior to the pavement epithelium, some of the cells of the outer layer of the door primordium divide periclinally to form central and terminal cells; these cells also enlarge to produce additional secretory trichomes on the surface near the aperture (Fig. 29--arrow, 30--arrow, 31--arrow, 96). More posteriorly, a row of large round cells develops which serves as a "door stop" (Fig. 96). Just anterior to the row of door stop cells, four trigger trichomes arise from the door outer surface (Fig. 119-122). During these changes, the external shape and appearance of the bladder change (Fig. 68, 69).

Prior to the differentiation of the door and pavement epithelium, the bladder surface is covered primarily with lenticular trichomes; however, by the time the differentiation of the internal structures begins, a second type of trichome develops on the bladder surface. This second type is smaller, two-lobed and spherical (Fig. 67, 70, 246, 247, 259-264). At the time of transition from lenticular shaped trichomes to the smaller spherical trichomes, the first periclinal divisions in the inner bladder epidermis occur to produce

the bifid and quadrifid trichomes. These latter two trichomes stain intensely with azure-B for nucleic acids, as do the door and pavement epithelium (Fig. 19).

At maturity, the bladder has changed orientation so that the aperture faces upward from the branch from which it was derived (Fig. 71); the bladder appears more pear-shaped (Fig. 72), and the inner and outer bladder wall cells are filled with starch (Fig. 11). Multicellular, branched antennae (Fig. 112), arise from the lateral margins of the outer epidermis at the anterior end of the bladder. In addition, the branches have elongated and bear spines which are highly refractive and anisotropic at maturity (Fig. 10).

Door

The door is semi-circular in shape with smaller cells in the central posterior region and along the free-margin (Fig. 121, 138). The door is two cell layers thick; the cells at the posterior end of the door have dense cytoplasm, whereas the cells at the anterior end are more vacuolate. The inner cell layer at the anterior end has large cells in which the interior walls are incomplete and the exterior walls are scalloped in appearance, whereas the anterior outer cells are much smaller (Fig. 119).

The door is covered by a cuticle on both sides and has three ill-defined regions which are ultrastructurally different. The most lateral posterior free margin of the door has fairly dense staining cells that are all similar in appearance, except that the

door edge cells are smaller (Fig. 140, 141). Plasmodesmata connect the anticlinal walls of the inner and outer layer of cells; however, no plasmodesmata occur in the wall between the two cell layers. Each cell has a large centrally located nucleus. The remainder of the cytoplasm contains numerous ribosomes, smooth endoplasmic reticulum (SER) with large cisternae, large dictyosomes, mitochondria, large starch filled plastids, and osmiophilic bodies. The SER is associated with large vesicles and the plasmalemma. The more anterior lateral cells are larger and more vacuolate.

About one-quarter of the way in from the lateral sides of the door, somewhat lens shaped wall thickenings occur in the anticlinal walls of both cell layers (Fig. 142-144). Plasmodesmata are common in these walls on either side of the thickenings but not in the wall between the two layers of door cells.

The cytoplasm of these cells has a large nucleus, RER, free ribosomes, numerous mitochondria, dictyosomes, occasional osmiophilic bodies, and plastids with little or no starch. Most of the cells have one to several vacuoles which contain particulate material.

Cells in the central door region have very large prominent wall thickenings in the anticlinal walls (Fig. 145, 146). Located between these thickenings are plasmodesmata. The cytoplasm of these cells has numerous vacuoles which contain various kinds of materials. Some vacuoles appear to have particulate material in suspension, while others are filled with amorphous membrane-containing material, or contain small vesicles which have fine, dense-staining granular

material. SER is present usually in association with the outer margins of the cells. Plastids with some starch, mitochondria, dictyosomes, free ribosomes, and osmiophilic bodies are randomly scattered in the cytoplasm.

Trichomes are not present on the inner surface of the door (Fig. 138). However, four multicellular trigger trichomes project from the lower quadrant of the aperture (Fig. 113, 116). These trigger trichomes, which are a single cell thick, are derived from the outer epidermal cells of the central region of the door (Fig. 115, 116, 121-124). At maturity, these cells have no cytoplasmic contents, appear anisotropic (Fig. 120), and shatter upon sectioning. However, young developing trigger trichomes have a full complement of cytoplasmic organelles (Fig. 125, 126). Centrally located in each cell is a nucleolus and intranuclear crystals. Large plastids, with some starch, and mitochondria are abundant. Dictyosomes are present but in fewer numbers than in the other cell types of the bladder. ER is commonly associated with the vacuoles (Fig, 125, arrow). The vacuoles appear to fuse with one another and numerous plasmodesmata connect the cells to each other.

In addition to the trigger trichomes, the outer door epidermal surface has shorter stalked trichomes of two different types surrounding or anterior to the trigger trichomes. Both of these types of trichomes display the three-celled structural pattern common to most of the trichomes of the bladder (excluding the trigger trichomes). One type has a terminal cell which is hammer-head shaped

while the other has a spherical shaped terminal cell (Fig. 116--arrow, 117, 121, 123). The hammer-head shaped trichomes in longitudinal section (Fig. 147) have a basal cell which is the equivalent of a door outer epidermal cell that has slightly elongated, a central cell, and terminal cell. The basal cell appears cytoplasmically much the same as the other door cells. The central cell has antinatal walls impregnated with dense material and an external cuticular coating continuous with the cuticle of the door surface. The cytoplasm of the central cell has a large nucleus, vacuoles, RER, free ribosomes, dictyosomes, mitochondria, and small multivesicular bodies either near or external to the plasmalemma. The terminal cell was poorly fixed in most instances; however, as in most of the other trichomes the cuticle is exfoliated.

Also located on the outer door surface is a row of short stalked round trichomes just posterior to the trigger trichomes (Fig. 103, 108, 119, 123). I have defined these as the door-stop cells, as they rest in a groove in the central pavement epithelial region when the door is closed. The row of door-stop cells appear to be joined at their terminal cells either by secretory material or cytoplasmic connections (Fig. 123).

Trichomes and antennae surrounding the aperture

Two multicellular branched antennae project from the epidermis above the outer margin of the bladder door (Fig. 113, 114, 115, 116). Smaller unbranched antennae arise from the lower outer margin of the bladder aperture (Fig. 113, 116).

Lining the outer margin of the bladder aperture are numerous long-stalked trichomes with elongate terminal cells (Fig. 23, 111, 113, 114, 116, 118, 123, 124). The trichomes are initiated from the anterior regions of the primordial door and pavement epithelium (Fig. 95, 96). All of these trichomes are similar in that they consist of three cells at maturity: a long basal stalk cell, a short central cell, and a large elongate terminal cell (Fig. 107, 109, 110, 127). When stained with PAS the terminal cells stain intensely for carbohydrate, whereas the central and basal cells stain only faintly (Fig. 21). Fresh, unstained trichomes do not autofluoresce under UV (Fig. 22); however, if aniline blue is added, fluorescence is observed in all three cells of the trichomes with the terminal cells fluorescing most intensely (Fig. 23). If these trichomes are sectioned either longitudinally or in cross-section, a thick wall is evident around the terminal cells and a densely staining material is present around the cells in the aperture region (Fig. 105, 106). With SEM, this external cell surface coat appears to be a secretory product (Fig. 118).

Ultrastructurally the basal cells of these trichomes are elongate and are extensions of the bladder epidermal cells from which they arise. The cytoplasm of the basal cell is quite dense with numerous arrays of ER and a large vacuole at the more distal end of the cell (Fig. 131). The more proximal cytoplasm of the basal cell is less densely stained and has smaller vacuoles. The distal anticlinal walls of the basal cell are covered by cuticle which is

continuous with the cuticle of the bladder wall epidermal cells. The distal periclinal wall is symplastically connected to the central cell by plasmodesmata.

The small central cell has anticlinal walls impregnated with dense material which are also covered by a cuticle (Fig. 130, 133). The cytoplasm of the central cell has numerous randomly arranged mitochondria, a centrally located nucleus, small vacuoles, occasional dictyosomes, and some RER. The RER appears in some places to be associated with the plasmalemma.

The terminal cell (Fig. 127, 132) is characterized by its elongate spherical shape and its highly-active appearing cytoplasm. The cell is surrounded by a thick homogeneous wall and an exterior thinner, densely staining wall. The thin cuticle is still evident but it is separated from the wall (Fig. 128). The cytoplasm has abundant parallel arrays of RER and numerous dictyosomes with from four to about fifteen stacks of cisternae (Fig. 128, 129). Various forms of vesicles are associated with the dictyosomes: large vesicles, coated vesicles, and small, dense vesicles. In addition, many mitochondria are present which are typically located either near the plasmalemma or RER. Very few plastids are present in these cells. The central cell region contains a large vacuole with a fine suspension of particulate material.

Pavement epithelium

The door when closed rests on the mature pavement epithelium which consists of a platform of glandular trichomes closely packed together. Each trichome has a basal cell, central cell, and two terminal cells (Fig. 97, 150). Generally, the terminal cells appear as only one cell when cut longitudinally (Fig. 99-104), but when sectioned tangentially or in cross-section they can clearly be seen as two cells (Fig. 97, 98).

Immature trichomes consist of three cell types which are cytoplasmically similar. The terminal cells have only a primary wall covered by a cuticle, and the central cell wall is weakly impregnated with dense material. The cytoplasm of the basal cell, central cell, and terminal cells contains conspicuous SER. In addition, numerous dense staining mitochondria, small vesicles, and vacuoles are present. Few, if any, plastids are present (Fig. 148, 149).

At maturity, the cells of the anterior portion of the pavement epithelium exfoliate their cuticles, whereas the more posterior trichomes of the pavement layer retain their cuticle (Fig. 99, 100--arrow, 104--arrow). The door rests in a groove in the middle region of the pavement epithelium, which can be seen as a slight depression where four or five rows of trichomes are depressed and appressed (Fig. 99, 100, 103, 104).

The three cells making up each trichome become more distinct from each other (Fig. 150). The basal cell of each trichome is

quite vacuolate, whereas the central cell and terminal cells have denser cytoplasms with small vacuoles (Fig. 97, 98, 101, 102).

The basal cell has RER which is frequently associated with the plasmodesmata of the anticlinal walls and the distal periclinal wall (Fig. 150, 154, 156). The proximal wall of the basal cell has no plasmodesmata (Fig. 150, 155).

The central cell has anticlinal walls impregnated with dense material which are also covered with a cuticle (Fig. 154). The proximal periclinal wall displays impregnated material at its juncture with the anticlinal walls but the material is not continuous throughout the periclinal wall (Fig. 156). Microtubules and small vesicles are associated with the plasmalemma adjacent to the impregnated walls (Fig. 153, 158). With phase contrast optics the central cells appear darker than the other cells of the pavement epithelium (Fig. 36) and with UV the impregnated walls autofluoresce yellow (Fig. 34, 35, 37).

The central cell cytoplasm is more dense than the basal cell but less dense than the terminal cell. Generally each central cell contains a large central nucleus, a few dense staining mitochondria, RER, and dictyosomes. Plasmodesmata occur in the periclinal walls but not in the anticlinal walls (Fig. 150, 152, 156).

The terminal cells are much more densely stained and of two main types, with some cells intermediate between the two. The terminal cells of the trichomes of the anterior pavement epithelium are long and somewhat globose shaped. Each has a centrally located

nucleus, scattered mitochondria, and a few plastids. The cytoplasm stains fairly uniform in density. Numerous dictyosomes (Fig. 156) are associated with coated vesicles, RER, and small vesicles (Fig. 151). Only a few small vacuoles are present. A very thick cell wall is produced at the distal end of these cells which is covered by cuticle (Fig. 150). It stains less densely than the impregnated wall of the central cell.

The second type of terminal cell is located in the more posterior trichomes of the pavement epithelium. These trichomes have similar basal and central cells when compared to the first type; however, the terminal cell is different in many respects (Fig. 157). A large central vacuole is present in each and the cell width increases the more posteriorly located its associated trichome is in the pavement epithelium. The cytoplasm is far more densely stained in the proximal end of the cell than in the distal end. Only a thin cell wall is present around these cells and their cuticles are exfoliated. A secretory product is released by the terminal cells and appears to be associated with the exfoliated cuticles to form the velum or seal between the closed door and pavement epithelium. The velum autofluoresces green under UV (Fig. 34, 37). When the door is open a remnant of the velum can be seen with the SEM as a thin sheet (Fig. 123). The nucleus, with intranuclear crystals, is located in the distal cytoplasm and is surrounded by numerous vesicles. The vesicles appear to be derived from ER whose cisternal size increases from the proximal end of the cell to the distal end.

The terminal cells become increasingly more vacuolate as the cuticle is exfoliated (Fig. 99, 100, 104).

Beneath the pavement epithelium is a mass of cells (Fig. 99, 100, 101, 102, 104). These cells are separated from the glandular trichomes of the pavement epithelium (Fig. 150) by thick walls with no plasmodesmata (Fig. 155). However, each cell in the mass is connected to adjacent cells by plasmodesmata (Fig. 159). Large mitochondria, ribosomes, vacuoles, and small vesicles are present in the cytoplasm. Large chloroplasts (Fig. 136), similar to those of the highly vacuolated bladder wall cells (Fig. 137), are evident.

Bifid and quadrifid trichomes

There are two specialized trichomes found on the inner surface of the bladder which have either two or four elongate cells and are termed bifids and quadrifids. The bifids are only found on the posterior margin of the pavement epithelium (Fig. 139, 216) and are initiated from the inner epidermal cells. After the first periclinal division, the future bifid consists of a basal cell which is part of the epidermal cell layer, and a single outer cell (Fig. 196). The basal cell is almost completely vacuolate with only a thin layer of cytoplasm around the cell periphery. The outer cell cytoplasm is characterized by numerous free ribosomes, a large central nucleus, few dictyosomes, ER, mitochondria, and plastids. The vacuoles of this cell contain small dark staining bodies about the size of ribosomes. The basal and outer cells are connected by plasmodesmata.

The outer cell, which will become the central cell of the mature bifid, divides periclinally to produce another cell which then elongates into the shape of a bowling pin (Fig. 197, 199, 200). Other than a difference in size and shape, this cell appears cytoplasmically similar to the outer cell from which it was derived. The newly produced elongate cell has numerous microtubules associated perpendicularly to the cell wall at the anterior end of the cell.

The elongate cell undergoes an anticlinal division to produce two new terminal cells (Fig. 198). The basal cell remains quite vacuolate. The central cell has numerous large mitochondria surrounding the centrally located nucleus. Microtubules are evident along the periclinal walls of the central cell and these walls appear to be slightly impregnated with a dense wall material. Some RER is present mostly at the distal part of the cell near the plasmalemma. The wall common to the central cell and the two terminal cells contains numerous plasmodesmata.

The two terminal cells have numerous mitochondria, plastids, dictyosomes, and RER which is frequently associated with the mitochondria. Small vesicles and vacuoles are also present. There are numerous microtubules associated with the plasmalemma all along the outer walls of the terminal cells. Two outer cell wall layers are present; an inner fibrous-like layer and an outer denser-staining layer.

The proximal portions of the terminal cells remain joined together (Fig. 225, 227); however, the distal regions of each cell

become increasingly more elongate (Fig. 201, 202, 219) and separate from each other (Fig. 226). At maturity, the two terminal cells are quite long and project into the bladder lumen (Fig. 50, 217, 218), perpendicular to the inner surface.

A third wall layer is laid down in the proximal region of each terminal cell. This layer lies between the outer primary wall layer and the inner fibrous layer. The third layer is sponge-like in appearance with numerous channels running through it (Fig. 219, 221, 222, 225, 228, 230). A constriction occurs (Fig. 219, 222, 228) mid-way between where it begins and ends (Fig. 221--arrow). In the constricted region, large aggregates of a substance are most obvious. This same substance appears to be present in the channels running through the wall. The cytoplasm in this region is more dense than the cytoplasm above or the cytoplasm adjacent to it in the central cell. Just above the constriction are numerous dense-staining mitochondria and the cell nucleus. More distally, the cytoplasm is characterized by large and small vacuoles, numerous arrays of RER, osmiophillic bodies, few plastids, and fewer mitochondria (Fig. 219, 220). This region has only a fibrous wall next to the plasma-lemma and a denser wall to the outside. No cuticle is present in this region (Fig. 219, 220). However, a cuticle is present over the wall surfaces where the sponge-like wall begins proximally (Fig. 221) and it is continuous from there to the bottom of each terminal cell (Fig. 222), over the central cell wall (Fig. 223), and on the surface lining the lumen (Fig. 223).

By the time the terminal cells have reached substantial length, the anticlinal walls of the central cell have become completely impregnated with the dense material (Fig. 219, 223, 224, 225) which autofluoresces yellow under UV (Fig. 51). The central cell is connected to both the terminal cells and the basal cell by numerous plasmodesmata (Fig. 219, 228, 229, 230). RER and small vesicles are frequently associated with the plasmodesmata.

The quadrifids are initiated from single epidermal cells lining the lumen of the bladder (Fig. 203). Each initial divides periclinally followed by a series of divisions to produce four cells (Fig. 170) which elongate (Fig. 46, 160). Each of these latter cells divides again producing a terminal cell which is initially quite short (Fig. 204) but elongates rapidly (Fig. 47, 48, 161-167, 205-207, 209-215). Further elongation of each of these cells (Fig. 166) is unequal so that two of the cells become longer than the other two cells (Fig. 168, 169, 172, 177-179, 189-193). The shorter cells lie more parallel to the inner bladder wall surface, whereas the two longer cells project more at a 45° angle to the inner bladder surface (Fig. 207, 209, 210, 212, 213). The two long terminal cells of the quadrifids are very similar to the two terminal cells of the bifids (Fig. 185, 186). As the cells of both the quadrifids and bifids elongate, they become increasingly more vacuolate (Fig. 171-193). At maturity, these cells contain a large vacuole with a small amount of dense cytoplasm at the base of each cell (Fig. 49, 50, 51). A single coin-shaped crystal (Fig. 52, 53)

is present in each vacuole. The crystals display constant movement in the living condition as viewed by polarization optics.

Quadrifids were observed in bladders which had trapped zooplankters and contained digestive material in the lumen (Fig. 194, 195). The terminal cells of the quadrifid contained a material similar in appearance to that of the digestive material in the lumen (Fig. 189-193).

More mature quadrifids have an obvious constriction in the region where the four terminal cells meet and attach to the central cell (Fig. 205, 206, 210, 213). The central cell is hexagonal to round in shape with a short round stalk to which the terminal cells are attached. The central cell can be seen best when the terminal cells are removed (Fig. 215).

The quadrifids are similar in ultrastructure to the bifids but have an additional wall layer in the terminal cells which traverses through the constricted region of the sponge-like wall. This additional wall layer is fibrous in appearance (Fig. 231, 232). Just as in the bifids, the constricted region contains aggregates of an amorphous substance. This material is always located along the marginal portion of the sponge-like wall (Fig. 231, 232, 235, 236). The cytoplasm of the most proximal end of the terminal cells is quite dense in contrast to the more distal cytoplasm (Fig. 231, 232, 234, 239). The cytoplasm has large arrays of RER (Fig. 231, 232) and SER (Fig. 234, 239). Many plasmodesmata connect the central cell with the four terminal cells (Fig. 231, 232, 234, 237, 239).

When the plasmodesmata are cut in cross-section the numerous channels surrounding them in the sponge-like wall layer can be seen as a vast web-like system of tubular elements running from the cell cytoplasm to the outer margin of the wall (Fig. 239).

The mature central cells have two quite distinct cytoplasmic conditions. This may be the result of separate functional activities that the trichomes are involved in at different times. For example, in some instances the cytoplasm had the same appearance as that described for the bifid central cells: a large central nucleus, some RER associated frequently with the plasmodesmata, vacuoles, and scattered mitochondria and plastids (Fig. 231, 232). A thick impregnated anticlinal wall is present which autofluoresces yellow under UV (Fig. 48, 49). The plasmalemma along this wall is moderately invaginated (Fig. 233). However, in other mature quad-rifids the plasmalemma is strongly invaginated deeply into the cytoplasm (Fig. 238, 239). The plasmalemma can be seen encircling mitochondria and lying close to the nucleus (Fig. 238).

The most distal region of the terminal cells of these quad-rifids also displays a highly active plasmalemma. Although the plasmalemma is not invaginated as in the central cells, it is folded over many times so that there are several layers of the plasmalemma under the cell wall (Fig. 241, 245). Associated with the plasmalemma and cell wall are numerous microtubules (Fig. 240, 242, 244). The cytoplasm frequently contains multivesicular bodies, numerous vesicles, RER, and dictyosomes (Fig. 240-245). The

dictyosomes and RER appear to be associated with the vesicles which contain material very similar to that of the fibrous cell wall next to the plasmalemma (Fig. 245).

The surface of the quadrifids is frequently covered with various substances. Even when the quadrifids appear too young to be functional, their surfaces are coated with a granular material (Fig. 204). Similar material is found on the inner bladder wall surface and may be debris accumulated during coating of the material for SEM.

At maturity, the quadrifids are either free or moderately free of surface materials (Fig. 209, 210, 214, 215) or coated with a variety of thread-like materials (Fig. 208, 211, 212), powdery-like materials (Fig. 206), or small scale-like coatings (Fig. 213). It is difficult to assess how much of this is caused from debris sucked into the bladder during the trapping process as the bladders are frequently filled with various materials present in the aquatic environment.

The surfaces of the inner bladder walls and young quadrifids were frequently observed to be covered with small coccus-bacteria-like bodies (Fig. 165--arrow, 201). These were also present on more mature quadrifids (Fig. 210).

Bladder outer surface trichomes

The surface trichomes observed on young bladders in the winter-buds are different in shape and ultrastructure from those on the surfaces of older bladders. All of the outer surface trichomes were

very difficult to fix and embed with good results. When these trichomes are poorly fixed there is a separation between the two terminal cells (Fig. 258). Consequently, the ultrastructural observations are limited.

The outer surface of the bladder is initially and predominately covered by short stalked trichomes which consist of a basal cell, central cell, and two lenticular shaped terminal cells. Occasionally smaller spherical trichomes are observed. Both types are identical to those on the young shoot (Fig. 64). The outer trichomes of the winter-bud bladders are produced by an elongation (Fig. 248) and division of the outer bladder wall cells. The smaller spherical trichomes have a vacuolate basal cell, a very dense central cell, and a terminal cell (Fig. 249, 250). The outer surface of these trichomes is covered by a cuticle which is exfoliated with the secretory product (Fig. 250). The cytoplasm contains a large central nucleus, scattered mitochondria, vacuoles which often contain dense staining material, numerous dictyosomes, and ER. These trichomes lack the usual impregnation of the central cell anticlinal wall layer.

The second type of surface trichome on young bladders has two lenticular shaped terminal cells. The central cell has impregnated anticlinal central cell walls (Fig. 251, 253). The cytoplasm of the large terminal cells displays characteristics indicative of a tremendous amount of activity. Dictyosomes are abundant with many stacks of cisternae (Fig. 251, 254) and associated with them are

numerous vesicles. These vesicles appear to come from the dictyosomes (Fig. 255, 256) and then fuse with the vacuoles (Fig. 254) or the plasmalemma (Fig. 256). The mitochondria are always found near the margins of these cells. A massive amount of material was always observed associated with the outer wall of the terminal cells (Fig. 251, 252, 254).

The outer surface trichomes of the older bladders have predominately spherical terminal cells and they are short-stalked, and slightly recessed in the outer bladder wall (Fig. 257, 259, 260, 265). These spherical trichomes are identical to those of the branch surfaces (Fig. 261, 262). Occasionally the lenticular-shaped trichomes described previously for the young bladders were also observed. The smaller spherical trichomes have the same basic structure of the majority of the other trichomes and consist of a basal cell, central cell, and terminal cell or cells. During early development (Fig. 263) the central cell is not impregnated and the cytoplasm has only small vacuoles. Plasmodesmata connect the three cells through the anticlinal walls. A cuticle covers the surface of these trichomes and is continuous with the cuticle of the outer bladder wall cells. The cytoplasm of the terminal cells have several dictyosomes with associated vesicles. Mitochondria, RER, small vacuoles, and free ribosomes are dispersed around the large central nucleus.

The walls between the two terminal cells and between the central cell and terminal cells become thicker and impregnated

with a denser staining material (Fig. 264, 271). The cytoplasm of the terminal cells begins to display increasing numbers of vesicles, ER with large cisternae, and vacuoles containing dense-staining material. Similar material can be seen in the cytoplasm. The dictyosome vesicles appear to fuse with the vacuoles and the ER is associated more with the plasmalemma (Fig. 264).

As the bladders develop, these lenticular trichomes become intermixed with newer trichomes that have smaller spherical terminal cells, a central cell, and a basal cell (Fig. 12, 70, 266-269). The lenticular-shaped trichomes which remain continue to have an apparent secretory product on the surface, whereas the newer spherical trichomes do not (Fig. 246, 247).

The lenticular trichomes of a young bladder (Fig. 38) do not fluoresce when stained with aniline blue (Fig. 39). Older bladders have predominately the smaller spherical trichomes which display a red autofluorescence (Fig. 40) and the central cell wall of the trichomes fluoresces yellow (Fig. 41, 43). If these same trichomes are stained with aniline blue for callose, a bright fluorescence of the walls of the terminal cells is apparent (Fig. 42, 269). The branches of the plant have similar trichomes which only autofluoresce red for chlorophyll in the unstained condition (Fig. 44). When these branches are stained for callose with aniline blue, however, the walls of the terminal cells also fluoresce positively for callose (Fig. 45).

In the mature surface trichome (Fig. 265) the basal cell contains a large central vacuole. The central cell has impregnated anticlinal walls (Fig. 265) and the periclinal walls have plasmodesmata with associated ER (Fig. 274, 275). In contrast to the other types of trichomes, the central cells of these trichomes have characteristic transfer wall projections on the periclinal walls (Fig. 265, 270, 272, 273). The central cell has numerous mitochondria located marginally near the plasmalemma (Fig. 268, 272, 273) and a large central nucleus with nucleolus and intranuclear crystals (Fig. 272, 273). The terminal cells have only a narrow band of cytoplasm which surrounds a large central vacuole (Fig. 265, 273). A thick inner wall, comparable to the region staining for callose, is present between the plasmalemma and a thinner outer wall.

DISCUSSION

No single investigation of Utricularia has combined field observations, chromatography, spectroanalysis, LM, TEM, and SEM to better understand the morphology, anatomy and developmental processes which occur in the plant; especially in the bladder traps. The objective of this investigation was to combine these tools and techniques to determine possible environmental factors which might be responsible for the gross morphological variation among plants from different aquatic habitats, and to observe microscopically bladder trap development in the plants grown under natural conditions.

Utricularia macrorhiza grows commonly in many Iowa aquatic habitats. The plants display varying degrees of morphological plasticity among the different habitats. Therefore, four aquatic habitats which appeared morphometrically different, as well as having U. macrorhiza populations that were morphologically different in gross appearance, were selected to determine what factors in the environment might bring about the plasticity displayed by the plants. In addition, an attempt was made to demonstrate whether the actual variation was due to the environmental conditions and therefore, phenotypic, or due to a genotypic response. Because of difficulty in maintaining both field transplants and growth chamber plants, it was impossible to assess whether any genotypic differences actually occur; however, other field data suggests that environmental

factors have a profound effect on the morphological expression of U. macrorhiza. If genotypic differences occur it is assumed that these would be most evident in comparing plants at Silver Lake Fen with plants from the other three habitats. This assumption is based on the fact that reproduction at the fen is predominately vegetative, whereas flowering and sexual reproduction is common in the other habitats. The lack of substantial flowering at Silver Lake Fen would result in a more limited gene pool.

Utricularia macrorhiza growing at Silver Lake Fen displays comparatively the greatest morphological variation. Certain environmental factors appear to be significantly involved in the reduced growth and inability to flower. The shallowness of the pools requires that the plants be in contact with the flocculent sludge in the bottom. The bladders become engorged with the flocculent sludge and cease to be functional. This prevents the trapping of sufficient numbers of zooplankters to provide the necessary animal nutriment to induce flowering. The actual nutrient supplied by animals, which allows for flowering, is unknown. However, Pringsheim and Pringsheim (1962) demonstrated in the laboratory the necessity of animal nutriment for flowering. The population which I observed at Silver Lake Fen of Utricularia macrorhiza is the first natural habitat reported where flowering is absent because of limited animal nutriment.

The plants at Silver Lake Fen were also smaller with shorter internodes and branches. As column 1 of Table 3 demonstrates, Silver Lake Fen (0.918) is negatively associated with phosphate

(-0.409) and iron (-0.433). This indicates that the phosphate and iron level at the fen is low, particularly in comparison to Three Corners Pond. Sorenson and Jackson (1968) demonstrated that Utricularia growing in poorly balanced inorganic media had shorter internodes than plants growing in well-balanced media. If the plants were fed paramecia the internode length increased. Furthermore, Lollar, Coleman, and Boyd (1971) demonstrated a greater uptake of ³²phosphorous from labeled ostracods in plants with bladders than in plants with no bladders. Therefore, the inability to trap adequate numbers of zooplankters which could supplement the nutrients available in the water, may also be responsible for the reduced internode and branch length.

The bladder color at Silver Lake Fen was red as opposed to being blue at the other three habitats. The bladder pigment was assumed to be anthocyanin by Gibbs (1929) but this has never been experimentally demonstrated. Therefore, the necessary chromatography and cytophotometry was carried out to verify that the pigment was indeed anthocyanin. The pH of the water in which the plants grow was not different enough to explain the pigmentation difference. However, two other factors could account for the color difference. Methylation of the hydroxyl groups of anthocyanin has a reddening effect, whereas oxidized anthocyanins are bluer (Harborne, 1964). It is highly possible that a methylation reaction occurs in the plants at the fen, because the water in which the plants grow contains unusually high levels of sulphate. The anaerobic substrate

would presumably give off methane. This combination could produce an excellent methylating agent (methyl sulphate). Provided that the plants absorb higher levels of sulphate and possess the necessary genetic coding for methylation of anthocyanin, methylation may be responsible for the red expression of the anthocyanin pigment. The red color may also be due to a pH difference in the cell sap which would be different from that of the surrounding water. This may also be related to the absorption of greater amounts of sulphate or other elements in the very hard water. A lower cell sap pH would cause a reddening of the anthocyanin.

Bladder pigmentation is always blue at the other three habitats, total hardness and sulphate levels are much lower, and other differences in the plant morphology and water chemistry are apparent. For example, the plants collected at Jemmerson Slough prior to August 1973 were more robust than those of any other habitat (Fig. 54). The statistical results provide very little explanation for the exceptional size of the plants and bladders. Phosphate and iron are negatively associated and therefore low at the slough, just as at Silver Lake Fen. However, a rich supply of zooplankters was available on which the plants could feed. The additional animal nutriment could be sufficient to allow for greater plant growth. It is probable that the statistical results for Jemmerson Slough are weaker because no compensation for plant death in 1973 was included in the program. Since Utricularia competes poorly with other aquatic vegetation, early death of the plants was probably

due to shading by Lemna and Typha. Furthermore, the increased growth in the Typha population would have placed greater demands on the available nutrients in the water and substrate. As Typha has a massive root system and Utricularia is rootless, the Typha plants would no doubt have an advantage in absorption of the available nutrients.

Statistically, Three Corners Pond shows a positive correlation for phosphate and iron, indicating over two seasons the amount of phosphate and iron available was significantly more than at any other habitat. The mature bladders were functional and always contained trapped zooplankters. Therefore, the size difference is probably not due to insufficient nutriment. The most noticeable difference in the habitat is greater water turbidity and water color. The positive correlation for these factors at Three Corners Pond may implicate reduced growth in plant length, and an increase in surface area by increased sub-branching, as a response to less available light. The light rays reaching the plants would be less because of the turbidity of the water and the higher organic content of the water as indicated by higher color readings.

Spring Lake had the smallest, most fragile plants. The negative correlation for phosphate, and the positive correlations for water color and, part of the time, for turbidity would in part explain the reduced growth. However, not included in the statistical program is another factor which is probably equal to if not more important than these correlations. The population of Utricularia observed at

Spring Lake was in a small ditch fed only by rain water, except during flooding of the lake early in the spring. As the ditch was quite small and contained many other vascular aquatic plants, the demand for available nutrients would be great and these nutrients not readily replaced. As the season became dryer the water level would proportionally drop and eventually stagnation of the water occurred. Probably as a consequence of these combined factors the plant growth was minimal and death the end result.

The field data has provided some insight into possible conditions which might influence morphological expression in Utricularia. No environmental study is ever complete. The complexity of every plant community involved innumerable environmental factors, all working together to produce a specific habitat type. This investigation touches upon only a few possibilities and many other factors are certainly involved in the morphological plasticity of Utricularia.

Benjamin (1848) and Pringsheim (1869) observed that the shoot tip was coiled within the winter-bud and surrounded by young "leaves" bearing bladders in various stages of development. Darwin (1875) and Meierhofer (1902) noted that the young bladders and branches had different shaped trichomes on their surfaces. Meierhofer (1902) indicated that the trichomes were involved in mucilage secretion. This investigation is in agreement with these previous observations and provides additional information regarding the anatomy and developmental processes within the winter-buds. The predominate form of trichome on the young shoot tip, branches, and bladders is

lenticular in shape, although smaller spherical trichomes are also present. Both secrete a PAS positive, carbohydrate mucilagenous-like material. The mucilage is quite thick by late September and no doubt, serves to protect the bud during dormancy in the winter. Buds collected in late September contained large quantities of starch which are stored until the following spring. The stored starch is apparently necessary for germination of the buds. Buds on the summer plants contained no starch.

Two investigators observed seasonal changes in the buds at the ultrastructural level. Vintéjoux (1970a) observed acid phosphatase localization in the cytoplasm of the winter-bud cells during the fall; whereas, acid phosphatase localization was in the vacuoles during germination. Acid phosphatases are a group of non-specific enzymes that hydrolyze a variety of organic esters to liberate phosphate ions (Hayat, 1973). In addition, Genevès and Vintéjoux (1967) enzymatically demonstrated that the intra-nuclear crystals of the winter-buds were protein in nature. Vintéjoux (1970b) observed that these protein crystals are quite prevalent in the nuclei of the meristematic cells of the winter-buds in the fall, but upon germination disappear or become less frequent except in the mucilagenous cells. She concluded that the protein may be used in histochemical activities during germination. Utricularia then possesses at least two stored materials necessary for germination; starch and protein.

The white thread-like vegetative propagules were observed on

plants at all four habitats. They were generally formed under stress conditions, such as crowded growth or low water levels. Darwin (1875) described tuberous vegetative propagules on U. montana. The vegetative propagules of U. macrorhiza in no way resemble those illustrated by Darwin, nor were such structures ever observed for U. macrorhiza. However, they were similar to the "air-shoots" described by Arber (1920). She termed them "air-shoots" because they had stomates and floated on the surface of the water. She also believed they supplied "air" to the plant. I do not agree with her interpretation, as the propagules contain large quantities of starch. In addition, Reinert and Godfrey (1962) reported they had gotten similar structures to germinate in the laboratory. Although they have a few stomates, their numbers seem hardly adequate to supply the plant with any substantial amount of air. Their function seems rather to be another means of vegetative reproduction; especially since they form when the plants are growing in very shallow water or under crowded growth conditions. They would be particularly important during the summer months when the apical buds are unprotected by thick mucilage, and contain little or no starch. Under stress conditions these apical buds would not easily survive, whereas the smaller starch-filled propagules with their mucilage coat (Fig. 18) might.

Bladders were observed from all locations and appeared to be anatomically the same. Therefore, the figures included in Appendix E represent a sampling of material from the four habitats. This is

because some stages and structures were better fixed from one habitat than another. This is best explained by the seasonal variation in molarity and buffering capacity of the water in which the plants were growing. These changes would be gradual in nature, and the plant membranes could adjust more easily than in plants taken from the field and emersed in a laboratory buffer or fixative. The best results were obtained using the natural water as a buffer for fixation, but results varied because the buffering capacity is not constant throughout the season or among habitats. Therefore, one habitat might have a better buffering capacity during one collecting period and another habitat a better buffering capacity at a different collection time.

Darwin (1875) and Meierhofer (1902) observed that young bladders were formed by a series of divisions and by invagination of the newly produced cell layers. My results agree with their studies and provide additional information with regard to bladder ontogeny. Cell division by the branch epidermal cells produces a mound of cells. Following the formation of the mound, unequal rates of division on opposite sides of the mound, and unequal cell enlargement are responsible for the invagination of the cell layers which form the bladder primordium. The processes of unequal division and cell enlargement remain important in determining the shape of the bladder until it is mature.

Changes in the relative amount of DNA and RNA were observed throughout bladder development. The initial mound stained uniformly

dense for nucleic acids. However, as the outer epidermal cells of the bladder primordium enlarged and became more vacuolate, the intensity of nucleic acid staining decreased, while only the inner epidermal layer of cells and the door and pavement epithelial regions stained intensely. The inner epidermal layer continues to stain intensely until the initiation of the quadrifids and bifids. These newly produced glandular trichomes then stain intensely for nucleic acids until their terminal cells have reached maturity.

With the exception of Compton (1909), previous investigators considered the bladder wall to be two cell layers thick. Compton observed that the wall was "about three cell layers thick," with the middle layer "disorganized." My observations agree more with those of Compton. The bladder wall is initially three layers thick but the middle layer ceases to divide before the outer and inner layers. This causes the cells of the middle layer to separate, resulting in large spaces between cells in the middle layer. This would give it the "disorganized" appearance noted by Compton.

Darwin (1875) observed that as the bladders matured in U. vulgaris they changed their orientation in relation to the branch from which they were derived. The same change in orientation was observed in U. macrorhiza. When the bladders are young their apertures face downwards toward the branch, but at maturity the aperture faces upward away from it. This change in orientation would place the aperture in a position more favorable to trap zooplankton.

Darwin (1875) also observed that a vascular bundle traversed

the stalk and divided once at the base of the bladder. One branch traversed the mid-ventral region of the bladder and the other branch traversed the mid-dorsal region. In the mid-dorsal region the vascular bundle divided again at the base of the pavement epithelium and into the anterior region of the door. Compton (1909) observed only phloem in the vascular bundle; however, Chandler (1910) and Arber (1920) reported "spiral elements." The vascular bundle of the bladder of U. macrorhiza is similar to Darwin's description. Spiral elements were observed, but rarely. The vascular bundle usually contained only phloem parenchyma, sieve tube elements with p-protein, and companion cells. These cells ultrastructurally resemble those described for Mimosa pudica (Esau, 1973). Like Mimosa, the companion cells have denser cytoplasm than the sieve tube elements and phloem parenchyma. In addition, the sieve tube elements and companion cells are connected by branched plasmodesmata (Fig. 135, arrow). In contrast to Esau's observations in Mimosa, plasmodesmata were also frequent between phloem parenchyma cells. No plasmodesmata connected the sieve tube elements to the phloem parenchyma, or the phloem parenchyma to the companion cells. The cytoplasmic constituents of the three cell types were similar to those in Mimosa. The companion cells have mitochondria, plastids, and many ribosomes. The parenchyma cells have cytoplasmic ribosomes in groups, dictyosomes, RER which is sometimes tubular, mitochondria, and plastids.

The door of the bladder is formed by an invagination of the inner and outer bladder epidermal cells. This agrees with Darwin's

(1875) observation that the door and pavement epithelium are extensions of the bladder wall. Not previously reported, is the relationship between the pavement epithelial cells and the outer layer of door cells. The cells of these two regions are continuous with one another at their lateral margins. Whereas, the inner cell layer of the door is continuous with the inner bladder wall layer (Fig. 89, 93).

The two cell layers of the door appear to act independently of one another. The inner cell layer of the door is never connected by plasmodesmata to the outer cell layer; although plasmodesmata connect the cells comprising the same layer to one another. Furthermore, trichomes develop only from the outer epidermis of the door and the inner epidermis becomes quite vacuolate before the outer epidermis.

Darwin (1875) observed that the door trichomes were formed before the quadrifids and bifids. My results indicate more specifically which trichome types are formed on the door and around the aperture margin at a particular time in development. The elongate, long stalked trichomes surrounding the aperture margin are initiated first. They occur anterior to the pavement epithelium (Fig. 95) and then at the anterior end of the door (Fig. 95, 96). The row of door stop trichomes are then initiated more centrally on the door. They form just prior to the initiation of the bifids, quadrifids, and terminal cells of the pavement epithelium (Fig. 19).

The mature bladder trap consists of a door, a pavement

epithelium, and the bladder proper which are composed of at least an inner epidermis and an outer epidermis. Thirteen types of trichomes are derived from the epidermal surfaces of the bladder. Ten of the trichome types are glandular and the other three are non-glandular. All of the ten types are anatomically similar in that each gland has a basal, central, and one, two or four terminal cells. However, these types vary in their morphology in respect to shape, size, cell wall modifications, and cytoplasmic characteristics which may be related to different functions.

The function of the U. macrorhiza bladder trap is to attract and trap aquatic organisms, digest the trapped organisms for nutrient, and to reset itself to trap again. Each type of trichome has a specific role in these three fundamental activities of the bladder. The door-associated, nonglandular trichomes consist of the branched and nonbranched multicellular antennae which form a funnel-like region to direct zooplankters to the nonglandular trigger trichomes. Glandular trichomes surrounding the aperture and on the surface of the door secrete a carbohydrate product which attracts the zooplankters more specifically to the trigger trichomes. When the zooplankters swim into the trigger trichomes the door is sprung and the organisms are sucked into the trap. The door closes on the pavement epithelium and the glandular door-stop trichomes, along with the anterior pavement epithelial cells and the velum, form a water-tight seal. In order for the bladder to trap again the water which has been sucked into the bladder lumen must be removed.

In addition, the trapped organisms must be digested and their digestive products absorbed. The absorption of water and digested products has been attributed to the inner epidermal glandular trichomes called quadrifids and bifids. The absorbed nutriments must have a pathway of transport for use in the plant body. The pattern of plasmodesmatal connections provides some insight to this pathway. Furthermore, the absorbed luminal water must be effectively removed from the bladder tissues to the outside in order for the bladder to reset itself. The most reasonable trichome type for this is the outer epidermis spherical trichomes. Table 4 summarizes the types of trichomes and the functions ascribed to them by previous investigators. The remaining discussion is concerned with the specific anatomical and functional aspects of the door, the pavement epithelium, and each of the trichome types found in U. macrorhiza.

Czaja (1923), Nold (1934) and Lloyd (1942) reported that the entire inner and outer bladder surfaces are covered by a cuticle except for the terminal cells of the trichomes. The door is no exception to these observations, as it has a thick cuticle on the both surfaces except for the terminal cells of the trichomes on the outer surface.

Withycombe (1924) described three ill-defined regions for the door; 1) a free marginal area that has small cells with thinner uniformly thick walls, 2) a more anterior central and lateral region of larger cells with some wall thickenings, and 3) a central posterior door region with more prominent wall thickenings. Lloyd (1942)

Table 4. Comparative review of the functions proposed for Utricularia trichomes

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
<u>Outer epidermal trichomes</u>				
1) Lenticular	+	-	Pringsheim (1869)	---
	+	+	Darwin (1875)	---
	+	+	Meister (1900)	mucilage secretion, protect plant from herbivores
	+	+	Meierhofer (1902)	mucilage secretion
	+	+	Beltz (1974) ^a	carbohydrate secre- tion, protection of winter-bud
2) Spherical (lacks endodermoid walls)	+	-	Beltz (1974) ^a	questionable carbo- hydrate secretion
3) Spherical (has endodermoid walls)	-	+	Darwin (1875)	---
	-	+	Meierhofer (1902)	mucilage secretion
	-	+	Nold (1934)	water transport
	-	+	Lloyd (1942)	no evidence for water transport no cuticle

^aPresent paper.

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
3) Spherical (has endodermoid walls)	-	+	Slinger (1954)	mucilage secretion
	-	+	Sculthorpe (1967)	mucilage secretion
	-	+	Sydenham and Findlay (1973)	possible active transport of Cl ⁻ ions
	-	+	Fineran and Lee (1974)	expel water
	-	+	Beltz (1974) ^a	water transport out of bladder, barrier to large molecular weight materials in habitat water
4) Unbranched antennae	-	+	Beltz (1974) ^a	mimick appearance of crustaceans
5) Branched antennae	-	+	Darwin (1875)	mimick appearance of crustaceans
	-	+	Withycombe (1924)	funnel-like region leading prey to aperture

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
5) Branched antennae	-	+	Beltz (1974) ^a	in agreement with the above
<u>Inner epidermis trichomes</u>				
6) Bifids,				
7) Quadrifids	-	+	Crouan (1858)	root hairs
	-	+	Darwin (1875)	absorbed decayed matter
	-	+	Cohn (1875)	remove water from lumen
	-	+	Goebel (1891)	absorbed decayed animal products
	-	+	Brocher (1911)	absorption
	-	+	Czaja (1923)	remove water from lumen
	-	+	Withycombe (1924)	remove water from lumen

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
6) Bifids,				
7) Quadrifids	-	+	Gibbs (1929)	digestive glands water absorption
	-	+	Nold (1934)	remove water from lumen
	-	+	Lloyd (1942)	water absorption
	-	+	Sydenham and Findlay (1973)	possible active transport of Na ⁺ and K ⁺ ions into lumen
	-	+	Fineran and Lee (1974)	water absorption and absorption of digestive products
	-	+	Beltz (1974) ^a	water absorption absorption of digested animal products

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
<u>Pavement epithelial trichomes</u>				
8a) Anterior trichomes	-	+	Lloyd (1929a, 1942)	exfoliate cuticles to form velum
	-	+	Fineran and Lee (1974) ^b	secrete mucilage to attract prey and to seal door
	-	+	Beltz (1974) ^a	exfoliate cuticles to form velum, along with secre- tory product
8b) Middle trichomes	-	+	Withycombe (1924)	mucilage secretion formation of groove for door to rest in
	-	+	Lloyd (1942)	groove for door to rest in, exfoliate cuticle to form velum

^bThey did not distinguish regions of pavement epithelium.

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
8b) Middle trichomes	-	+	Fineran and Lee (1974) ^b	secrete mucilage to attract prey and to seal door
	-	+	Beltz (1974) ^a	form groove for door to rest in, no exfoliation of cuticle
8c) Posterior trichomes	-	+	Lloyd (1942)	act independently of other regions
	-	+	Fineran and Lee (1974) ^b	secrete mucilage to attract prey and to seal door
	-	+	Beltz (1974) ^a	cushion for door to rest on

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
<u>Door trichomes</u>				
9) Hammer-head,				
10) Short stalked spherical, and				
11) Long stalked aperture trichomes	-	+	Darwin (1875)	absorptive glands
	-	+	Goebel (1891)	secrete mucilage
	-	+	Meierhofer (1902)	secretory
	-	+	Luetzelburg (1910)	secrete sugar-like material to attract prey
	-	+	Withycombe (1924)	secretory
	-	+	Gibbs (1929)	secrete sugar-like material to attract animals

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
<u>Door trichomes</u>				
9) Hammer-head,				
10) Short stalked spherical, and				
11) Long stalked aperture trichomes	-	+	Hada (1930)	secrete sugar-like material to attract animals
	-	+	Beltz (1974) ^a	secrete carbohydrate product and possible carbohydrate storage
12) Door-stop trichomes	-	+	Beltz (1974) ^a	secretory product which joins cells together and may at- tract velum to sur- face to aid in formation of a seal rest in the pavement epithelial groove

Table 4. (continued)

Trichome type	Presence on immature bladders	Presence on mature bladders	Investigators	Proposed function
13) Trigger trichomes	-	+	Darwin (1875)	called them bristles indicated no function
	-	+	Brocher (1911)	release door when stimulated
	-	+	Withycombe (1924)	sensory and cause door to respond
	-	+	Hada (1930)	transparent so that animals can't see them and swim into them triggering door
	-	+	Lloyd (1942)	release door when stimulated
	-	+	Sydenham and Findley (1973)	involved in door turgor loss
	-	+	Beltz (1974) ^a	release door when stimulated

described the door has having "two hinges" which swing independently of one another. The ultrastructural observations of this investigation are in agreement with Withycombe's description of three door regions. The arrangement of these regions supports Lloyd's "two hinge" theory. The most anterior inner door wall cells have scalloped exterior walls, and their anticlinal walls appear to break down along with the cytoplasm (Fig. 119). This region would be comparable in location to Lloyd's "upper hinge." The scalloped external walls and few anticlinal walls would allow the upper door region to be quite flexible. More posterior to this region are the two regions with wall thickenings that project inwardly into the cell. These are most pronounced in the region surrounding the trigger trichomes. This would be comparable to Lloyd's "lower hinge." The wall ingrowths would provide for the greatest flexibility and support in the trigger area.

Sydenham and Findlay (1973) have demonstrated that turgor changes in the door cells are always associated with stimulation of the trigger trichomes. They reported that loss of turgor is rapid and only necessary in a few cells to fire the trap on the first stimulus. When the trichomes were triggered repeatedly it required more time and a loss of turgor in more cells to allow the door to open. This phenomenon may be related to the location of the wall thickenings and to certain cytoplasmic characteristics of the cells nearest the trigger trichomes. First the prominent wall thickenings would provide a greater cell surface area than is present

in the cells further from the trigger trichomes. The central cells also have numerous tubular arrays of SER which are arranged perpendicularly to and in close association with the plasmalemma. These cytological factors could account for the more rapid ability to lose turgor. Tabular SER is present in the other cell regions of the door but displays a different arrangement. In the door cells of the posterior free margin, the SER is most frequently associated with the plasmodesmata. In the inner cells with smaller thickenings the SER is associated with the plasmalemma but not to the same degree it is in the cells surrounding the trigger trichomes.

Branched and unbranched antennae arise from epidermal cells at the lateral edges of the aperture. The branched antennae have been previously described by Darwin (1875) and Withycombe (1924) for U. vulgaris. An assortment of antenna types occurs in various species of Utricularia (Taylor, 1964). Darwin (1875) concluded that the antennae function to mimic the appearance of crustacea or to serve as a funnel to the aperture and trigger trichomes. His conclusions still seem reasonable, as no secretory product or surface trichomes are present on the surface of the antennae cells. Therefore, no secretory attractant to zooplankton is likely.

Darwin (1875) first illustrated the trigger trichomes of Utricularia, which he called "bristles." Merl (1921) and Withycombe (1924) described the trigger trichomes as extensions of four of the outer door layer epidermal cells while Withycombe further described them as bicellular in U. vulgaris. They are multicellular

in U. macrorhiza. When immature, the trigger trichomes have large plastids and numerous plasmodesmata between the cells. SER is associated with the plasmodesmata and the vacuoles. The vacuoles fuse. Frequently, large intranuclear crystals were observed. These cells are empty at maturity; therefore, the cellular events observed in the immature trigger trichomes are most likely involved in cell enlargement, strengthening of the cell walls which are anisotropic at maturity, and eventually the breakdown of the cell cytoplasm.

Darwin (1875) described three types of outer door surface trichomes, which correspond somewhat to the spherical trichomes, hammer-head-shaped trichomes, and door-stop trichomes I have described for U. macrorhiza. Darwin believed these trichomes were absorptive; whereas, Goebel (1891), Meierhofer (1902), Luetzelburg (1910), and Hada (1930) all believed they were secretory. My results are also more indicative of a secretory role by all three trichome types. With SEM a thin to relatively thick surface film was observed which may be secreted by the trichomes. The most noticeable "secretory product" was on the door-stop trichomes and caused many of them to be stuck together.

The door surface trichomes have a structural pattern similar to the majority of the other trichomes of the bladder. The similarity that exists among the bladder trichomes has been previously observed by Withycombe (1924), Gibbs (1929), and Lloyd (1942). This pattern consists of a basal cell, central cell, and terminal cell(s). The antennae and trigger trichomes are the only trichomes

to vary from this pattern. The anticlinal central cell wall is always impregnated with an electron dense material, except in the small spherical trichomes on the outer immature bladder and branch surfaces.

The impregnated walls appear very similar to the casparian strips of root endodermal cells described by Bonnett (1968) in Convolvulus arvensis. Similar walls have been observed in Drosera tentacles by Williams and Pickard (1974). They have termed cells with such walls "endodermoid cells." Williams and Pickard suggested that the endodermoid walls in Drosera control the movement of materials from the stalk into the secretory cells and the movement of digestive nutrients from the head cells into the stalk. Fineran and Lee (1974) have reported a "heavily impregnated" wall in Utricularia monanthos, a terrestrial form, which is impervious to the movement of lanthanum nitrate. Williams and Pickard (1974) suggested that such endodermoid walls "facilitate the spread of a receptor potential." On the basis of cytological evidence, they believed that the plasmalemmas of the endodermoid cells lie in the path of current flow between the proximal cells and the surface of the cap cells in Drosera. Current is restrained from flowing between endodermoid cells because of the associated cuticle on the outer surface of the endodermoid wall. Therefore, the generator potential or receptor potential probably occurs in the endodermoid layer.

In Utricularia it is possible that similar electrical patterns occur comparable to those in Drosera. No experimental evidence

exists at present. However, the cytological results indicate that the system could be similar. For example, any action potential generated in the terminal cell or cells must cross to the basal cell via the plasmalemma and protoplast since apoplastic movement would presumably be broken by the resistant endodermoid cuticle. If the basal cells were more negatively charged, when they were depolarizing, the endodermoid cells would receive a positive charge through their plasmodesmatal connections. As a consequence, the terminal cells would become more positive with respect to the basal cells. The opposite could also be true, where the basal cell might be more positive and then the terminal cell would become more negative. This theory is further supported by Sydenham and Findlay (1973) who measured membrane potential differences in Utricularia. In addition, they have shown that sodium and potassium ions are transported into the bladder lumen, whereas, chloride ions are actively transported in the opposite direction.

The trichomes surrounding the aperture of the bladder have the same basic structural pattern of a basal cell, central cell, and terminal cell. An obvious secretory product is evident on the surface of the terminal cells. This is a carbohydrate material, as it stains positive for PAS. Goebel (1891) and Lloyd (1942) believed this secretory product was "sugar-like" and attracted animals to the aperture region. These glands do not autofluoresce under UV, but when stained with aniline blue they fluoresce intensely. Aniline blue, especially coupled with UV, has been considered specific for

callose, a β -1, 3-glucan which is related to laminarine (Waterkeyn and Bienfait, 1970). β -1, 3-glucose linked molecules are also present in the reserve polysaccharides of Euglena, Ochromonas, and Perenema (Kreger and Meeuse, 1952, Clarke and Stone, 1960, and Archibald et al., 1963). Laminarin is a reserve polysaccharide of sublittoral brown algae. It is difficult to understand the presence of callose in these carbohydrate secreting glands. It is possible that what fluoresces is not callose, but a closely related reserve polysaccharide. This might explain why the fluorescence appears to come from the cytoplasm rather than from the cell walls. It is also possible that this positive reaction is due to artifact or tissue damage. However, I am reluctant to accept such a possibility as identical results were observed on several occasions using new stain and plants. If the substance is callose, then callose may be more common than previously believed and may serve a number of unknown functions.

The cells of the trichomes surrounding the margin of the aperture indicate a high degree of cytoplasmic activity. The basal cell of these trichomes displays cytoplasmic polarity with the more distal cytoplasm being most dense, and having a proliferation of ER. This would suggest the movement of materials to the more distal end of the cell where some cellular products are passed on to the central cell via the plasmodesmata.

The central cell has the same type of endodermoid wall previously discussed for the door surface trichomes. Again it is

probably involved in the establishment of an ion gradient system and the movement of materials from the basal cell to the terminal cell. The central cell cytoplasm is dominated by a central nucleus, surrounded by numerous mitochondria, many ribosomes, vacuoles partially filled with dense granular material, and some RER. The abundance of ribosomes would indicate a high degree of protein synthesis and stored protein could be the material in the vacuoles. RER only has been observed at the distal end of the cell in association with the plasmalemma and numerous small vesicles. Similar vesicles were seen in the proximal end of the cell and these were in association with the dictyosomes. Such vesicles may migrate across the cell to the distal end carrying cellular products from the basal cell which were passed through the plasmodesmata, or may transport dictyosome derived products.

The terminal cell possesses by far the greatest number of dictyosomes and vast arrays of parallel RER. As these trichomes secrete carbohydrate, the dictyosomes are most likely responsible for its production. It is difficult to see any established pattern between the dictyosomes and RER. The dictyosome associated vesicles appear to transport the dictyosome product directly to the plasmalemma without it passing through the RER. The mitochondria display a peripheral polarity which implicates them in providing the necessary energy for the transport of the secretory product across the plasmalemma. A cuticle is present on the terminal cell, but as the secretory product builds up the cuticle is pushed away. The

outer portion of the primary wall is partially impregnated with closely packed particles which have been described for the outer bladder surface glands of U. monanthos by Fineran and Lee (1974) and called by them, "cutin cystoliths." These cutin cystoliths appear similar to the particles in the outer portion of the terminal cell walls in U. macrorhiza.

The door when closed rests in a groove in the pavement epithelium as described by Withycombe (1924) and Lloyd (1936). Withycombe observed that the pavement epithelium was composed of closely appressed trichomes and along with Lloyd (1942) implicated these trichomes in mucilage secretion. Lloyd (1929a) and later Gibbs (1929) observed that a thin veil was formed by the exfoliated cuticles of the anterior pavement epithelial terminal cells. Lloyd (1929a) termed this veil the "velum" and reported that it served to seal the small gap between the door and pavement epithelium, thereby keeping the bladder water-tight when the door was closed. Fineran and Lee (1974) observed that the terminal cells of the pavement epithelium actively secrete mucilage, while the central cells have wall ingrowths and have highly impregnated anticlinal distal periclinal walls. My observations are in agreement with Withycombe (1924), Lloyd (1929a, 1936, 1942), and Gibbs (1929). However, they only partially agree with Fineran and Lee (1974). The pavement epithelium is composed of numerous trichomes closely appressed together. In the central region of the pavement epithelium these trichomes are both appressed and depressed to form the groove. Each

trichome has a basal cell, central cell, and two terminal cells. Previous investigators have only observed a single terminal cell. In most longitudinal sections of U. macrorhiza only a single terminal cell was apparent; however, when the pavement epithelial layer was cut in cross-section or tangentially, two terminal cells are clearly seen.

In immature trichomes, the cytoplasms of all three cell types are similar, but at maturity each is quite distinct. The basal cells have a large central vacuole. Plasmodesmata connect the basal cells to each other and also to the central cell above. However, the basal cells are not connected to the cells beneath them by plasmodesmata. ER is frequently associated with the plasmodesmata and can be seen continuous from one cell to the next. Thus, materials may move symplastically from one basal cell to the next and from the basal cells to the central cells.

The central cells of the pavement epithelium have a large central nucleus, mitochondria, dictyosomes, ribosomes, and RER. The central cell is connected by plasmodesmata to the terminal cells. The RER is associated with the plasmodesmata and with the plasmalemma along the anticlinal walls. The anticlinal walls are heavily impregnated and are comparable to the endodermoid walls in Drosera and those previously discussed for the door surface trichomes. The plasmalemma undulates along the wall but is closely appressed to it. Microtubules, RER, and dictyosome vesicles are associated with the plasmalemma and are possibly responsible for

the deposition of the materials which impregnate the wall; these are probably lignin and waxes. The proximal periclinal wall of the central cell is partially impregnated but not all the way through the wall. If the central cell is cut in a median longitudinal fashion the central portion of the wall is not impregnated. Conversely, Fineran and Lee (1974) have shown the distal periclinal wall to be impregnated; but not the proximal wall. I have never observed any impregnation of the distal periclinal wall. Furthermore, they have shown transfer walls in the central cell and I have never observed them in the pavement epithelial trichomes. This appears to be at least a species difference and may represent a difference characteristic between aquatic forms such as U. macrorhiza and terrestrial forms such as U. monanthos.

In U. macrorhiza the terminal cells vary depending on their location in the pavement epithelium. The centrally and posteriorly located trichomes always had dense cytoplasm in the terminal cells with relatively small vacuoles. A thick distal wall which stains intensely for PAS is present between the cuticle and plasmalemma of each terminal cell. The cuticle was never observed to be exfoliated from these cells. Withycombe (1924) and Lloyd (1942) reported that all of the trichomes of the pavement epithelium secrete mucilage. Furthermore, Fineran and Lee (1974) stated that they "actively secrete mucilage which attracts prey and seals the doorway." My results do not completely support their observations. The anterior most trichomes, which exfoliate their cuticles are

responsible for the formation of the velum (seal) and appear to secrete a product. However, the trichomes making up the groove where the closed door rests, and the trichomes posterior to it, never appear to release a secretory product. Because of their location behind the closed door it seems unlikely that they would secrete a product to attract prey, as the prey would either be on the other side of the closed door or already trapped within the bladder lumen. These central to posterior trichomes are actively involved in the deposition of the thick wall material and have numerous dictyosomes, vesicles, and ER with large cisternae. The cisternae of the ER and the vesicles appear to contain similar material (Fig. 15) which is probably produced by the dictyosomes, and transported by the vesicles and ER to the plasmalemma for wall deposition. It would appear that these trichomes function merely as a cushion for the door to rest on. The door-stop trichomes would rest in the groove and the tip of the door would rest on the posterior pavement epithelial trichomes.

The anterior trichomes then form the velum which fills the slight gap between the groove and door-stop cells. The secretory product of the door-stop cells which joins them to one another may also facilitate adherence of the velum to the door-stop trichome surfaces and thus also aid in the creation of the watertight seal.

The anterior trichomes of the pavement epithelium have large central vacuoles and display cytoplasmic polarity. The proximal cytoplasm is quite dense, whereas the distal cytoplasm is less

dense and contains the nucleus. The secretory product appears to be produced by the dictyosomes and transported by the SER.

Two types of specialized trichomes are found on the inner bladder surface. These were termed bifids and quadrifids by Darwin (1875). He observed that the bifids were located only on the posterior margin of the pavement epithelium. Darwin (1875) described the quadrifids as having two long terminal cells and two short terminal cells in Utricularia vulgaris. The same is true in U. macrorhiza; however, the four terminal cells are of equal length in some species. For example, Fineran and Lee (1974) have shown that the terminal cells of the quadrifids of U. monanthos are equal in length. Darwin (1875) and Abel and Denffer (1961) observed that the long terminal cells are always oriented toward the stalk. This agrees with my observations for U. macrorhiza.

Goebel (1891) and Lloyd (1942) reported that the terminal cells lacked cuticle on their surfaces. I have observed cuticle on the lower surfaces of the terminal cells but this disappears more distally.

Darwin (1875) observed a small particle in the terminal cells which displayed Browning movement. It is curious that no mention of this particle is made in later papers. Darwin believed these particles were unusual nuclei. I have observed these particles in Utricularia macrorhiza and they are actually small birefringent, crystal-like bodies. A single crystal-like body is suspended in

the vacuole of each terminal cell and displays constant movement. Attempts to isolate these bodies for identification were unsuccessful because of their small size.

No explanation exists for the specific location of the bifids on the back of the pavement epithelium or for their variation from the quadrifids in having only two terminal cells, as they are otherwise anatomically similar to the quadrifids. The bifids and quadrifids have been implicated in the same function by previous investigators. Darwin (1875) found that both the bifids and quadrifids absorb decayed matter. He further demonstrated that they absorb ammonium nitrate and thus concluded their function was absorptive rather than secretory. In Utricularia vulgaris, Goebel (1891) observed oil droplets in the quadrifids of bladders containing decayed animal matter, and no oil droplets in those with no trapped animals. I have observed the same phenomenon in U. macrorhiza. Withycombe (1924) demonstrated that the quadrifids and bifids absorb water by using aqueous methylene blue stain.

Gibbs (1929) and Luetzelburg (1910) speculated that the quadrifids might be responsible for the secretion of a digestive enzyme. Luetzelburg (1910) demonstrated the presence of benzoic acid in the bladder lumen and believed it inhibited bacterial decay. Arber (1920), Hada (1930), and Sculthorpe (1967) all state that no digestive enzyme is present and that bacterial decay seems most reasonable. However, neither Arber (1920) nor Sculthorpe (1967) provide any experimental evidence to support their statements. Hada's (1930) paper

is written in Japanese with a short English summary. His summary indicates that he observed, following animal death, the number of bacterial cells greatly increases. He believed that animal death was due to crushing of the animals by pressure exerted by the bladder walls when they resumed the concave position. I do not know what experimental evidence he provides in his paper as this information was not included in the English summary.

My results support the role of bacteria in digestion of trapped animals. No satisfactory answer exists for the cause of death, as some organisms remain alive and others die rapidly within 10 minutes to 12 hours. Euglena survives and reproduces within the bladders as initially observed by Gibbs (1929). Other green algae such as Phacus die rapidly. The suggestion by Hada (1930) that the animals are killed by being crushed does not seem reasonable as I have observed trapped organisms moving about freely after the walls have become concave. There may be several factors which cause death for different organisms. Lack of oxygen, intolerance to the acidic condition of the lumen, or perhaps the secretion of a yet unidentified toxic substance. Once death occurs bacterial decay is a definite possibility for the breakdown of the organisms so that the plant can absorb the necessary nutrients. I have observed small coccoid bacteria. They are present in the bladder lumen long before the bladders are functional and frequently appear on the surfaces of developing quadrifids, bifids, and pavement epithelial trichomes, and on the bladder inner epidermal surface. These bacteria are also evident

on the inner epidermal surface after bladder maturity and are present in great numbers around digested organisms. Other bacterial types were rarely observed in the bladder lumen, although they must be brought in during the trapping process. It may be that the coccoid bacteria are specially adapted to survive in the bladder, whereas other bacteria are not and thus are effectively eliminated.

Meierhofer (1902) published the most accurate and extensive developmental sequence for the quadrifid trichomes in Utricularia vulgaris. He observed that the inner epidermal cells elongated and protruded beyond the normal limits of the epidermis. These cells divided to produce the basal cells and the basal cells in turn divided to produce four terminal cells. In U. macrorhiza the quadrifids originate from inner bladder wall epidermal cells and the bifids originate from the epidermal cells of the posterior margin of the pavement epithelium. The developmental sequence is similar for both types of trichomes. The epidermal cells undergo periclinal divisions to produce outer cells. Each epidermal cell elongates slightly and becomes quite vacuolate and is comparable to the basal cell of other trichomes in Utricularia. This basal cell is connected to the newly produced outer cell and adjacent bladder wall epidermal cells by plasmodesmata. The outer cell has a large central nucleus, dictyosomes, ER, and numerous ribosomes. The dictyosomes and ER appear to be implicated in the synthesis and deposition of cell wall material. The vacuoles of these cells contain small dark granules which may be stored protein. The outer cell

undergoes division to produce another cell which elongates into a bowling pin shape. Cytoplasmically this cell appears very similar to the one from which it was derived and is connected to it by plasmodesmata. Numerous microtubules are associated with the cell wall and are apparently involved in the deposition of cell wall material. This cell divides anticlinally to produce two cells if the trichome is a bifid or four cells if it is a quadrifid. The newly produced terminal cells have numerous mitochondria, plastids, RER, dictyosomes, and microtubules. The dictyosomes are associated with vesicles which appear to transport material to the RER. The RER is also seen associated with the microtubules along the plasma-lemma. A second fibrous-like wall layer is being laid down.

The proximal regions of the terminal cells remain joined together, but the distal regions separate and become more elongate. In the bifids the two terminal cells elongate equally and project into the bladder lumen perpendicular to the surface. The terminal cells elongate unequally in the quadrifids, with two becoming longer than the other two. The longer terminal cells project at an angle to the bladder surface, whereas the shorter terminal cells lie more parallel to the surface.

A third wall layer is laid down in the proximal region of each terminal cell which lies between the outer primary wall layer and the inner fibrous wall layer. The wall is sponge-like in appearance with the numerous channels. The lower portion of the terminal cell is constricted.

Fineran and Lee (1974) have shown that a tracer substance, lanthanum nitrate, is absorbed by these trichomes and they believe that the trichomes absorb digested products and water during resetting of the bladder trap. They reported the presence of a transfer wall in the central cell which facilitates absorption by increasing the surface area of the plasmalemma. I never observed a transfer wall in these cells at any stage of development. The plasmalemma of the central cell was highly undulate in some sections. Such undulations may facilitate absorption in U. macrorhiza without the presence of a transfer wall like that observed in U. monanthos.

The sponge-like wall contains large aggregates of a substance I believe to be the absorbed product of animal digestion. The presence of this material is most noticeable in the constricted region. Such material has not been reported previously at the ultrastructural level but may be comparable to the oil-like droplets observed by Goebel (1891) at the LM level. Fineran and Lee (1974) did not mention the presence of the sponge-like wall in the proximal terminal cells and it is not shown in any of their micrographs.

The cytoplasm of the terminal cells suggests a highly active system for the movement of materials across the plasmalemma. The plasmalemma is folded over in layers under the fibrous cell wall. This would provide an increased surface area for absorption or, possibly, secretion. Numerous microtubules, multivesicular bodies, and dictyosome vesicles are associated with the plasmalemma.

RER runs the entire length of the terminal cell. The proximal part of the cell is surrounded by the sponge-like appearing wall and contains the greatest number of mitochondria, and the cell nucleus. Because of this polarity of organelles it would seem most reasonable that these cells are involved in absorption. The digestive products could be absorbed more easily in the distal region of the cell where the cuticle is absent. The absorbed products may be transported by way of migration of the multivesicular bodies and vesicles to the proximal region of the cell where the mitochondria supply the necessary energy for their transport to the constricted region by way of the channels in the sponge-like appearing wall.

The RER may be associated with the transport and production of enzymes which could aid in the conversion of the digestive products into more easily transportable substances. Beneath the constricted region, where the greatest accumulation of material occurs, the cytoplasm is quite dense with RER. Here the stored material, when needed, may be converted for transport to the central cell and on through the bladder to the remainder of the plant for nutrition.

The digestive nutrient products are probably transported from the quadrifids and bifids by plasmodesmatal connections to the vascular tissue where they are carried to the remainder of the plant body. These cells have also been implicated in water absorption, and water probably takes the shortest route out of the bladder by passing from the quadrifids and bifids across the wall

cells and out the spherical surface trichomes as suggested by Nold (1934). This seems most reasonable since the remainder of the inner and outer bladder surfaces are covered by cuticle.

Darwin (1875) observed that the outer surface trichomes of young bladders were of different sizes and shapes. Meierhofer (1902) further observed that the trichomes of young bladders were mucilage secreting trichomes. Slinger (1954) and Sculthorpe (1967) agreed that the outer surface trichomes secreted mucilage. In U. macrorhiza there are three main types of surface trichomes: 1) the lenticular shaped carbohydrate-secreting trichomes; 2) the small spherical carbohydrate coated trichomes; and 3) the spherical non-carbohydrate secreting trichomes. Each of these types of trichomes has two terminal cells. Rarely, trichomes with a single terminal cell were observed. In contrast, during a visit to Dr. Fineran's laboratory in New Zealand (December, 1973), Dr. Fineran, his graduate student May Lee, and I found that the predominate outer surface trichome type in U. monanthos has a single terminal cell and only rarely were two terminal cells observed. This comparative observation provides many interesting questions regarding the evolution of Utricularia species, sepecially since the species studied by Fineran and Lee (1974) was terrestrial and found in a relatively isolated place (South Island, New Zealand), and my species was aquatic and fairly universal in distribution throughout North America.

The young bladder and branch surfaces are predominantly covered

by short stalked trichomes with a basal cell, central cell, and two lenticular terminal cells. These trichomes always had large intranuclear crystals in all four cells. The basal cell is quite vacuolate and connected to the central cell by plasmodesmata. Neither the central cell nor the two terminal cells fixed well. It is therefore, difficult to assess them in detail. However, the central cell has the usual impregnated endodermoid anticlinal walls. The terminal cell walls are partially impregnated with the cutin cystoliths. The cytoplasm of the terminal cells display a great deal of activity. Numerous dictyosomes are present with very large associated dictyosome vesicles. These vesicles appear to migrate to the plasmalemma for transport of the carbohydrate secretory product. The mitochondria are always located along the periphery of the cell and also appear to be involved in the highly active secretory process.

Small spherical trichomes are also present on the young bladders and branches. The exact function of these trichomes is unclear. They have an associated secretory product on the surface of their terminal cells and the cuticle can be seen to be exfoliated sometimes. However, the cytoplasm of these cells does not display the usual characteristics of highly secretory cells. In addition, no impregnation of the anticlinal walls of the central cell was observed. It may be that these trichomes do not secrete any product on their surfaces, but rather the secretory product comes from adjacent lenticular trichomes.

The older bladders and branches have spherical short-stalked trichomes with two terminal cells. These trichomes do not have any apparent secretory product on their surfaces and are implicated in the active transport of water out of the bladder. A thin cuticle is present on the outer surface which may possess small pores similar to those found in the cuticle of the secretory cells of Drosera (Williams and Pickard, 1974).

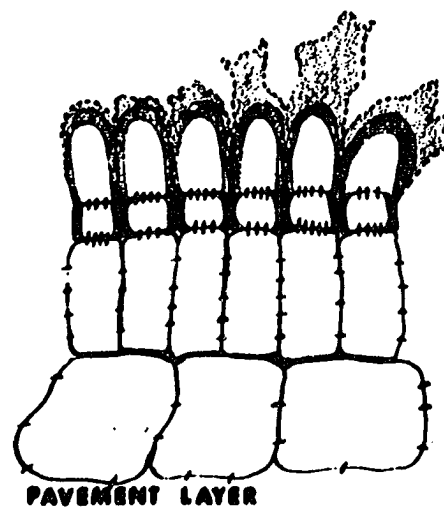
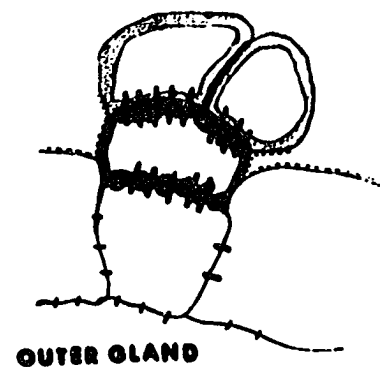
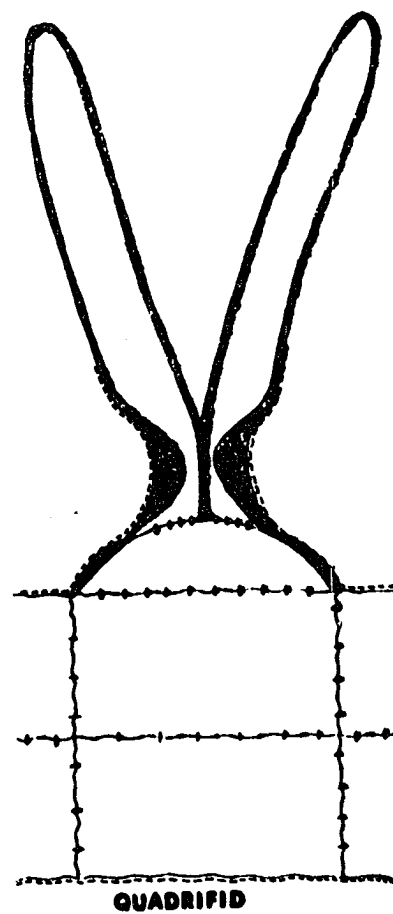
These trichomes are initiated by a periclinal division of some of the outer surface epidermal cells. This division produces an outer cell which undergoes further division to produce two terminal cells. The two terminal cells are involved in the deposition of a thick wall, partially impregnated with cutin cystoliths. The cytoplasm is filled with numerous vesicles and ER which appears involved in the transport of the wall material.

The terminal cells then become quite vacuolate with a much reduced protoplast. The central cell has endodermoid anticlinal walls and periclinal transfer walls. These were the only trichomes observed that had these wall ingrowths. Mitochondria were associated predominately with the distal periclinal transfer wall and must facilitate the active transport of water out of the trichomes.

When these trichomes were observed under UV no autofluorescence of the outer terminal cell walls occurs, only the highly impregnated central walls autofluoresced. However, when stained with aniline blue the thick walls of the terminal cells fluoresced intensely. Such fluorescence with aniline blue is considered a positive

indication of callose (Currier, 1957). In this instance the presence of callose is more explainable than in the trichomes surrounding the bladder aperture. According to Heslop-Harrison (1964, 1966) the most reasonable explanation for callose walls during microsporogenesis, is to function as a molecular filter, permitting the passage of basal nutrients into the microspore mother cell but not larger molecules. A similar principal may apply to the callose walls of the outer trichome terminal cell wall. Since they are surrounded by the water of the aquatic habitat and must transport water to the outside, it may be essential to have a wall that prevents the movement of larger molecules in the surrounding water from entering the cell. Identical trichomes occur on the mature branch surfaces and they also display a positive fluorescence for callose when stained with aniline blue. The function of these trichomes on the branches is unknown and no extensive study of them was done during this investigation. I can, therefore, only speculate that just as in their counterparts on the bladder surface, the callose wall serves as a barrier to the entrance of large molecules which might upset the ionic transport system of the trichomes.

Illustration 4 compares the structural similarities of the following trichome types: the quadrifids and bifids, the spherical outer surface water-transporting trichomes, the pavement epithelial trichomes, and the trichome type surrounding the aperture. All four trichome types have a similar structural pattern of basal, central, and terminal cells. The central cell anticlinal walls



ILLUST. 4. COMPARISON OF TRICHOMES

are always impregnated. This impregnation is probably lignin and waxes. Under UV they always autofluoresce bright yellow, similar to lignified spiral thickenings. The endodermoid walls are always covered by cuticle. Lüttge (1971) stated that the most plausible explanation for such endodermoid walls is to prevent apoplastic transport or they must be considered a "peculiar feature of unknown selective value." Variations occur in the periclinal central cell walls depending on the trichome type.

Intranuclear crystals were observed in all cells of the bladder except the quadrifids and bifids. The crystals have been suggested to have a protein storage function. Unzelman and Healey (1972) have reviewed these nuclear inclusions and indicate they are frequently found in protein secreting cells, or protein-carbohydrate secreting trichomes. Their absence in the quadrifids and bifids may be supportive of the idea that these trichomes do not secrete enzymes as previously suggested. However, further observations will have to be made to substantiate that these intranuclear crystals never occur in quadrifids or bifids.

Joseph Priestly said that "in completing one discovery we never fail to get an imperfect knowledge of others of which we could have no idea before, so that we cannot solve one doubt without creating several new ones." Such is certainly true of this investigation. There are, I am certain, far more partially answered or unanswered questions that have come from this investigation than have been answered. It has, nonetheless, added substantial new information

to our understanding of Utricularia and its response to the environment, and in addition, to a better understanding of the developmental processes which occur during bladder ontogeny.

SUMMARY

Utricularia macrorhiza Le Conte was collected from four different aquatic locations in Iowa. Environmental conditions, including water chemistry, were monitored for each location over two growing seasons and morphological differences in plants from each habitat were recorded. The environmental and morphological data were statistically analyzed to determine possible correlations between environmental conditions and morphological variation. Water depth, available numbers of zooplankters, functional ability of the bladders to trap, water color, water turbidity, and nutrient levels in the water were all significantly associated with plant morphology.

Pressed herbarium specimens and chemically preserved specimens were sent to Dr. Peter Taylor at Royal Botanic Gardens, Kew, England for taxonomic verification. All specimens were determined to be Utricularia macrorhiza Le Conte.

Plants were also prepared for developmental studies of the bladder trapping organs with the light microscope (LM), transmission electron microscope (TEM), and scanning electron microscope (SEM). Bladder ontogeny was followed from the time of initiation to bladder maturity. Histochemistry was carried out at the LM level at various stages of development for carbohydrates and nucleic acids. Fluorescence microscopy was used to identify the location and changes during development in the presence or formation of endodermoid walls,

RNA, cuticle, and callose. Various stages of development were also observed using SEM. Combined information from LM, TEM, and SEM resulted in a better understanding of the anatomy and functional aspects of the bladder traps. Thirteen different trichome types were observed during the ontogenic studies. These trichomes were divided into two major groups: nonglandular trichomes and glandular trichomes. The nonglandular trichomes consist of multicellular branched and unbranched antennae and the unbranched trigger trichomes. The glandular trichomes consist of outer bladder surface trichomes of which there are two spherical types and one lenticular type; long-stalked aperture associated trichomes, several types of door surface associated trichomes (short-stalked trichomes, hammer-head trichomes, and door-stop trichomes), the pavement epithelial trichomes, and inner bladder surface trichomes called bifids and quadrifids.

Each type of glandular trichome is similar in morphology in that it has one or more terminal cells, a central cell with endodermoid anticlinal walls, and a basal cell. Variations in trichome morphology occur in cell size, number, and shape. Wall specializations are specific for each trichome and apparently are associated with function.

The lenticular outer surface trichomes are involved in carbohydrate secretion, while the spherical outer surface trichomes are implicated in the secretion and absorption of various ions which appear to be associated with water transport out of the bladder trapping organ. The aperture associated trichomes, as well as some

door surface associated trichomes, also secrete carbohydrate materials. Some of the carbohydrate material may serve as an attractant to lure prey nearer the trigger trichomes and the secretory product of the door-stop trichomes serves to help seal the gap between the closed door and pavement epithelium. Some of the pavement epithelial trichomes exfoliate their cuticles and release a secretory product to form an additional seal, the velum, and serve as a resting place for the closed door. The inner bladder surface quadrifids and bifids appear to be responsible for the absorption of digested animal materials and in the absorption and secretion of various ions which appear to be associated with water transport. The directional movement of products through the various glandular trichomes seems to be controlled, in part, by the presence or absence of plasmodesmata, endodermoid walls, cuticle, and cytoplasmic organization.

Combined field and microscopic observations have provided further information regarding the necessity of animal nutriment for flowering. One natural habitat was observed to have Utricularia plants which never flowered, apparently due to an inability to trap adequate amounts of zooplankton. Flowering occurred in the other habitats and an abundance of zooplankters were observed trapped in the bladders. Coccus bacteria have been implicated in the breakdown of trapped animals, prior to absorption, for use by the plant. All of these results give a more complete picture of plasticity in Utricularia macrorhiza and the influence of environmental conditions.

In addition, a better understanding of bladder anatomy and its relation to the trapping mechanism has been provided.

LITERATURE CITED

- Abel, P., and D. Von Denffer. 1961. Über den Zusammenhang zwischen Zell--und Organpolarität bei der Ausbildung des Zellteilungsmusters auf der Blaseninnenwand von Utricularia vulgaris. Beitr. Biol. Pfl. 37:77-84.
- Allen, Ruth McV. 1959. A study of Utricularia olivacea. Bartonian 29:1-2.
- Arber, Agnes. 1920. Water plants: A study of aquatic angiosperms. Univ. Press, Cambridge. (Repr. 1963, with introduction by W. T. Stearn, as *Historiae Naturalis Classica*, 23. Cramer, Weinheim).
- Archibald, A. R., W. L. Cunningham, D. J. Manners, J. R. Stark, and J. F. Ryley. 1963. Studies on the metabolism of the protozoa. 10. The molecular structure of the reserve polysaccharides from Ochromonas malhamensis and Peranema trichophorum. Biochem. J. 88:444-451.
- Baker, J. G. 1884. Contributions to the flora of Madagascar--Part II. Monopetalae. J. Linn. Soc. (Bot.) 20:159-236.
- _____. 1886. Further contributions to the flora of Madagascar--Second and final part. J. Linn. Soc. (Bot.) 21:407-455.
- Barnhart, J. H. 1915. Segregation of genera in Lentibulariaceae. Mem. N.Y. Bot. Gard. 6:39-64.
- Bell, C. R. 1967. Plant variation and classification. Fundamentals of botany series, W. A. Jensen and L. G. Kavaljian, (eds.). Wadsworth Pub. Co., Inc., Belmont, Calif.
- Benjamin, L. 1847. Neue Gattungen und Arten des Utricularieen nebst einer neuen Eintheilung der Gattung Utricularia. Linnaea 20:297-320.
- _____. 1848. Ueber den Bau und die Physiologie der Utricularien. Bot. Zeit. Jahrb. 6:1-5, 17-23, 45-50, 57-61, 81-86.
- Bentham, G. 1869. Flora Australiensis: A description of the plants of the Australian Territory. Vol. 4 L. Reeve and Co., London.
- Benson, L. 1957. Plant classification. D. C. Heath and Co., Boston.

- Bonnett, H. T. 1968. The root epidermis: Fine structure and function. J. Cell. Bio. 37:199-205.
- Bosser, J. 1956. Un nouveau genre Malgache de Lentibulariaceae. Le Naturaliste Malgache 8:27-30.
- _____. 1958. Sur deux nouvelles Lentibulariacées de Madagascar. Le Naturalists Malgache 10:21-29.
- Brocher, F. 1911. Le problème de l'Utriculaire. Ann. Biol. Lacustre 5:33-46.
- Bruce, E. A. 1934. Tropical African plants, XI. Bull. Misc. Inf., Kew 1933:475.
- Buchenau, F. 1865. Morphologische Studien an deutschen Lentibularieen. Bot. Zeit. Jahrb. 23:61-66, 69-71, 77-80, 85-91, 93-99.
- Bünning, E. 1953. Entwicklungs und Bewegungsphysiologie der Pflanze. Springer-Verlag, Berlin, Göttingen, Heidelberg. 318-319 pp.
- Burrell, W. H., and W. G. Clarke. 1911. Botanical rambles in West Norfolk, with notes on the genus Utricularia. Trans. Norfolk and Norwich Nat. Soc. 9, Part 2:263-268.
- Büsgen, M. 1888. Ueber die Art und Bedeutung des Thierfangs bei Utricularia vulgaris L. Ber. deutsch. Bot. Ges. 6:55-63.
- Cain, S. A. 1944. Foundations of plant geography. Harper and Bros., New York. 261 pp.
- Carr, L. G. 1940. Further notes on coastal flora elements in the bogs of Augusta County, Virginia. Rhodora 42:86-93.
- Chandler, B. 1910. Utricularia emarginata Benj. Ann. Bot. 24: 549-555.
- Chapman, A. W. 1883. Flora of the southern United States. 2nd ed. Ivison, Blakeman, Taylor, and Co., New York. pp. 282-283.
- Chevalier, A. A. 1911. Lentibulariaceae. Bull. Soc. Bot. Fr.--Mémoires 20:186-188.
- _____. and F. Pellegrin. 1917. Lentibulariaceae. Bull. Soc. Bot. Fr.--Mémoires 8:276-277.

- Clarke, A. E. and B. A. Stone. 1960. Structure of the paramylon from Euglena gracilis. Biochim. Biophys. Acta 44:161.
- Cohn, F. 1875. Ueber die Function der Blasen von Aldrovanda und Utricularia. Beitr. Biol. Pflanzen 1:71-92.
- Cooley, W. W. and P. P. Lohnes. 1971. Multivariate data analysis. Wiley. New York. 364 pp.
- Compton, R. H. 1909. The morphology and anatomy of Utricularia brachiata Oliver. New Phytol. 8:117-130.
- Core, E. L. 1955. Plant taxonomy. Prentice-Hall, Inc., Englewood Cliffs, N.J.
- Correll, D. S., and H. B. Correll. 1972. Aquatic and wetland plants of the southwest United States. U.S. Govt. Printing Office, Washington, D.C.
- Crouan, F. 1858. Observations sur un mode particulier de propagation des Utricularia. Bull. Soc. Bot. Fr. 5:27-29.
- Currier, H. B. 1957. Callose substance in plant cells. Amer. J. Bot. 44:478-488.
- Czaja, A. Th. 1922. Die Fangvorrichtung der Utriculariablase. Bot. Zeit Jahrb. 14:705-729.
- _____. 1923. Ein allseitig geschlossenes, selektivpermeables System. Ber. deutsch. Bot. Ges. 40:381-385.
- Czech, G. 1952. Neue raxa, Kombinationen und vorkommen in Süd Afrika. Mitt. Bot. Staatssamml. München 1:344.
- Darwin, C. 1875. Insectivorous plants. John Murray Co., London.
- Dawson, G. 1960. Sinopsis de la especies Argentinas del genero Utricularia. J. Biol. Soc. Argent. 8:139-159.
- Dean, B. 1890. Report on the supposed fish-eating plant. Commissioners of Fisheries of the State of N.Y., Rept. 18:183-197.
- De Candolle, A. 1844. Lentibularieae. Prodromus Systematis Naturalis Regni Vegetabilis Vol. 8, 1-33 pp.
- Diannelidis, T. 1948. Beitrag zur Elektrophysiologie pflanzlicher Drüsen. Phyton 1:7-23.

- _____. and K. Umrath. 1953. Aktionsströme der Blase von Utricularia vulgaris. Protoplasma 42:58.
- Edgeworth, M. P. 1848. Description of a new genus of Lentibulariae. Proc. Linn. Soc. 1:351.
- Ekambaram, T. 1916. Irritability of the bladders in Utricularia. Agric. J. India 11 (special no.):72-79.
- _____. 1918. Utricularia flexuosa Vahl. Bot. Bull. Presidency Coll., Madras. Sept. 1918:1-21.
- _____. 1924. A note on the mechanism of the bladders of Utricularia. J. Indian Bot. Soc. 4:73-74.
- Esau, Katherine. 1973. Comparative structure of companion cells and phloem parenchyma cells in Mimosa pudica L. Ann. Bot. 37: 625-632.
- Exell, A. W., and A. Rozeira. 1958. Supplement to the flora of S. Tomé and Príncipe Islands. 6a. Conferencia Internacional dos Africanistas Ocidentais (Ilha de São Tomé, 1956) 3:85.
- Eyles, D. E., and J. L. Robertson. 1944. A guide and key to aquatic plants of the Southeastern United States. Public Health Bull. No. 286. Washington, D.C. U.S. Govt. Printing Office.
- Fassett, N. C. 1969. A manual of aquatic plants. Third ed.: appendix by E. C. Ogden. McGraw-Hill Book Co., New York.
- Fernald, M. L. 1941. Another century of additions to the flora of Virginia. Rhodora 43:642-645.
- _____. 1946. Contributions from the Gray Herbarium of Harvard University, No. CLX. Technical studies on North American plants. Rhodora 48:60.
- _____. 1950. Gray's manual of botany, 8th ed. American Book Co., New York.
- Fernandes, A. 1947. Utricularia subulata L. na flora Portuguesa. Anuario Soc. Bot. 13:5-16.
- Fineran, B. A. and May S. L. Lee. 1974. Ultrastructure of glandular hairs in traps of Utricularia monanthos. Eighth International Congress on Electron Microscopy, Canberra, 2:600-601.

- Fries, R. E. 1916. Wissenschaftliche Ergebnisse der Schwedischen Rhodesia-Kongo-Expedition 1911-12. Bot. Untersuch. 1:297-302.
- _____. 1924. Beiträge zur Kenntnis der Flora des Kenia, Mt. Aberdare und Mt. Elgon. Notizbl. Bot. Gart. Berl. 8:703.
- Gardner, G. 1846. Travels in the interior of Brazil, 1836-1841. London, 527, 528, 562 pp.
- Genevès, L., and Colette Vintéjoux. 1967. Sur la présence et l'organisation en un réseau tridimensionnel d'inclusions de nature protéique dans les noyaux cellulaires des hibernacles d'Utricularia neglecta L. (Lentibulariaceae). C. R. Acad. Sci. Paris 264:2750-2763.
- Gibbs, R. D. 1929. The trap of Utricularia. Torreyia 29:85-94.
- Gleason, H. A. 1931. Botanical results of the Tyler-Duida Expedition. Bull. Torr. Bot. Club 58:277-286.
- _____. 1952. Illustrated flora of the Northeastern United States and adjacent Canada. N.Y. Botanical Garden, New York.
- _____. 1968. The New Britton and Brown illustrated flora of the Northeastern United States and adjacent Canada. Vol. 3: The sympetalous dicotyledoneae. Hafner Publishing Co., Inc., New York.
- _____ and A. Cronquist. 1963. Manual of vascular plants of Northeastern United States and adjacent Canada. D. Van Nostrand Co., Inc., Princeton, N.J.
- Glück, H. 1902. Ueber die systematische Stellung und geographische Verbreitung der Utricularia ochroleuca R. Hartman. Ber. deutsch. Bot. Ges. 20:14-156.
- _____. 1906. Biologische und morphologische Untersuchungen über Wasser-und Sumpfgewächse. II. Untersuchungen über die mitteleuropäischen Utricularia--Arten, über die Turionbildung bei Wasserpflanzen, sowie über Ceratophyllum. Gustav Fisher Verlag, Jena 41:108, 213, 216-217, 335, 339, 348, 350, 352-353, 355.
- _____. 1913. Contributions to our knowledge of the species of Utricularia of Great Britain with special regard to the morphology and geographical distribution of Utricularia ochroleuca. Ann. Bot. 27:607-620.

- Goebel, K. 1889. Arbeiten aus dem botanischen Institut zu Marburg. IV. Der Aufbau von Utricularia. Flora Bot. Zeit. 72:291-297.
- _____. 1891. Morphologische und Biologische studien. V. Utricularia. Ann. du Jardin Bot. de Buitenzorg 9:41-126.
- _____. 1904. Morphologische und biologische Bemerkungen. 15. Regeneration bei Utricularia. Flora Bot. Zeit. 93:98-126.
- Goeppert, H. R. 1847. Ueber die Schläuche von Utricularia vulgaris und einen Farbestoff in denselben. Bot. Zeit. Jahrb. 5: 721-726.
- Haas, T. P. 1947. Observations on Utricularia inflata and Utricularia cleistogamea, a contribution to the biology of Utricularia. Amer. J. Bot. 34:583-584 (Abstr.).
- Hach Chemical Company. 1965. DR-EL Manual, fourth ed. Edited by Hach Chemical Co., Ames, Iowa. 53 pp.
- Hada, Y. 1930. The feeding habits of Utricularia. Trans. Sapporo Nat. Hist. Soc. 11:175-183.
- Harborne, J. B. 1964. Biochemistry of phenolic compounds. Academic Press, New York. 618 pp.
- Harborne, J. B. 1958. Spectral methods of characterizing anthocyanins. Biochem. J. 70:22-28.
- Harder, R. 1963. Blütenbildung durch tierische Zusatznahrung und andere Faktoren bei Utricularia exoleta R. Braun. Planta 59:459-471.
- Hartman, R. 1857. De Svenska arterna av slägtet Utricularia. Bot. Not. 2:25-32.
- Hayat, M. A. 1973. Electron microscopy of enzymes. I. Principles and methods. Van Nostrand Reinhold, Co., New York. 201 pp.
- Hegner, R. W. 1926. The interrelations of protozoa and the utricles of Utricularia. Biol. Bull. Woods Hole 50:239-270.
- Heslop-Harrison, J. 1964. Cell walls, cell membranes and protoplasmic connections during meiosis and pollen development, in H. F. Linskens (ed.) Pollen physiology and fertilisation. Amsterdam: North Holland. 39-47 pp.

- _____. 1966. Cytoplasmic continuities during spore formation in flowering plants. *Endeavour* 25:65-72.
- Hoehne, F. C., and J. G. Kuhlmann. 1918. Utriculáias do Rio de Janeiro e seus arredores. *Mém. Inst. Butantan* 1:5-26.
- Holland, M. 1868. (Utricularia). *Quart. Mag. High Wycombe Nat. Hist. Soc.* July:5.
- Hovelacque, M. 1888. Recherches sur l'appareil végétatif des Bignoniacées, Rhinanthacées, Orobanchées et Utriculariées. Recherches dans l'Appareil Vegetale, *Lib. Acad. Med. Paris*. 675 pp.
- Hutchinson, J., and J. M. Dalziel. 1931. Flora of west tropical Africa. 2:231-236.
- Im Thurn, E. F., and D. Oliver. 1887. The botany of the Roraima expedition of 1884. *Trans. Linn. Soc. London, Ser. II*, 2: 249-300.
- Irmisch, T. 1858. Botanische Mittheilungen. I. Ueber Utricularia minor. *Flora Bot. Zeit.* 41:33-37.
- Jensen, W. A. 1962. Botanical histochemistry. W. H. Freeman Co., San Francisco, Calif.
- Kamienski, Fr. 1877. Vergleichende Untersuchungen über die Entwicklungsgeschichte der Utricularien. *Bot. Zeit. Jahrb.* 35:761-776.
- _____. 1890. Recherches sur la famille der Lentibulariées (Utriculariées). *Zap. Novoross. Obsch. Est.* 12:179-210.
- _____. 1894. Neue und unbeschriebene Arten der Gattung Utricularia. *Ber. deutsch. Bot. Ges.* 12:3-7.
- _____. 1904. Lentibulariaceae Africanæ. *Bot. Jahrb.* 33:92-113.
- Kausik, S. B. 1938. Pollen development and seed formation in Utricularia coerulea L. *Beih. Bot. Zbl.* 58:365-378.
- _____ and M. V. S. Raju. 1956. Variations in development of proembryo in Utricularia coerulea L. *Curr. Sci. India* 25: 296-297.
- Khan, R. 1954. A contribution to the embryology of Utricularia flexuosa Vahl. *Phytomorphology* 4:80-117.

- Killian, C. 1953. Contribution à l'étude de biologie de quelques Utricularia tropicaux. Bull. I. F. A. N. 15:72-82.
- Kondo, K. 1971a. Germination and developmental morphology of seeds in Utricularia cornuta Michx. and Utricularia juncea Vahl. Rhodora 73:541-547.
- _____. 1971b. Chromosome number of Utricularia resupinata B. D. Green (Lentibulariaceae). J. Jap. Bot. 46:26-29.
- _____. 1972a. A comparison of variability in Utricularia cornuta and Utricularia juncea. Amer. J. Bot. 59:23-37.
- _____. 1972b. Chromosome number of Utricularia subulata L. J. Jap. Bot. 47:31-32.
- Kreger, D. R. and B. I. D. Meeuse. 1952. X-ray diagrams of Euglena-paramylon, of the acid insoluble glucan of yeast cell walls and of laminarin. Biochim. Biophys. Acta 9:699-700.
- Kumazawa, M. 1967. An experimental study on the seedling of Utricularia pilosa Makino. Phytomorphology 17:494-498.
- Kurz, Luise. 1959. Anatomische und entwicklungsphysiologische Untersuchungen an Utricularia. Beitr. Biol. Pflanzen 35: 111-135.
- Le Conte, J. 1823. Observations on the North American species of the genus Utricularia. Ann. Lyc. Nat. Hist. N.Y. 1:72-79.
- Lewis, W. H., H. L. Stripling, and R. G. Ross. 1962. Chromosome numbers for some Angiosperms of the southern United States and Mexico. Rhodora 64:147-161.
- Lloyd, F. E. 1929a. The mechanism of the water-tight door of the Utricularia trap. Plant Physiol. 4:87-102.
- _____. 1929b. The resistance of the door of the Utricularia trap to water-pressure. Proc. Linn. Soc. (Jan. 17).
- _____. 1930. The structure of the trap of Utricularia capensis. J. Bot. Soc. S. Afr. 16:5-10.
- _____. 1931a. The range of structural and functional variation of the "traps" of Utricularia. Proc. 5th Internat. Bot. Congr., Cambridge, 1930:450-451.
- _____. 1931b. The range of structural and functional variation in the traps of Utricularia. Flora 125:260-276.

- Lloyd, F. E. 1932a. The range of structural and functional variety in the traps of Utricularia and Polypompholyx. *Flora* 126:303-328.
- _____. 1932b. Is the door of Utricularia an irritable mechanism? *Canad. J. Res.* 7:386-425.
- _____. 1933. The structure and behavior of Utricularia purpurea. *Canad. J. Res.* 8:234-252.
- _____. 1935. Utricularia. *Biol. Rev.* 10:72-110.
- _____. 1936. Notes on Utricularia, with special reference to Australia. *Victorian Nat.* 53:91-112.
- _____. 1942. The carnivorous plants. *Chronica Botanica Co.*, Waltham, Mass.
- _____ and G. Taylor. 1947. Some new species of Utricularia. *Contr. Gray Herb.* 165:82-90.
- Lollar, Anne Q., D. C. Coleman, and C. E. Boyd. 1971. A carnivorous pathway of phosphorous uptake by Utricularia inflata. *Arch. Hydrobiol.* 69:400-404.
- Luetzelburg, P. 1910. Beiträge zue Kenntniss der Utricularien. *Flora Bot. Zeit.* 100:145-212.
- Lüttge, U. 1971. Structure and function of plant glands. *Ann. Rev. Plant Phys.* 22:23-43.
- Mason, L. 1957. A flora of the marshes of California. 733-741 pp.
- Matheson, R. 1930. The utilization of aquatic plants as aids in mosquito control. *Amer. Nat.* 64:56-86.
- Meierhofer, H. 1902. Beiträge der Anatomie und Entwicklungsgeschichte der Utricularia--Blasen. *Flora Bot. Zeit.* 90:84-113.
- Meister, F. 1900. Beiträge zur Kenntnis der europäischen Arten von Utricularia. *Mém. de l'Herbier Boissier, Chambessey*, No. 12, 40 pp.
- Merl, E. M. 1915. Beiträge zur Kenntnis der Utricularien und Genliseen. *Flora Bot. Zeit.* 108:127-200.
- _____. 1921. Biologische Studien über die Utriculariablase. *Flora Bot. Zeit.* 115:59-74.

- Merl, E. M. 1925. Beiträge zur Kenntnis der brasilianischen Utricularien. Flora Bot. Zeit. 118-119:386-392.
- Merz, M. 1897. Untersuchungen über die Samenentwicklung der Utricularieen. Flora Bot. Zeit. 84:69-87.
- Meyer, E. H. F. 1837. Commentariorum de plantis Africae Australioris. Universitäts Königsberg, Königsberg. 281-282 pp.
- Mildbraed, J. 1922. Wissenschaftliche Ergebnisse der Zweiten Deutschen Zentral-Afrika-Expedition 1911-12. Botanik 2:146.
- Mohan Ram, H. Y., H. Hanada, and J. P. Nitsch. 1972. Studies on growth and flowering in axenic culture of insectivorous plants. III. Effects of photoperiod, ethrel morphactin, and a few other growth substances and metabolic inhibitors on Utricularia inflexa. Z. Pflanzenphysiol. 68:235-253.
- Mollenhauer, H. H. 1964. Plastic embedding mixtures for electron microscopy. J. Stain Technol. 39:111.
- Moyle, J. B. 1945. Some chemical factors influencing the distribution of aquatic plants in Minnesota. Amer. Mid. Nat. 34:402-420.
- Muenscher, W. C. 1944. Aquatic plants of the United States. Comstock Publishing Co., Inc., Ithaca, New York. 374 pp.
- Nieuwland, J. A. 1914. Critical notes on new and old genera of plants. Amer. Mid. Nat. 3:189-190.
- Nold, R. H. 1934. Die Funktion der Blase von Utricularia vulgaris. Beih. Bot. Zbl. 52:415-448.
- Oliver, D. 1860. Descriptions of new species of Utricularia from South America, with notes upon the genera Polypompholyx and Akentra. J. Linn. Soc. Bot. 4:169-176.
- _____. 1867. On the Lentibularieae collected in Angola by Dr. Welwitsch, A. L. S., with an enumeration of the African species. J. Linn. Soc. Bot. 9:144-156.
- Pellegrin, F. 1914. Contribution à l'étude de la flore de l'Afrique occidentale française: Lentibulariées. Bull. Soc. Bot. Fr. 61:13-21.
- _____. 1930. Lentibulariacées. Fl. Gen. de l'Indo-Chine. Le Comte. 4:467-487.

- Perrier de la Bathie, H. 1955a. Lentibulariacées. Fl. Madagascar et des Comores. Humbert. 1-22 pp.
- _____. 1955b. Revisions des Lentibulariacées de Madagascar. Mem. Inst. Sci. Madag., Sér. B, 5:187-200.
- Phillips, E. P. 1917. A contribution to the flora of the Leribe Plateau and Environs, etc. Ann. S. Afr. Mus. 16:230.
- Pringsheim, N. 1869. Über die Bildungsvorgänge am Vegetationskegel von Utricularia vulgaris. Monatsbêr. d. k. preuss. Akad. D. Wiss., Berlin 1869:92-116.
- _____. 1888. Ueber die Entstehung der Kalkincrustationen an Süßwasserpflanzen. Jahrb. Wiss. Bot. 19:138-154.
- Pringsheim, E. G., and O. Pringsheim. 1962. Axenic culture of Utricularia. Amer. J. Bot. 49:898-901.
- _____ and _____. 1967. Kleiner Beitrag zur Physiologie von Utricularia. Z. Pflanzenphysiol. 57:1-10.
- Prior, S. 1939. Carnivorous plants and the man-eating tree. Field Mus. Bot. Lflt. no. 23.
- Reese, G. 1952. Ergänzendende Mitteilungen über die Chromosomenzahlen mitteleuropäischer Gefasspflanzen. Ber. deutsch. Bot. Ges. 64:250-257.
- Reinert, G. W., and R. K. Godfrey. 1962. Reappraisal of Utricularia inflata and U. radiata (Lentibulariaceae). Amer. J. Bot. 49:213-220.
- Reinsch, P. 1859. Ueber den Bau und die Entwicklung der Blätter und der Schläuche von Utricularia vulgaris L. Denkschr. der kgl. bayer. bot. Ges. zu Regensburg. 4:368-402.
- Ridley, H. N. 1888. On the foliar organs of a new species of Utricularia from St. Thomas, West Africa. Ann. Bot. 2:305-308.
- Roszbach, G. G. 1939. Aquatic Utricularias. Rhodora 41:113-128.
- Schenck, H. 1887. Beiträge zur Kenntniss der Utricularien. Utricularia montana Jacq. und Utr. Schimperii nov. spec. Jahrb. Wiss. Bot. 18:218-235.

- Schimper, A. F. W. 1882. Notizen über insektenfressende Pflanzen. Bot. Zeit. Jahrb. 40:241-243.
- Schrank, R. 1824. Botanische Beobachtungen. Flora Bot. Zeit. 7:1-9.
- Schultz, F. 1873. Beiträge zur Flora der Pfalz. Flora. Bot. Zeit. 56:247-251.
- Sculthorpe, C. D. 1967. The biology of aquatic vascular plants. Edward Arnold Publishers, London. 610 pp.
- Shumacher, G. 1960. Further notes on the occurrence of desmids in Utricularia bladders. Castanea 25:62-65.
- Skutch, A. F. 1928. The capture of prey by the bladderwort: A review of the physiology of the bladders. New Phytol. 27: 261-297.
- Slinger, J. 1954. The morphology and anatomy of Utricularia transrugosa Stapf. Bothalia 6:385-406.
- Smith, J. E. 1819. Utricularia. Rees, Cyclopaedia. 37 pp.
- Sorenson, D., and W. T. Jackson. 1968. The utilization of paramecia by the carnivorous plant Utricularia gibba. Planta 83:166-170.
- Spruce, R. 1908. Notes of a botanist on the Amazon and Andes, during the years 1849-1864. Wallace, London.
- Spurlock, B. O., M. S. Skinner, and A. A. Kattine. 1966. A simple rapid method for staining epoxy-embedded specimens for light microscopy with the polychromatic stain Paragon-1301. Amer. J. Clin. Pathol. 46:252-258.
- Stapf, O. 1906. Lentibularieae. Flora of Tropical Africa. Dyer, London 4:468-499, 574-575.
- _____. 1908. Eine neue Utricularia von Kingagebirge. Bot. Jahrb. Syst. Pflanzengesch. Pflanzengeogr. 40:60.
- _____. 1912. Diagnoses Africanæ, L. Bull. Misc. Inf. Kew 1912:331.
- _____. 1916. Diagnoses Africanæ, LXV. Bull. Misc. Inf. Kew 1916:41.

- Stempak, J. G., and R. T. Ward. 1964. An improved staining method for electron microscopy. *J. Cell. Biol.* 22:697-701.
- Stephens, Edith L. 1923. Carnivorous plants of the Cape Peninsula. *J. Bot. Soc. S. Africa* 9:20-24.
- _____. 1938. Notes on three South African terrestrial *Utricularia*ae. *J. S. Afr. Bot.* 4:47-51.
- Steyermark, J. A. 1953. Botanical exploration in Venezuela, III. *Fieldiana: Bot.* 28:534-553.
- Suessenguth, K. 1951. *Lentibulariaceae* in Suessenguth and Merxmüller, a contribution to the flora of the Marandellas District, Southern Rhodesia. *Trans. Rhod. Sci. Assoc.* 43: 118-120.
- Sydenham, P. H., and G. P. Findlay. 1973. The rapid movement of the bladder of *Utricularia* sp. *Austral. J. Biol. Sci.* 26: 1115-1126.
- Sylvén, N. 1909. Die Genliseen und *Utricularien* des Regnell'schen herbariums. *Ark. Bot.* 8:1-48.
- Taylor, P. 1954. *Lentibulariaceae*: Brenan and collaborators, plants collected by the Vernay Nyasaland expedition of 1946. *Mem. N.Y. Bot Gard.* 9:15-17.
- _____. 1961. Notes on *Utricularia*. *Mitt. Bot Staatssamml. München* 4:95-106.
- _____. 1963. New taxa and combinations in West African *Lentibulariaceae* and *Gentianaceae*. *Taxon* 12:293-294.
- _____. 1964. The genus *Utricularia* L. (*Lentibulariaceae*) in Africa (south of the Sahara) and Madagascar. *Kew Bull.* 18:1-245.
- _____. 1967. *Lentibulariaceae*: Botany of the Guayana Highland, Part VII. *Mem. N.Y. Bot Gard.* 17:201-228.
- Treat, Mary. 1875. Plants that eat animals. *Gardener's Chronicle* 6 March 1875:303-304 pp.
- _____. 1876. Is the valve of *Utricularia* sensitive? *Harper's New Monthly Mag.* 52:382-387.

- Treviranus, L. C. 1848. Noch etwas über die Schläuch der Utricularien. Bot. Zeit. Jahrb. 6:444-448.
- Ule, E. 1898. Ueber Standortsanpassungen einiger Utricularien in Brasilien. Ber. deutsch. Bot. Ges. 16:308-314.
- Unzelman, J. M., and P. L. Healey. 1972. Development and histochemistry of nuclear crystals in the secretory trichome of Pharbitis nil. J. Ultrastruct. Res. 39:301-309.
- Uttal, L. J. 1956. Notes on Utricularia biflora and U. fibrosa. Rhodora 58:41-43.
- Van-Tieghem, P. 1868. Anatomie de l'Utriculaire commune. Bull. Soc. Bot. Fr. 15:158-162.
- Vintéjoux, Colette. 1970a. Comportement des inclusions intranucleaires cristallines dans les cellules méristématiques et différenciées, au cours de la germination des hibernacles d'Utricularia neglecta L. (Lentibulariacées). C. R. Acad. Sci. Paris, Ser. D. 270:2438-2440.
- _____. 1970b. Localisation d'une activité phosphatasique acide dans les inclusions cytoplasmiques des feuilles d'hibernacles chez l'Utricularia neglecta L. (Lentibulariacées). C. R. Acad. Sci. Paris, Ser. D. 270:3213-3216.
- Wagner, V. A. 1928. The resting buds of Utricularia stellaris. Trans. Roy. Linn. Soc. S. Afr. 16:204.
- Waterkeyn, L. and A. Bienfait. 1970. On a possible function of the callosic special wall in Ipomoea purpurea (L) Roth. Grana 10:13-20.
- Weatherby, C. A. 1945. Utricularia inflata var. minor in Canada. Rhodora 47:235.
- Wight, R. 1849. Conspectus of Indian Utriculariae. Kew Gard. Misc. 1:372-374.
- Williams, S. E. and Barbara G. Pickard. 1974. Connections and barriers between cells of Drosera tentacles in relation to their electrophysiology. Planta 116:1-16.
- Withycombe, C. L. 1916. Observations on the bladderwort. Knowledge 13:238-241.
- _____. 1924. On the function of the bladders in Utricularia vulgaris. J. Linn. Soc. Bot. 46:401-413.

ACKNOWLEDGEMENTS

It would be impossible to thank all the many individuals who have contributed to this accomplishment but the author wishes to thank, particularly, the following: my parents, Mr. and Mrs. Robert L. Beltz who first exposed me to the beauty of life and its complexity and who have shared both the pleasures and agonies of this accomplishment with loving support; my uncle, Mr. Jay L. Morris and grandparents, Mr. and Mrs. O. Morris and Mrs. Rosa Beltz for their love and financial support.

Special acknowledgement is extended to Dr. Clay Dine, who has throughout my life been a constant source of encouragement. I wish to thank the following friends who have through professional assistance and personal encouragement contributed to this accomplishment: Rhonda Riggins-Pimentel, Ken Laser, Cal Wiemers, Glen Crum, Don Roeder, Dean Roosa, Robert Cecich, Mike Cousens, Woody Begres, Don Bechtel, Valerie Nelson, Lou Facto, Ray Franklin, Rev, Evans Moreland, Jim and Evangeline Guest, and Bill McCracken.

Additional thanks is extended to Dr. Harry T. Horner, Jr., my major professor, who has more than any contributed to my professional growth, always willing to consider what was most important to my welfare and future happiness, and commendable in his patience in dealing with my individual idiosyncrasies; Dr. Nels Lersten and Dr. Lois Tiffany who have also taken exceptional interest in my endeavors and served both as friends and professional advisors; Dr. Roger Bachmann and Dr. Cecil Stewart who have willingly served on my

committee and have assisted me on numerous occasions; Dr. Fred Smith and Dean D. J. Zaffarano for their endeavors in obtaining financial support for my research; Dr. John Dodd for use of his growth chambers and Hach Chemical Portable Engineers Laboratory; Dr. Jean Wooten and Dr. Donald Nevins for assistance with the anthocyanin determination; Dr. Richard Pimentel for assistance with the statistical analysis; Dr. Duane Isely and Dr. Peter Taylor for their assistance with the taxonomy; Dr. Ruth Wildman and Dr. George Knaphus for their encouragement and professional interest; Chris Pearson for help with the maintenance of my plants in my absence; Mr. Paul Elsner for his maintenance and advice regarding equipment, and especially for his assistance, along with Dr. Horner's with the SEM results; and Mrs. Anita Serovy for typing the manuscript.

I wish to thank the following organizations for financial support or use of equipment: Sigma Xi, McBride Fellowships, Iowa State Research Dissertation Assistanceships, and all of the SEM companies listed in the Materials and Methods section.

Lastly, I am grateful to all those who helped me medically and through personal support following my automobile accident. I am grateful that I could live and fulfill the confidence they displayed in my ability to return and complete my graduate program. Although I accept responsibility for the expression of this work, much of it belongs to those named and many more too numerous to mention . . . I thank you all.

The influence of natural environmental conditions on the
growth, distribution, and morphological expression of
the carnivorous, aquatic plant Utricularia macrorhiza
Le Conte, with light and electron microscopic
observations of bladder ontogeny

by

Carolyn Kay Beltz

Volume 2 of 2

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Botany and Plant Pathology
Major: Botany (Morphology)

Approved:

Harry T. Homer, Jr.
In Charge of Major Work

W. J. Smith
For the Major Department

D. J. Z. J. J. J.
For the Graduate College

Iowa State University
Ames, Iowa

1974

Copyright © Carolyn Kay Beltz, 1975 All rights reserved.

TABLE OF CONTENTS

	Page
APPENDIX A: TABLES FOR THE LITERATURE CITED	160
APPENDIX B: FIELD METHODS	175
APPENDIX C: LABORATORY TECHNIQUES	179
APPENDIX D: KEY TO FIGURES	188
APPENDIX E: FIGURES	191

APPENDIX A:
TABLES FOR THE LITERATURE CITED

Table A1. Survey of species and investigators

<u>Utricularia</u> species	Investigators
<hr/>	
<u>U. adpressa:</u>	Taylor, P. 1967; Kamienski, F. 1890
<u>U. affinis:</u>	Goebel, K. 1889, 1891; Kamienski, F. 1890; Merz, M. 1897.
<u>U. albida:</u>	Lloyd, F. E. 1936
<u>U. albiflora:</u>	Lloyd, F. E. 1936.
<u>U. albina:</u>	Lloyd, F. E. 1932a,b.
<u>U. alpina:</u>	Taylor, P. 1967.
<u>U. Amazonasana:</u>	Steyermark, J. A. 1953.
<u>U. amethystina:</u>	Taylor, P. 1967.
<u>U. angolensis:</u>	Kamienski, F. 1904.
<u>U. appendiculata:</u>	Bruce, E. A. 1934.
<u>U. arenaria:</u>	Oliver, D. 1867.
<u>U. arenicola:</u>	Steyermark, J. A. 1953.
<u>U. augustifolia:</u>	Oliver, D. 1860.
<u>U. aureolimba:</u>	Steyermark, J. A. 1953.
<u>U. aureomaculata:</u>	Steyermark, J. A. 1953; Taylor, P. 1967.
<u>U. ayacuchae:</u>	Steyermark, J. A. 1953.
<u>U. baoulensis:</u>	Chevalier, A. 1911; Pellegrin, F. 1914.
<u>U. baumii:</u>	Kamienski, F. 1904; Pellegrin, F. 1914.
<u>U. benjaminiana:</u>	Kamienski, F. 1904; Taylor, P. 1967.
<u>U. bicolor:</u>	Oliver, D. 1860; Sylvén, N. 1909; Merl, E. M. 1915.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. bifida</u> :	Goebel, K. 1891; Merz, M. 1897.
<u>U. biflora</u> :	Uttal, L. J. 1956.
<u>U. biloba</u> :	Lloyd, F. E. 1936.
<u>U. bipartita</u> :	Chapman, A. W. 1883.
<u>U. bisquamata</u> :	Schrank, R. 1824.
<u>U. bolivarana</u> :	Steyermark, J. A. 1953.
<u>U. brachiata</u> :	Compton, R. H. 1909.
<u>U. brachyceras</u> :	Kamienski, F. 1904; Stephens, E. L. 1938.
<u>U. bremii</u> :	Hartman, R. 1857; Buchenau, F. 1865; Schultz, F. 1873; Meierhofer, H. 1902; Glück, H. 1913.
<u>U. bryophila</u> :	Ridley, H. N. 1888; Goebel, K. 1889, 1891; Kamienski, F. 1904; Pellegrin, F. 1914.
<u>U. calliphysa</u> :	Lloyd, F. E. 1936.
<u>U. calycififa</u> :	Taylor, P. 1967.
<u>U. campbelliana</u> :	Merl, E. M. 1915; Lloyd, F. E. 1936; Steyermark, J. A. 1953; Taylor, P. 1967.
<u>U. capensis</u> :	Oliver, D. 1867; Kamienski, F. 1904; Lloyd, F. E. 1931a,b, 1932a,b, 1936; Stephens, E. L. 1938.
<u>U. capilliflora</u> :	Lloyd, F. E. 1936.
<u>U. chiribiquiyensis</u> :	Taylor, P. 1967.
<u>U. clandestina</u> :	Darwin, C. 1875; Kamienski, F. 1890.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. cleistogama</u> :	Treat, Mary 1875; Haas, T. P. 1947.
<u>U. coerulea</u> :	Schrank, R. 1824; Oliver, D. 1867; Kamienski, F. 1890; Goebel, K. 1891, 1904; Lloyd, F. E. 1936; Kausik, S. B. 1938; Kausik, S. B. and M. V. S. Raju 1956.
<u>U. colorata</u> :	Sylvén, N. 1909; Merl, E. M. 1915.
<u>U. conferta</u> :	Kamienski, F. 1904.
<u>U. congesta</u> :	Steyermark, J. A. 1953.
<u>U. cornuta</u> :	Schimper, A. F. W. 1882; Chapman, A. W. 1883; Lloyd, F. E. 1931a,b, 1936; Kondo, K. 1971a,b, 1972a,b.
<u>U. cucullata</u> :	Sylvén, N. 1909; Taylor, P. 1967.
<u>U. cuspidata</u> :	Steyermark, J. A. 1953.
<u>U. cyanea</u> :	Lloyd, F. E. 1936.
<u>U. cymbantha</u> :	Oliver, D. 1867; Lloyd, F. E. 1936.
<u>U. deightonii</u> :	Lloyd, F. E. 1936; Lloyd, F. E. and G. Taylor 1947.
<u>U. delicata</u> :	Kamienski, F. 1904.
<u>U. delicatula</u> :	Lloyd, F. E. 1936.
<u>U. diantha</u> :	Oliver, D. 1867.
<u>U. dichotoma</u> :	Kamienski, F. 1890; Merl, E. M. 1915; Lloyd, F. E. 1936.
<u>U. diploglossa</u> :	Oliver, D. 1867; Kamienski, F. 1904.
<u>U. dregei</u> :	Kamienski, F. 1904.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. dunstanii</u> :	Lloyd, F. E. 1936
<u>U. dunsenii</u> :	Sylvén, N. 1909; Merl, E. M. 1915, 1925.
<u>U. ecklonii</u> :	Oliver, D. 1867; Stephens, E. L. 1938; Taylor, P. 1954.
<u>U. elachista</u> :	Goebel, K. 1891; Merz, M. 1897.
<u>U. elevata</u> :	Kamienksi, F. 1904.
<u>U. emarginata</u> :	Sylvén, N. 1909; Chandler, B. 1910; Merl, E. M. 1915.
<u>U. engleri</u> :	Kamienksi, F. 1904.
<u>U. erectiflora</u> :	Taylor, P. 1967.
<u>U. exilis</u> :	Oliver, D. 1867; Kamienksi, F. 1904; Pellegrin, F. 1914; Taylor, P. 1954.
<u>U. exoleta</u> :	Goebel, K. 1891, 1904; Merz, M. 1897; Kamienksi, F. 1904; Glück, H. 1913; Pellegrin, F. 1914; Lloyd, F. E. 1936; Bünning, E. 1953; Pringsheim, E. and O. Pringsheim 1962, 1967; Harder, R. 1963.
<u>U. fasciculata</u> :	Kamienksi, F. 1890.
<u>U. fernaldiana</u> :	Lloyd, F. E. and G. Taylor 1947.
<u>U. fibrosa</u> :	Chapman, A. W. 1883; Uttal, L. J. 1956.
<u>U. fimbriata</u> :	Taylor, P. 1967.
<u>U. firmula</u> :	Oliver, D. 1867; Kamienksi, F. 1904; Pellegrin, F. 1914.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. flaccida</u> :	Taylor, P. 1967.
<u>U. flexuosa</u> :	Goebel, K. 1891; Kamienski, F. 1904; Ekambaram, T. 1916, 1918, 1924; Merl, E. M. 1921; Skutch, A. F. 1928; Lloyd, F. E. 1936; Bünning, E. 1953.
<u>U. foliosa</u> :	Oliver, D. 1860; Kamienski, F. 1904; Sylvén, N. 1909; Pellegrin, F. 1914; Merl, E. M. 1915; Shumacher, G. J. 1960; Taylor, P. 1967.
<u>U. fontana</u> :	Treviranus, L. C. 1848.
<u>U. geminiloba</u> :	Kamienski, F. 1890; Merl, E. M. 1915.
<u>U. gibba</u> :	Chapman, A. W. 1883; Kamienski, F. 1890; Gibbs, R. D. 1929; Lloyd, F. E. 1931a,b, 1932; Shumacher, G. J. 1960; Taylor, P. 1967; Sorenson, D. R. and W. T. Jackson 1968.
<u>U. gibbseae</u> :	Lloyd, F. E. 1931.
<u>U. globulariaefolia</u> :	Sylvén, N. 1909; Merl, E. M. 1915; Lloyd, F. E. 1931a,b, 1936.
<u>U. graniticola</u> :	Pellegrin, F. 1914; Chevalier, A. and F. Pellegrin 1917.
<u>U. guyanesis</u> :	Taylor, P. 1967.
<u>U. hamiltoni</u> :	Lloyd, F. E. 1936.
<u>U. heterochroma</u> :	Steyermark, J. A. 1953; Taylor, P. 1967.
<u>U. hispida</u> :	Taylor, P. 1967.
<u>U. holtzii</u> :	Lloyd, F. E. 1936.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. hookeri</u> :	Treviranus, L. C. 1848; Lloyd, F. E. 1932a,b.
<u>U. humboldtii</u> :	Im Thurn, E. F. and D. Oliver 1887; Kamienski, F. 1890; Lloyd, F. E. 1936; Steyermark, J. A. 1953; Taylor, P. 1967.
<u>U. hydrocarpa</u> :	Kamienski, F. 1890; Taylor, P. 1967.
<u>U. hydrocotylodes</u> :	Lloyd, F. E. and G. Taylor 1947.
<u>U. ibarensis</u> :	Baker, J. G. 1886; Kamienski, F. 1904.
<u>U. inaequalis</u> :	Treviranus, L. C. 1848.
<u>U. incerta</u> :	Kamienski, F. 1904.
<u>U. inflata</u> :	Benjamin, E. 1848; Treviranus, L. C. 1848; Chapman, A. W. 1883; Kamienski, F. 1890, 1894; Weatherby, C. A. 1945; Haas, T. P. 1947; Reinert, G. W. and R. K. Godfrey 1962; Taylor, P. 1967.
<u>U. inflexa</u> :	Benjamin, L. 1848; Treviranus, L. C. 1848; Kamienski, F. 1894, 1904; Merz, M. 1897; Mohan Ram, H. Y., H. Harada, and J. P. Nitsch 1972.
<u>U. intermedia</u> :	Benjamin, L. 1848; Hartman, R. 1857; Crouan, F. 1858; Buchenau, F. 1865; Schultz, F. 1873; Meierhofer, H. 1902; Glück, H. 1913; Hada, Y. 1930; Lloyd, F. E. 1936; Kurz, L. 1959.
<u>U. jamesoniana</u> :	Oliver, D. 1860; Ridley, H. N. 1888; Kamienski, F. 1890; Taylor, P. 1967.
<u>U. juncea</u> :	Taylor, P. 1967; Kondo, K. 1971a,b, 1972a,b.
<u>U. kirkii</u> :	Lloyd, F. E. 1931a,b, 1936.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. kuhlmanni</u> :	Merl, E. M. 1915.
<u>U. lasiocaulis</u> :	Lloyd, F. E. 1936.
<u>U. lateriflora</u> :	Kamienksi, F. 1890; Merl, E. M. 1915; Lloyd, F. E. 1932a,b, 1936.
<u>U. lawsoni</u> :	Lloyd, F. E. 1936.
<u>U. laxa</u> :	Taylor, P. 1967.
<u>U. lehmanni</u> :	Oliver, D. 1867.
<u>U. linarioides</u> :	Oliver, D. 1867; Kamienksi, F. 1904.
<u>U. lindmanii</u> :	Sylvén, N. 1909.
<u>U. lingulata</u> :	Baker, J. G. 1884.
<u>U. livida</u> :	Oliver, D. 1867; Kamienksi, F. 1904.
<u>U. lloydii</u> :	Lloyd, F. E. 1932a,b, 1936.
<u>U. longecalcarata</u> :	Oliver, D. 1867; Kamienksi, F. 1904.
<u>U. longiciliata</u> :	Oliver, D. 1860; Sylvén, N. 1909; Merl, E. M. 1915; Lloyd, F. E. 1932a,b, 1936; Taylor, P. 1967.
<u>U. longifolia</u> :	Goebel, K. 1889, 1891, 1904; Ule, E. 1898; Kamienksi, F. 1890; Sylvén, N. 1909; Merl, E. M. 1915; Lloyd, F. E. 1931a,b.
<u>U. lundii</u> :	Sylvén, N. 1909.
<u>U. macrorrhiza</u> :	Le Conte, J. 1823.
<u>U. malmeana</u> :	Sylvén, N. 1909; Merl, E. M. 1915.
<u>U. manni</u> :	Oliver, D. 1860.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. maxima</u> :	Kamienski, F. 1890.
<u>U. menziesii</u> :	Treviranus, L. C. 1848; Kamienski, F. 1890; Lloyd, F. E. 1936.
<u>U. meyeri</u> :	Sylvén, N. 1909.
<u>U. micropetala</u> :	Oliver, D. 1867; Kamienski, F. 1904; Pellegrin, F. 1914; Killian, C. 1953; Taylor, P. 1963.
<u>U. minor</u> :	Benjamin, L. 1848; Hartman, R. 1857; Crouan, F. 1858; Irmisch, T. V. 1858; Buchenau, F. 1865; Schultz, F. 1873; Darwin, C. 1875; Kamienski, F. 1890, 1904; Meierhofer, H. 1902; Goebel, K. 1904; Glück, H. 1913; Barnhart, J. H. 1915; Lloyd, F. E. 1929a,b, 1936; Hada, Y. 1930; Reese, G. 1952; Pringsheim, E. and O. Pringsheim 1967.
<u>U. modesta</u> :	Sylvén, N. 1909; Merl, E. M. 1915.
<u>U. monanthos</u> :	Lloyd, F. E. 1936.
<u>U. montana</u> :	Oliver, D. 1860; Schenck, H. 1887; Kamienski, F. 1890; Goebel, K. 1889, 1891, 1904; Lloyd, F. E. 1936.
<u>U. moorei</u> :	Lloyd, F. E. 1936.
<u>U. muelleri</u> :	Kamienski, F. 1894, 1904.
<u>U. multicaulis</u> :	Lloyd, F. E. 1936.
<u>U. myriocista</u> :	Taylor, P. 1967.
<u>U. nana</u> :	Sylvén, N. 1909; Merl, E. M. 1915; Lloyd, F. E. 1932a,b; Taylor, P. 1967.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. naviculata</u> :	Taylor, P. 1967.
<u>U. neglecta</u> :	Benjamin, L. 1848; Hartman, R. 1857; Buchenau, F. 1865; Schultz, F. 1873; Darwin, C. 1875; Kamienski, F. 1904; Glück, H. 1913; Skutch, A. F. 1928; Reese, G. 1952; Genevès, L. and C. Vintéjoux 1967; Vintéjoux, C. 1970.
<u>U. nelumbifolia</u> :	Ule, E. 1898; Merl, E. M. 1915.
<u>U. neottiioides</u> :	Oliver, D. 1860; Kamienski, F. 1890; Merz, M. 1897; Sylven, N. 1909; Lloyd, F. E. 1932a,b, 1936; Taylor, P. 1967.
<u>U. nervosa</u> :	Kamienski, F. 1890.
<u>U. nigrescens</u> :	Syiven, N. 1909.
<u>U. novae-zealandiae</u> :	Lloyd, F. E. 1936.
<u>U. obtusa</u> :	Syiven, N. 1909.
<u>U. ochroleuca</u> :	Hartman, R. 1857; Glück, H. 1902, 1913; Reese, G. 1952; Pringsheim, E. and O. Pringsheim 1967.
<u>U. odontosepala</u> :	Stapf, O. 1912.
<u>U. odontosperma</u> :	Taylor, P. 1954.
<u>U. oligosperma</u> :	Benjamin, L. 1848; Oliver, D. 1860; Merz, M. 1897; Sylven, N. 1909.
<u>U. olivacea</u> :	Lloyd, F. E. 1936; Allen, R. McV. 1959; Taylor, P. 1967.
<u>U. oliverana</u> :	Steyermark, J. A. 1953; Taylor, P. 1967.
<u>U. oliveri</u> :	Kamienski, F. 1894, 1904.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. orbiculata</u> :	Oliver, D. 1867; Kamienski, F. 1890; Goebel, K. 1891; Lloyd, F. E. 1932a,b, 1936.
<u>U. orinosensis</u> :	Steyermark, J. A. 1953.
<u>U. palatina</u> :	Benjamin, L. 1848; Oliver, D. 1860.
<u>U. palatum</u> :	Kamienski, F. 1890.
<u>U. pallens</u> :	Sylvén, N. 1909.
<u>U. papillosa</u> :	Stapf, O. 1916.
<u>U. paradoxa</u> :	Lloyd, F. E. 1936; Lloyd, F. E. and Taylor, G. 1947.
<u>U. parkeri</u> :	Baker, J. G. 1884; Kamienski, F. 1904.
<u>U. peltata</u> :	Oliver, D. 1860; Kamienski, F. 1890; Sylvén, N. 1909; Merl, E. M. 1915; Lloyd, F. E. 1936.
<u>U. peltatifolia</u> :	Pellegrin, F. 1914; Chevalier, A. and F. Pellegrin, 1917; Killian, C. 1953.
<u>U. pentadactyla</u> :	Taylor, P. 1954.
<u>U. picta</u> :	Kamienski, F. 1890.
<u>U. pilifera</u> :	Chevalier, A. 1911; Pellegrin, F. 1914.
<u>U. pobeguinii</u> :	Pellegrin, F. 1914.
<u>U. pollichii</u> :	Schultz, F. 1873.
<u>U. porphyrophylla</u> :	Kamienski, F. 1890.
<u>U. prehensilis</u> :	Oliver, D. 1867; Kamienski, F. 1904; Pellegrin, F. 1914.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<hr/>	
<u>U. puberula</u> :	Lloyd, F. E. 1932a,b.
<u>U. pubescens</u> :	Oliver, D. 1867; Taylor, P. 1967.
<u>U. pulcherrima</u> :	Sylvén, N. 1909; Merl, E. M. 1915.
<u>U. purpurea</u> :	Oliver, D. 1860; Treat, Mary 1876; Chapman, A. W. 1883; Merz, M. 1897; Lloyd, F. E. 1932a,b, 1936.
<u>U. pusilla</u> :	Kamienksi, F. 1904; Sylvén, N. 1909; Merl, E. M. 1915; Taylor, P. 1967.
<u>U. pygmaea</u> :	Lloyd, F. E. 1936.
<u>U. quelchii</u> :	Taylor, P. 1967.
<u>U. quinquerradiata</u> :	Oliver, D. 1860; Kamienksi, F. 1894; Sylvén, N. 1909; Merl, E. M. 1915.
<u>U. radiata</u> :	Reinert, G. W. and R. K. Godfrey 1962.
<u>U. reflexa</u> :	Oliver, D. 1867; Kamienksi, F. 1904; Pellegrin, F. 1914.
<u>U. regnelli</u> :	Sylvén, N. 1909.
<u>U. rehmannii</u> :	Kamienksi, F. 1904.
<u>U. reniformis</u> :	Goebel, K. 1891; Ule, E. 1898; Sylven, N. 1909; Merl, E. M. 1915, 1925; Lloyd, F. E. 1931a,b, 1936.
<u>U. resupinata</u> :	Sylvén, N. 1909; Lloyd, F. E. 1932a,b; Taylor, P. 1967; Kondo, K. 1971a,b.
<u>U. reticulata</u> :	Oliver, D. 1867; Goebel, K. 1891.
<u>U. riccioides</u> :	Chevalier, A. 1911; Pellegrin, F. 1914.
<u>U. rigida</u> :	Pellegrin, F. 1914; Lloyd, F. E. 1936.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. rosea</u> :	Goebel, K. 1891.
<u>U. sandersoni</u> :	Oliver, D. 1867.
<u>U. sandwithii</u> :	Taylor, P. 1967.
<u>U. sanguinea</u> :	Kamienski, F. 1904.
<u>U. scandens</u> :	Taylor, P. 1963.
<u>U. schimperii</u> :	Schenck, H. 1887.
<u>U. schinzii</u> :	Kamienski, F. 1904.
<u>U. sematophora</u> :	Stapf, O. 1908.
<u>U. setacea</u> :	Treviranus, L. C. 1848.
<u>U. singeriana</u> :	Oliver, D. 1867; Lloyd, F. E. 1936.
<u>U. spartea</u> :	Baker, J. G. 1884; Kamienski, F. 1904.
<u>U. spicata</u> :	Sylvén, N. 1909; Merl, E. M. 1915
<u>U. spiralis</u> :	Oliver, D. 1867; Kamienski, F. 1904; Pellegrin, F. 1914; Taylor, P. 1963.
<u>U. sprengelii</u> :	Kamienski, F. 1904.
<u>U. spruceana</u> :	Oliver, D. 1860; Sylvén, N. 1909; Merl, E. M. 1915.
<u>U. squamosa</u> :	Kamienski, F. 1890.
<u>U. stanfieldii</u> :	Taylor, P. 1963.
<u>U. stellaris</u> :	Shrank, R. R. 1824; Benjamin, L. 1848; Treviranus, L. C. 1848; Wight, R. 1849; Oliver, D. 1867; Kamienski, F. 1890, 1894, 1904; Goebel, K. 1891; Merz, M. 1897; Pellegrin, F. 1914; Wagner, V. A. 1928.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<u>U. striata</u> :	Benjamin, L. 1848.
<u>U. striatula</u> :	Pellegrin, F. 1914; Killian, C. 1953.
<u>U. subpeltata</u> :	Steyermark, J. A. 1953.
<u>U. subulata</u> :	Schrank, R. 1824; Oliver, D. 1867; Chapman, A. W. 1883; Kamienski, F. 1904; Sylven, N. 1909; Pellegrin, F. 1914; Fernandes, A. 1947; Taylor, P. 1967; Kondo, K. 1972a,b.
<u>U. tenuissima</u> :	Taylor, P. 1967.
<u>U. tepuiana</u> :	Steyermark, J. A. 1953.
<u>U. ternata</u> :	Sylvén, N. 1909.
<u>U. thomasi</u> :	Lloyd, F. E. and G. Taylor 1947.
<u>U. thonningii</u> :	Pellegrin, F. 1914; Lloyd, F. E. 1936.
<u>U. tortilis</u> :	Oliver, D. 1867.
<u>U. transrugosa</u> :	Slinger, J. 1954.
<u>U. tribracteata</u> :	Kamienski, F. 1904.
<u>U. trichophylla</u> :	Oliver, D. 1860; Taylor, P. 1967.
<u>U. tricolor</u> :	Taylor, P. 1967.
<u>U. tridentata</u> :	Sylvén, N. 1909; Merl, E. M. 1915.
<u>U. triloba</u> :	Sylvén, N. 1909.
<u>U. triphylla</u> :	Ule, E. 1898.
<u>U. tubulata</u> :	Lloyd, F. E. 1936.
<u>U. turumiquiransis</u> :	Steyermark, J. A. 1953.

Table A1. (continued)

<u>Utricularia</u> species	Investigators
<hr/>	
<u>U. uliginosa</u> :	Merl, E. M. 1915.
<u>U. unifolia</u> :	Oliver, D. 1860.
<u>U. venezuelana</u> :	Steyermark, J. A. 1953.
<u>U. villosula</u> :	Pellegrin, F. 1914.
<u>U. viscosa</u> :	Oliver, D. 1860; Sylven, N. 1909; Merl, E. M. 1915; Taylor, P. 1967.
<u>U. volubilis</u> :	Merl, E. M. 1915.
<u>U. vulgaris</u> :	Goeppert, H. R. 1847; Benjamin, L. 1848; Hartman, R. 1857; Crouan, F. 1858; Reinsch, P. 1859; Oliver, D. 1860; Pringsheim, N. 1869; Schultz, F. 1873; Cohn, F. 1875; Darwin, C. 1875; Kamienski, F. 1877, 1890; Büsgen, M. 1888; Goebel, K. 1891, 1904; Merz, M. 1897; Meierhofer, H. 1902; Brocher, F. 1911; Merl, E. M. 1915; Withycombe, C. L. 1924; Diannelidis, T. 1948; Reese, G. 1952; Kurz, L. 1959; Schumacher, G. J. 1960; Abel, P. and D. Von Denffer 1961.
<u>U. wallichiana</u> :	Oliver, D. 1867; Kamienski, F. 1890; Lloyd, F. E. 1936.
<u>U. warburgi</u> :	Goebel, K. 1891.
<u>U. warmingi</u> :	Kamienski, F. 1894; Merl, E. M. 1925.
<u>U. welwitschii</u> :	Oliver, D. 1867; Kamienski, F. 1904; Lloyd, F. E. 1932a,b, 1936.

APPENDIX B:
FIELD METHODS

Table B1. Collection methods

Plants were collected by hand from various water depths

Water samples were collected in glass bottles

Plants were floated on paper and pressed in a standard herbarium
press

Plants were placed in glass bottles or plastic bags and stored
in styrofoam ice chests for transport

Plants were fixed in FAA (formalin-acetic acid-alcohol) for
transport and storage

Table B2. Water chemistry

Water temperature recorded

Water depth measured

Total hardness measured--Hach Method (Hach, 1965)

Pipette 10 ml water sample into titration flask
 Add 3 drops of Hardness I to sample--swirl
 Add contents of one ManVer II powder pillow
 Titrate sample with TitraVer Solution until color changes
 from pink to blue
 Titrate slowly to end point
 Total hardness of the water in ppm as CaCO_3 equals the
 number of ml of TitraVer used multiplied by 100

Iron measured--Hach method (Hach, 1965)

Measure 25 ml water sample into clean colorimeter bottle
 Add contents of one FerroVer Powder Pillow--swirl
 If iron is present an orange color develops
 Let sample stand for 2 min., but no longer than 5 min., before
 measuring color
 Fill clean colorimeter bottle with untreated water sample
 and place it in the light cell
 Insert the Iron (Phenanthroline) Meter Scale in the light cell
 and use the 4445 color filter
 Press light switch and adjust the light control for a reading
 of zero ppm
 Place prepared sample in light cell
 Press light switch and read ppm Iron

pH value measured--Hach method (Hach, 1965)

Measure 25 ml sample of water into clean colorimeter bottle
 Add 1 ml of Wide Range Indicator--swirl
 Fill a colorimeter bottle with some of the original water
 sample and place it in light cell
 Place Wide Range pH Scale into meter and insert color filter
 no. 4048
 Press light switch and adjust the light control so that the
 meter reads at far right end of scale
 Place the colorimeter bottle containing prepared sample into
 the light cell, press light switch and read scale
 For colors green, blue, and violet read from right to left on
 upper scale
 For colors red, orange, and yellow read from left to right on
 lower scale

Table B2. (continued)

 Phosphate (Ortho) measured--Hach method (Hach, 1965)

Measure two 25 ml water samples respectively into two clean colorimeter bottles
 Temperature of sample should be 24 C
 Add 15 drops of Ammonium Molybdate to each sample--swirl
 Add contents of one StannVer Powder Pillow to one colorimeter bottle sample--swirl
 If phosphate is present a blue color develops after 10 min.
 Place colorimeter bottle minus StannaVer in light cell
 Insert Phosphate Meter Scale in the meter and use color filter 5330
 Press light switch, adjust the light control for a meter reading of zero ppm
 Place prepared sample in light cell, press light switch, and read ppm phosphate

Turbidity measured--Hach method (Hach, 1965)

Place Turbidity scale in the meter and use color filter no. 4445
 Fill colorimeter bottle with demineralized water and place in light cell
 Press light switch and adjust the light control for a meter reading of zero Jackson Turbidity Units
 Fill a colorimeter sample bottle with sample being tested and place it in the light cell
 Press the light switch and read the turbidity in Jackson Turbidity Units

Color measured--Hach method (Hach, 1965)

Fill colorimeter bottle with colorless water and place it in light cell
 Insert Color Meter Scale in the meter and use the no. 5543 color filter
 Adjust light control for a meter reading of zero units
 Fill colorimeter bottle with the sample, place it in the light cell and read the units of apparent color

APPENDIX C:
LABORATORY TECHNIQUES

Table C1. Paraffin embedding method I

Bladders dissected from brahcnas

Entire bladders fixed in FAA (formalin-acetic acid-alcohol) at
4 C for 12 hr

Hydrate to water

Rinse in water (3X, 20 min each)

Dehydration--Tertiary butyl alcohol (TBA) series (15-20 min each)

Step 1--25 ml TBA, 125 ml 95% ethanol, 100 ml water

Step 2--35 ml TBA, 100 ml 95% ethanol, 65 ml water

Step 3--50 ml TBA, 100 ml 95% ethanol, 50 ml water

Step 4--100 ml TBA, 100 ml 95% ethanol, 1% Eosin added
for surface stain

Step 5--150 ml TBA, 50 ml 100% ethanol

Step 6--100% TBA (3X, 20 min each)

Infiltration with 56-57 C Tissuemat

Add Tissuemat chips periodically over 24 hr (60 C)

Remove lid from container

Add Paraplast chips over next 24 hr

Decant and add pure melted Paraplast (3X over next 24 hr)

Pour in blocks and arrange bladders

Trim and mount blocks

Section at 10 μ m

Mount

Stain

Table C2. Paraffin embedding method II

Bladders dissected from branches

Entire bladders fixed in FAA (formalin-acetic acid-alcohol) at
4 C for 12 hr

Hydrate to water

Rinse in water (3X, 20 min each)

Dehydration and infiltration with 56 C Tissuemat using an
Autotechnicon automatic tissue processor at NADL. The
steps on the Autotechnicon set as follows:

- 95% ethanol (1 hr)
- absolute ethanol (2X, 1 hr)
- 1:1--ethanol : n-butanol (1 hr)
- n-butanol (2X, 1 hr)
- n-butanol + Tissuemat (3 hr)
- Tissuemat (6 hr)
- Tissuemat (8 hr)

Infiltrated material was poured in pill boxes and the boxes floated
on ice to speed hardening of the Tissuemat

Trim and mount blocks

Section at 10 μ m

Mount on slides

Stain

Table C3. Total water insoluble carbohydrates--Periodic acid-Schiff reaction (PAS)

FAA fixed bladders

Tissuemat embed, section at 10 μ m

Mount slides with Haupt's adhesive

Hydrate to water

0.5% periodic acid solution in distilled water, room temperature
(15-30 min)

Rinse in running tap water (10 min)

Stain in Schiff's reagent^a, 4 C in dark (15 min)

Rinse in tap water (10-20 sec)

Dip in 2% sodium bisulfite (1-2 min)

Rinse in running tap water (10 min)

Dehydrate to xylene

Mount in Permount or Piccolyte

^aSchiff's reagent: Add 0.5 g basic fuchsin and 0.5 g sodium metabisulfite to 100 ml of 0.15 N HCl and shake occasionally over several hours. Add 300 mg of decolorizing charcoal, shake, and filter. All glassware should be rinsed in HCl first before using.

Table C4. Nucleic acids--Azure B with RNAase extraction

FAA fixed bladders

Tissuemat embed, section at 10 μ m

Mount 4 slides with Haupt's adhesive, alternate sections

Remove Tissuemat to absolute ethanol

Coat slides with collodion

Hydrate to distilled water

Extraction procedure (2 hr)

Slide #1--Distilled water, room temperature

Slide #2--Distilled water, 37 C

Slide #3--0.1% RNAase, pH 6.8, 37 C (adjust pH with 1 N NaOH)

Slide #4--Distilled, water, pH 6.8, 37 C

Distilled water rinse (3X, 5 min each)

Stain in 0.25 mg/ml Azure B in citrate buffer, pH 4.0, 2 hr, 50 C

Rinse in running tap water (3X, 5 min each)

Blot slides dry

Pure tertiary butyl alcohol (TBA), 10-15 min

1 TBA : 1 Xylene (5 min)

Xylene (2X, 5 min each)

Mount in Permount or Piccolyte

RNA will stain purple to dark blue, DNA will stain blue green

Table C5. Acridine orange fluorescence technique

Dissect bladders from branches or cut branches in small pieces
Stain in pH 6 buffered^a 0.1% acridine orange fluorescent stain^b
Rinse in pH 6 phosphate buffer (1/15 M KH_2PO_4 and 1/15 M Na_2HPO_4
mixed to obtain pH 6)
Differentiated in 1 M CaCl_2 for 3 to 10 sec.
Briefly rinse in buffer, mount wet with cover glass, and examine
with bright-field fluorescence microscopy
Use barrier filter 490 μ -470 μ and lamphouse filters BG38 and UG1

^aPhosphate buffer:

- (1) KH_2PO_4 : 4.53 gm/500 ml distilled water
(2) K_2HPO_4 : 2.36 gm/500 ml distilled water
Mix 115 ml of (1) with 20 ml of (2) to get pH 6

^b0.1% Acridine orange stain:

Stock solution: 500 mg acridine orange/500 ml distilled
water

Staining solution: 10 ml of stock acridine orange to
90 ml of 1/15 M phosphate buffer

Table C6. Callose--Aniline blue-fluorescence technique

Sectioned material

FAA fixed bladders

Tissuemat embed, section 10 μm

Mount slides using Haupt's adhesive

Hydrate to water and rinse 10 min

Stain slides 15 min in 0.005% aniline blue in 0.15 M K_2HPO_4 at pH 8.4

Add cover slips over a drop of stain solution

Observe preparations using a fluorescence microscope equipped with a mercury lamp, lamp housing filters BG38 and UG1, and barrier filter 430 μ

Callose fluoresces yellow-green to blue

Fresh material

Place entire bladders or branches in a well of stain for 15 min

Place bladders or branches in a drop of stain solution

Add cover slip

Observe as above

Table C7. Araldite-Epon embedding method (modification of Mollenhauer, 1964)

Dissect bladders from branches

Fix in 2-3% glutaraldehyde in 0.025 M phosphate buffer or filtered pond water, pH 7.0-8.0 at 4 C for 12 hr)

Buffer rinse (3X, 20 min each)

Dehydration (20 min each)

30% acetone
60% acetone
90% acetone
100% acetone (3X, 30 min each)

Infiltration with Araldite-Epon^a on a rotary turntable

Acetone:	3 drops Araldite-Epon every 10 min for 1 hr
4 Acetone :	1 Araldite-Epon 2 hr
3 " :	" 2 hr
1 " :	" 4 hr
1 " :	" 10 hr
Pure Araldite-Epon	10 hr

Pour into aluminum weighing pans

Polymerization

12-24 hr at 37 C
12-24 hr at 45 C
42-72 hr at 60 C

Trim blocks

Section

^aAraldite-Epon mixture:

Araldite 502	14.0 gm
Epon 812	16.0 gm
DDSA (dodecenyl succinic anhydride)	30.0 gm
DMP-30 accelerator	1.5 ml/dilution

Table C8. Material preparation for SEM

Dissect bladders from branches or cut branches in small pieces

Fix in 2-3% glutaraldehyde in 0.025 M phosphate buffer, pH 7.0-8.0
at 4 C for 12 hr

Buffer rinse (3X, 20 min each)

Dehydration (20 min each)

25% ethanol

50% ethanol

70% ethanol

95% ethanol

absolute ethanol (2X, 20 min each)

Replace ethanol with Freon TF:

4 ethanol : 1 Freon TF

3 ethanol : 1 Freon TF

2 ethanol : 1 Freon TF

1 ethanol : 1 Freon TF

1 ethanol : 2 Freon TF

1 ethanol : 3 Freon TF

pure Freon TF (3X, 30 min each)

Dry in critical point apparatus using liquid carbon dioxide as the
transitional fluid

Mount dried material on stubs with silver tape and paint

Coat with carbon and 12 inches of gold in vacuum evaporator

APPENDIX D: KEY TO FIGURES

A	- anterior
AD	- anterior door region
AP	- absorbed product
AT	- aperture trichome
B	- bifid
BA	- branched antennae
BC	- basal cell
BP	- bladder primordium
BT	- bacteria
CA	- callose
CB	- carbohydrate secretory product
CC	- central cell
CD	- central door region
CH	- channels
CPC	- companion cell
CR	- crystal
CS	- cutin cystoliths
CT	- cuticle
CW	- cell wall
D	- door
DO	- dorsal
DS	- door-stop trichome

DT	- dictyosome
E	- endodermoid wall
ER	- endoplasmic reticulum
FW	- fibrous wall
HT	- hammer-head trichomes
INC	- intranuclear crystals
IW	- inner bladder wall layer
LD	- lateral door region
LM	- lumen
LT	- lenticular trichome
M	- mitochondrion
MB	- microbody
MT	- microtubule
MVB	- multivesicular body
MW	- middle bladder wall layer
N	- nucleus
NO	- nucleolus
O	- osmiophilic body
OW	- outer bladder wall layer
P	- posterior
PD	- plasmodesmata
PE	- pavement epithelium
PFM	- posterior free margin of door
PL	- plastid
PM	- plasmalemma

pP	- p-protein
PP	- phloem parenchyma
PW	- primary wall
Q	- quadrifid
RER	- rough endoplasmic reticulum
S	- starch
SER	- smooth endoplasmic reticulum
SLW	- sponge-like wall
ST	- spherical trichome
STE	- sieve tube element
TC	- terminal cell
TO	- trapped organism
TT	- trigger trichome
TW	- transfer wall
UAT	- unbranched antennae
V	- velum
VB	- vascular bundle
VC	- vacuole
VN	- ventral
VS	- vesicle
WS	- bladder wall layer space
WT	- wall thickening

APPENDIX E: FIGURES

Fig. 1. Utricularia macrorhiza flowering at Jemmerson
Slough.



Fig. 2. Utricularia macrorhiza at Spring Lake floating free above substrate.

Fig. 3. Utricularia macrorhiza at Silver Lake Fen floating in very shallow water so that it is in contact with substrate. White, thread-like vegetative propagules are common (arrow).

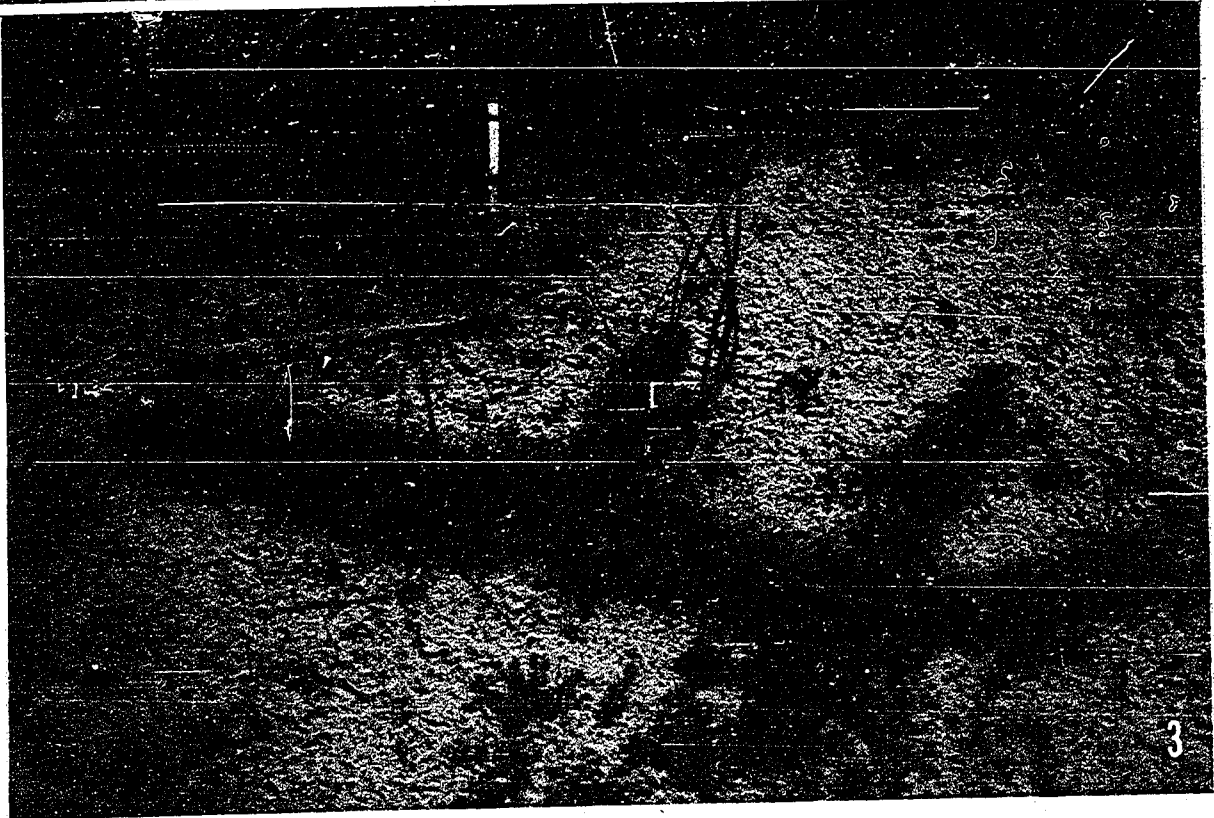
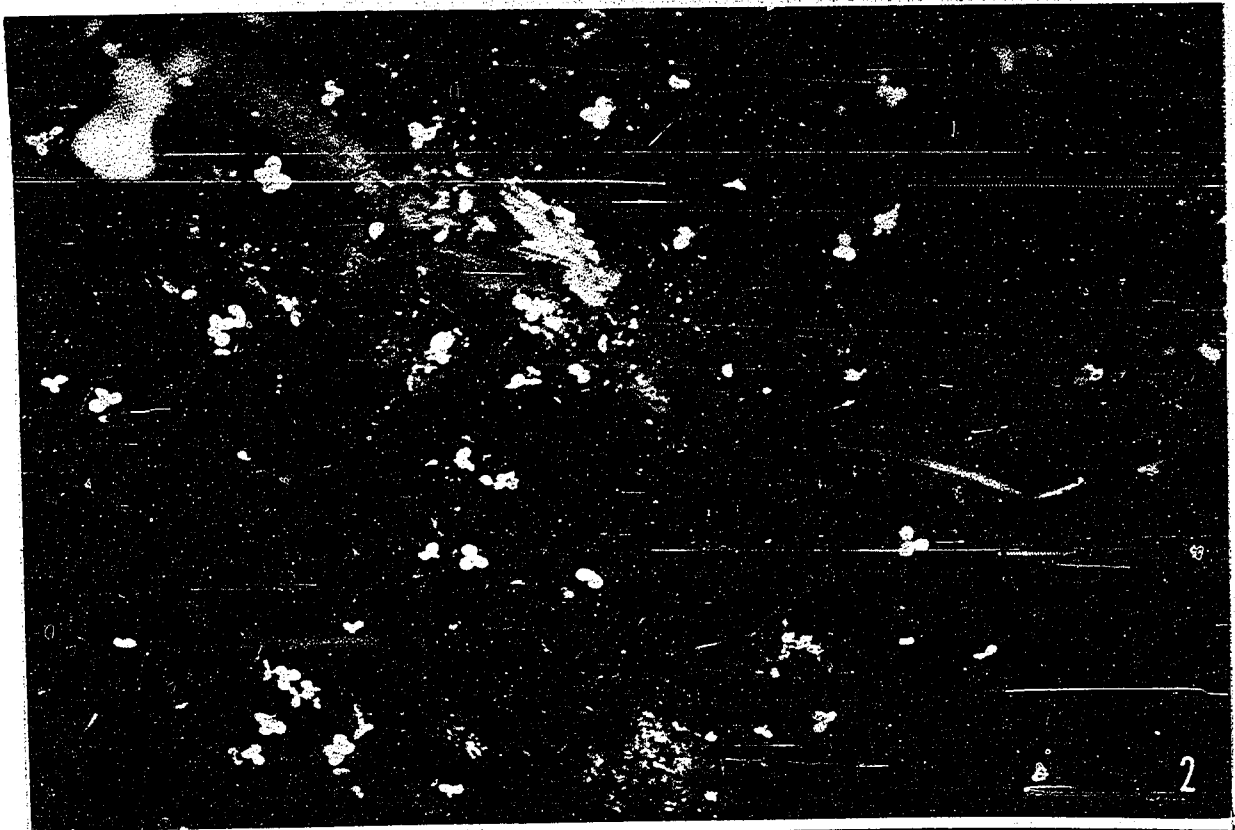


Fig. 4. Three Corners Pond, arrow points to location of Utricularia macrorhiza at periphery of pond.

Fig. 5. Silver Lake Fen, showing small shallow pools which make up fen and certain Utricularia macrorhiza.

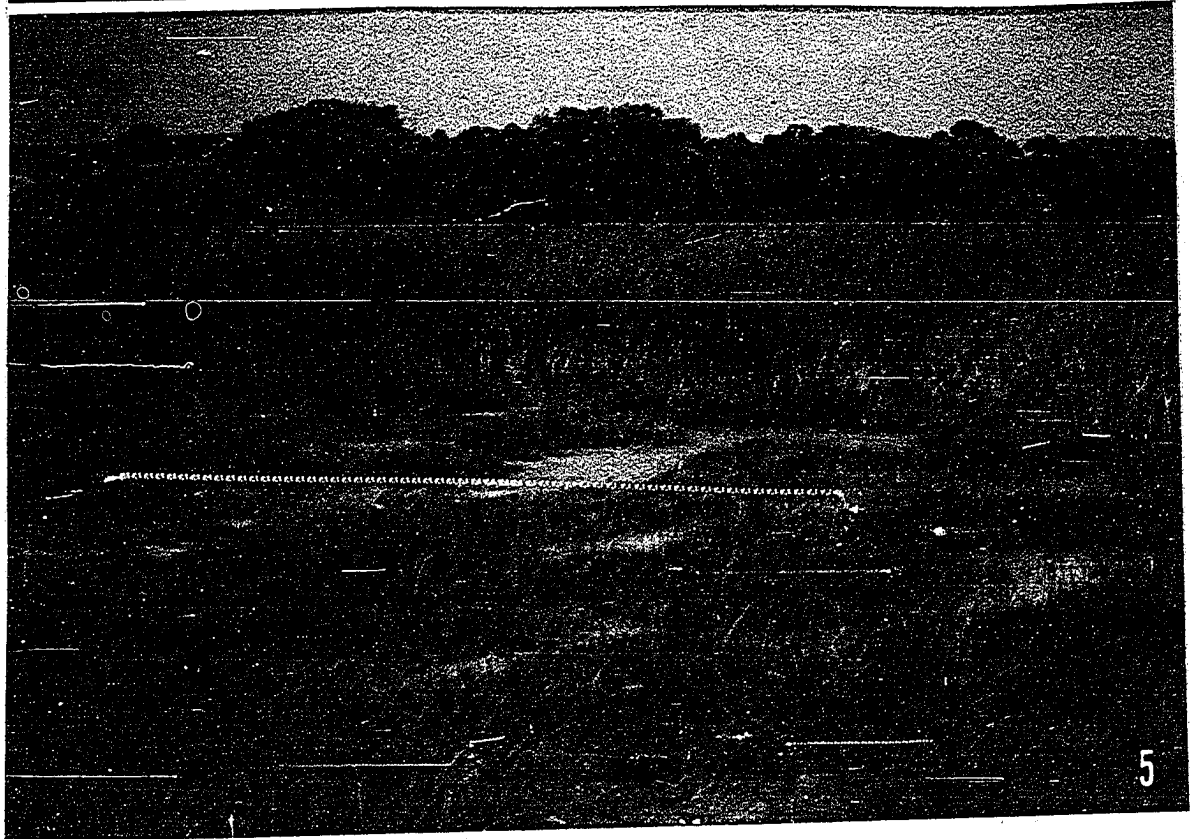
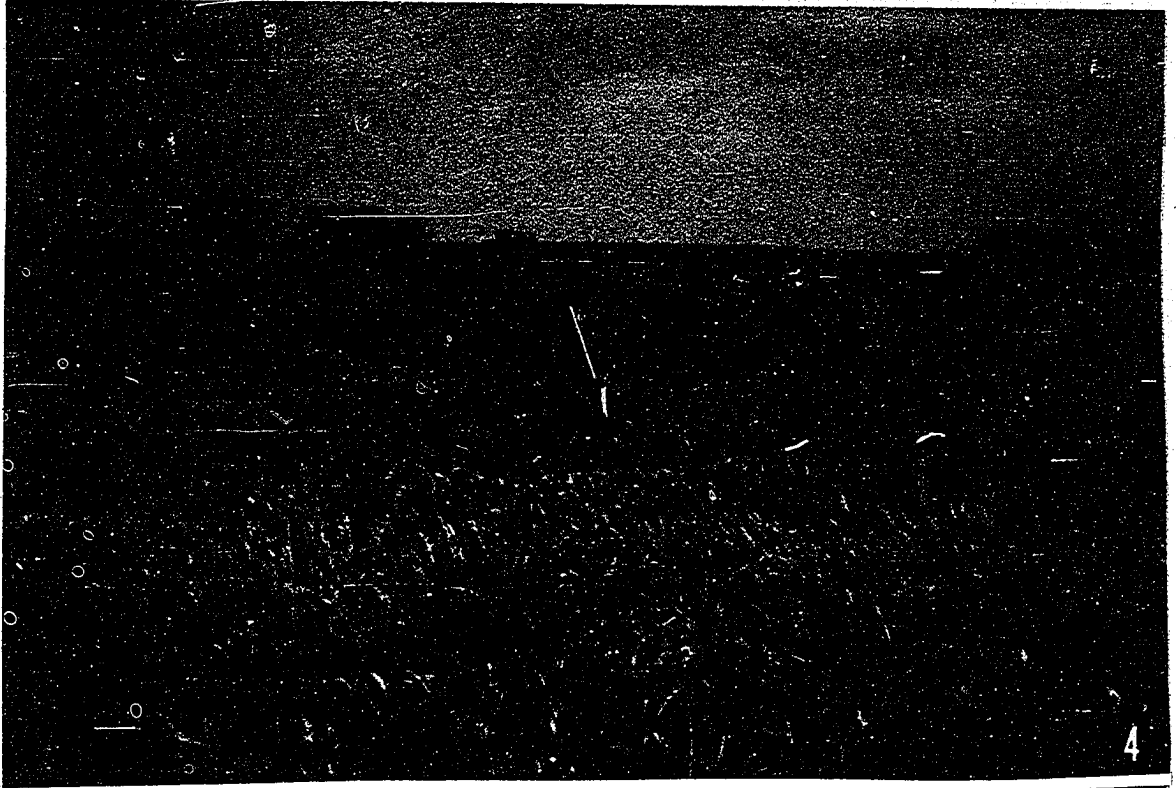
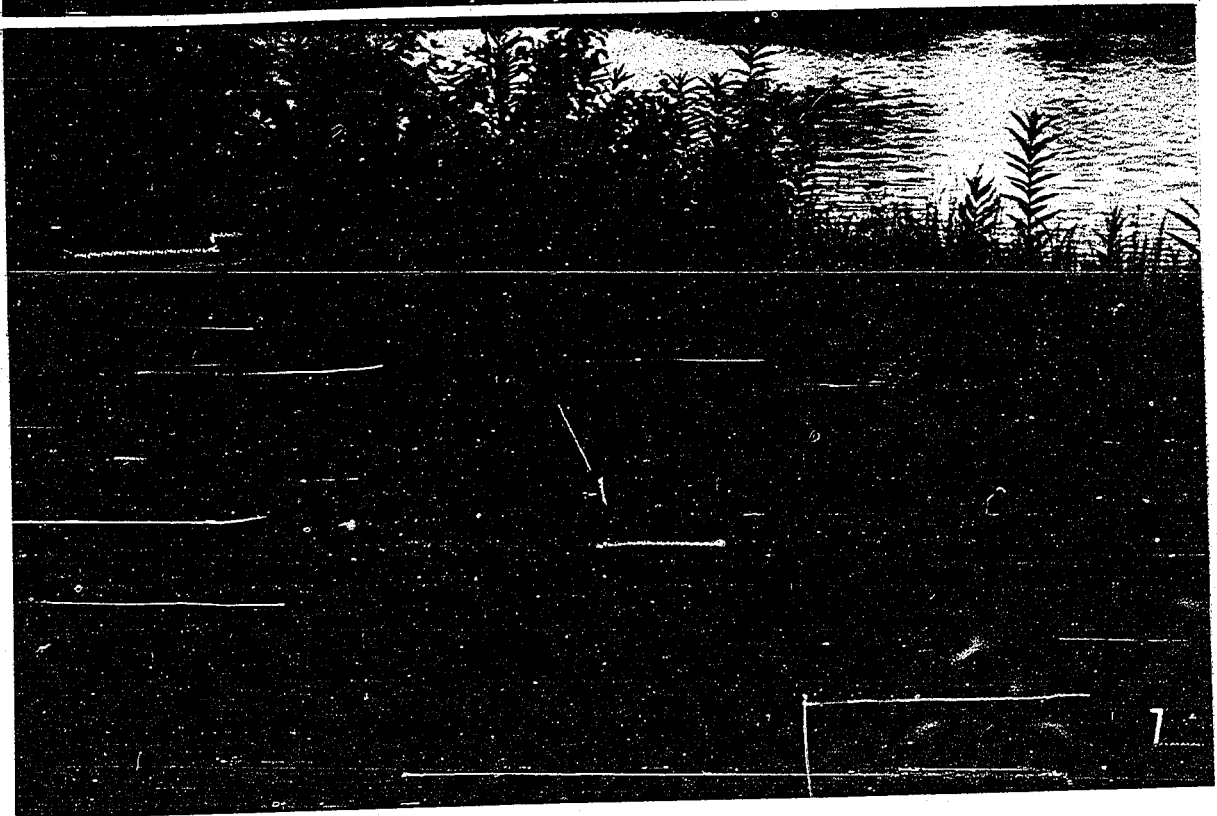
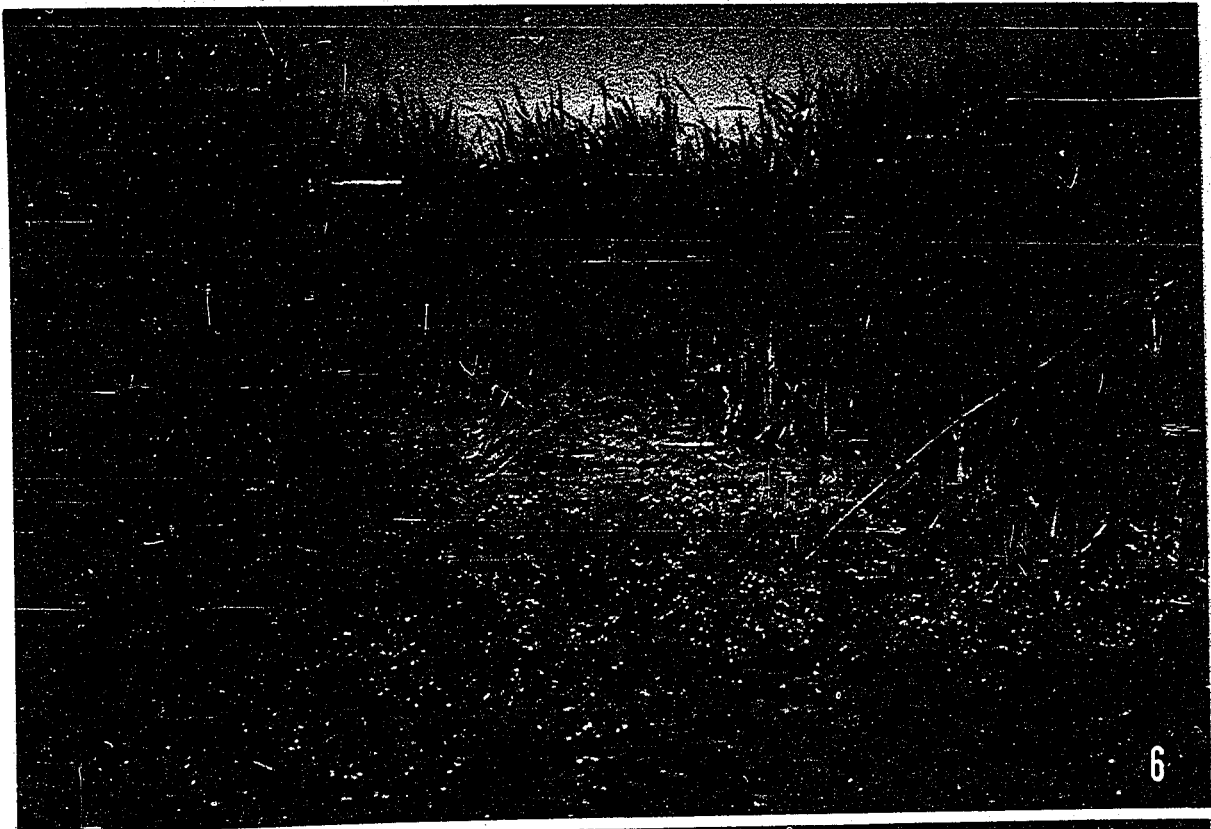
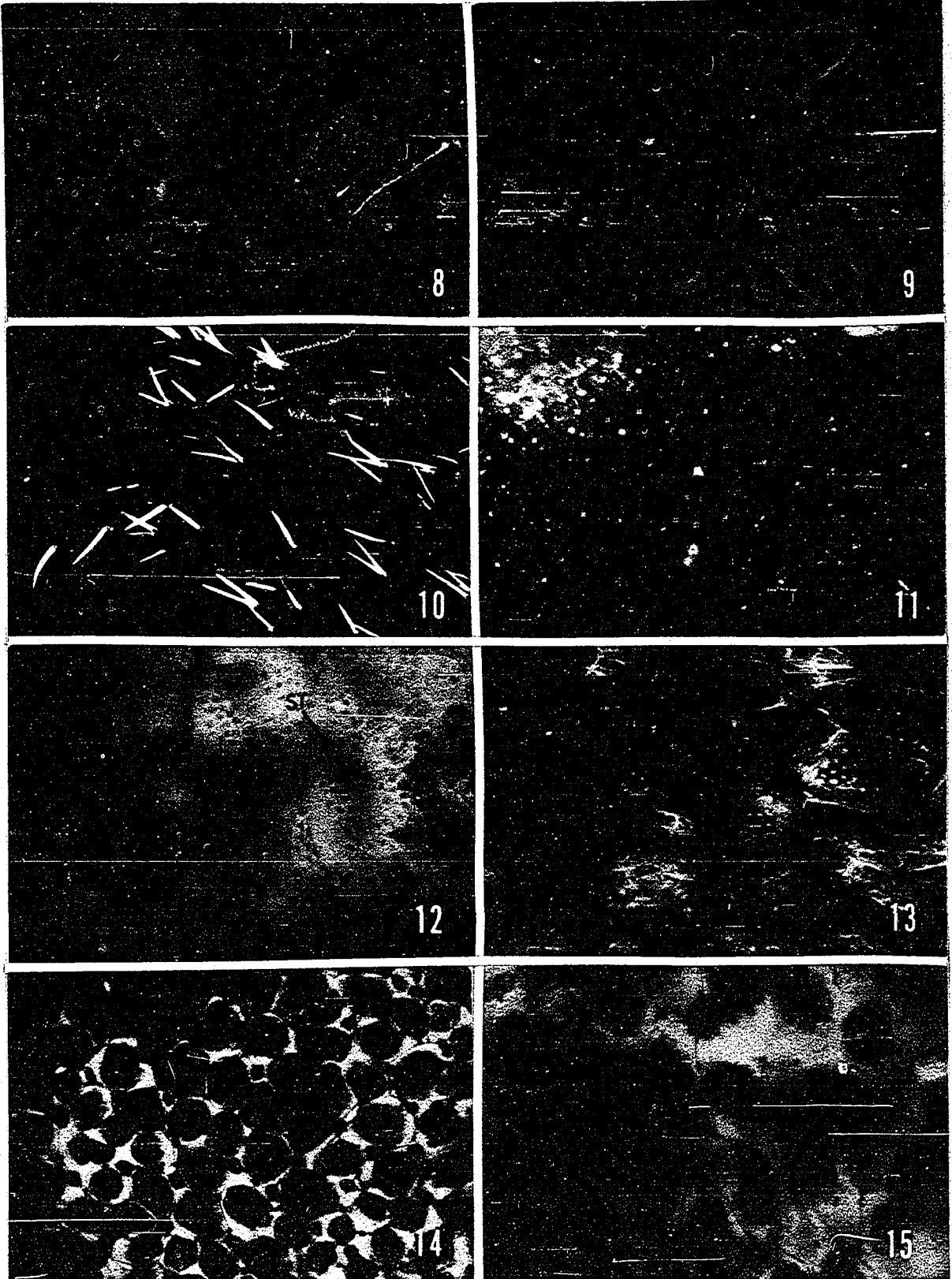


Fig. 6. Jemmerson Slough with numerous flowering plants of Utricularia macrorhiza growing between dense Typha populations.

Fig. 7. Small ditch at Spring Lake where Utricularia macrorhiza grows. A few plants are in flower (arrow).



- Fig. 8. Young bladders shown using polarized light. Vascular bundle is birefringent (arrow). X 242 (SPRING)
- Fig. 9. Fresh specimen of young shoot tip under phase contrast optics showing young bladders and branches. X 242 (SLOUGH)
- Fig. 10. Mature branch under polarized light showing birefringent spines on branch surfaces. X 99 (SPRING)
- Fig. 11. Mature bladder wall under polarized light showing starch in bladder wall cells and red anthocyanin pigmentation. X 99 (SILFEN)
- Fig. 12. Outer bladder wall under phase contrast optics showing red anthocyanin pigmentation and spherical trichomes. X 391 (SILFEN).
- Fig. 13. Bladder wall under phase contrast optics showing blue anthocyanin pigmentation of mature bladder. X 391 (SLOUGH)
- Fig. 14. Bladder wall under phase contrast optics showing blue anthocyanin in older bladder. X 241 (SLOUGH)
- Fig. 15. Bladder wall under phase contrast optics showing blue anthocyanin pigmentation in bladder that is no longer functional. X 391 (SLOUGH)



- Fig. 16. Developing bladder primordia embedded in paraffin and stained for nucleic acids with azure B. Bladder primordia stain most intense with azure B. X 241 (SLOUGH)
- Fig. 17. Developing bladders embedded with paraffin and stained with azure B for nucleic acids. Outer wall and inner wall cells still stain intensely, but middle layer of cells has stopped dividing. Door and pavement epithelial primordia also stain intensely. X 99 (SLOUGH)
- Fig. 18. Section through tip of a small vegetative propagule; plastic embedded and stained with PAS. Note abundance of starch and carbohydrate secretory product. X 242 (SILFEN)
- Fig. 19. Developing bladder embedded in paraffin and stained with azure B for nucleic acids. Door, pavement epithelium, trichomes surrounding aperture, and cells produced by first division of inner epidermis to produce bifids and quadrifids stain intensely. X 242 (SLOUGH)
- Fig. 20. Longitudinal section through a branch. Paraffin section is stained with PAS and arechymatous tissue of branch displays large amounts of starch, while trichomes on outer surface show a positive reaction for a carbohydrate secretory product. X 99 (SPRING)
- Fig. 21. PAS stained trichomes which surround aperture indicating presence of a large amount of carbohydrate in their terminal cells. X 391 (SPRING)
- Fig. 22. Fresh specimen under UV showing aperture region of a mature bladder. Trichomes surrounding aperture do not autofluoresce. Only chlorophyll in wall cells fluoresces. X 242 (SLOUGH)
- Fig. 23. Same as Fig. 22 only stained with aniline blue. Under UV aperture trichomes fluoresce intensely. X 242 (SLOUGH)

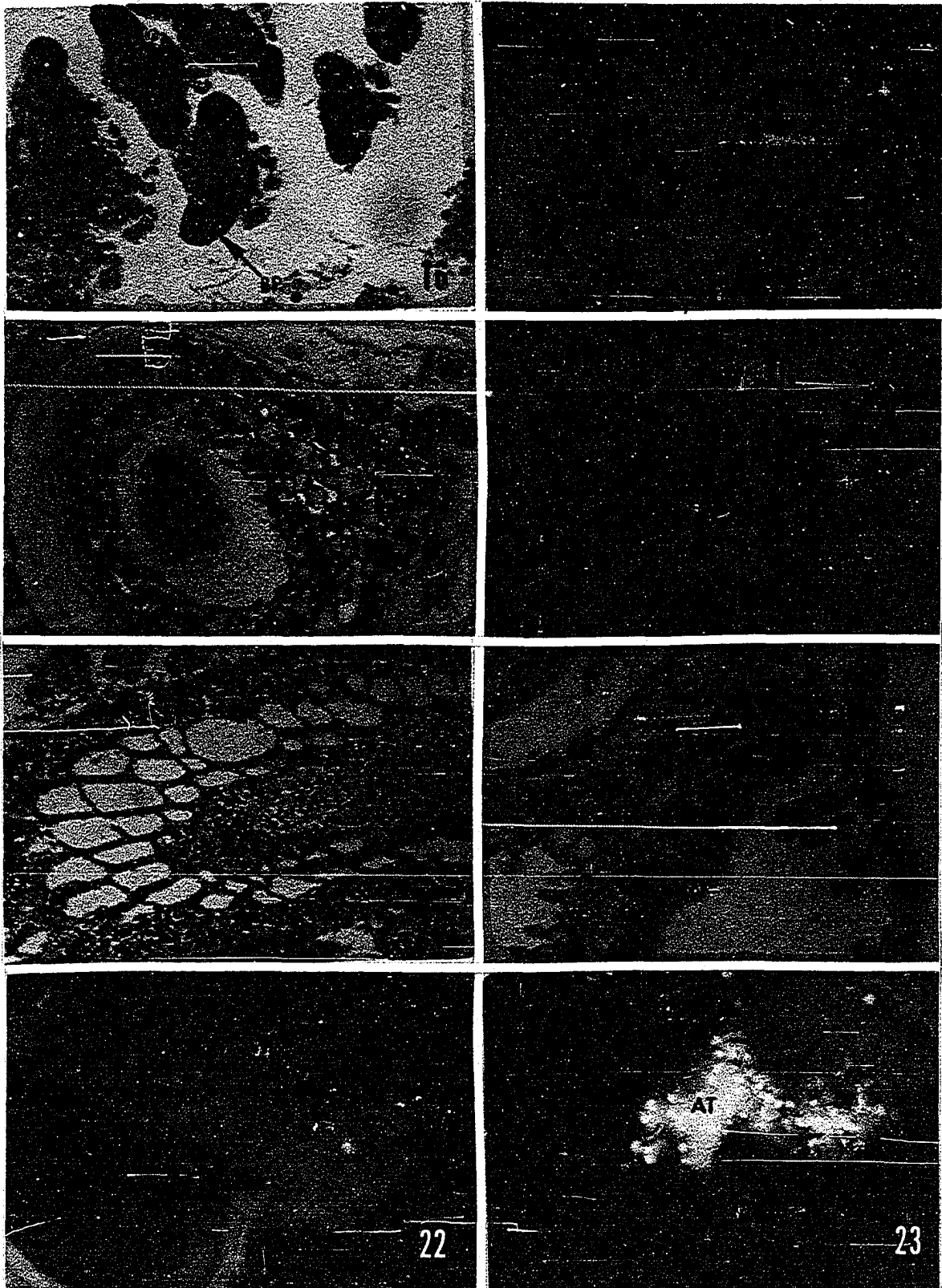
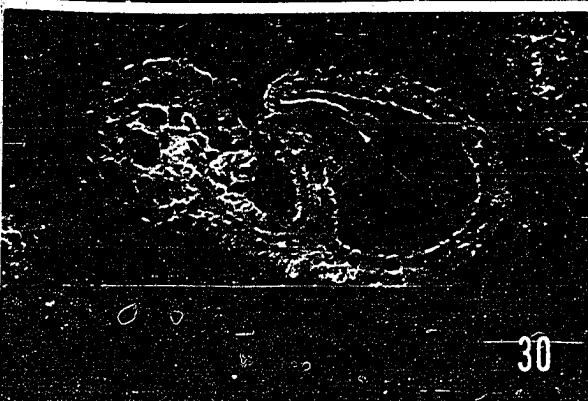
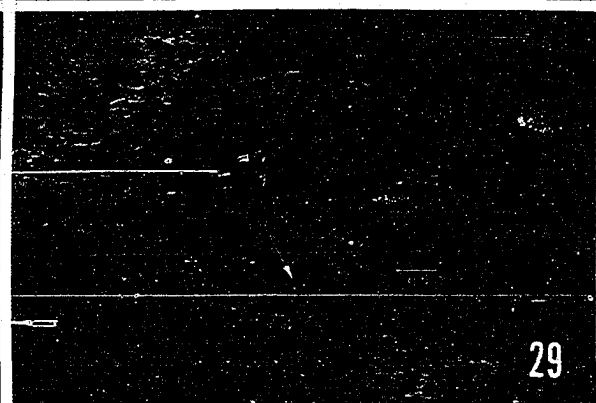
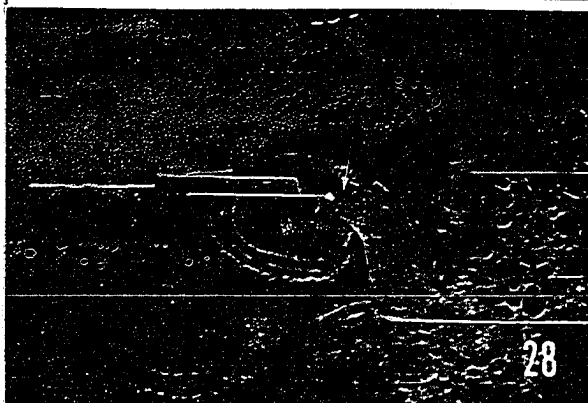
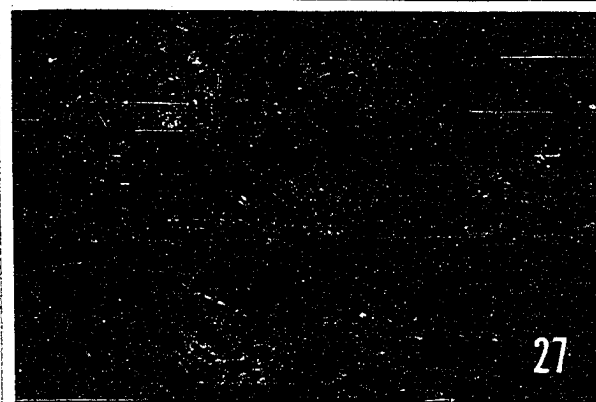
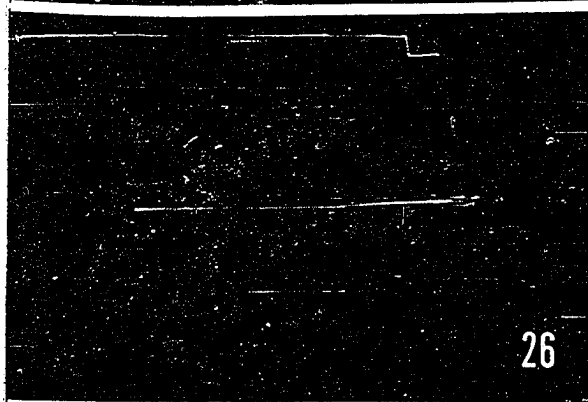
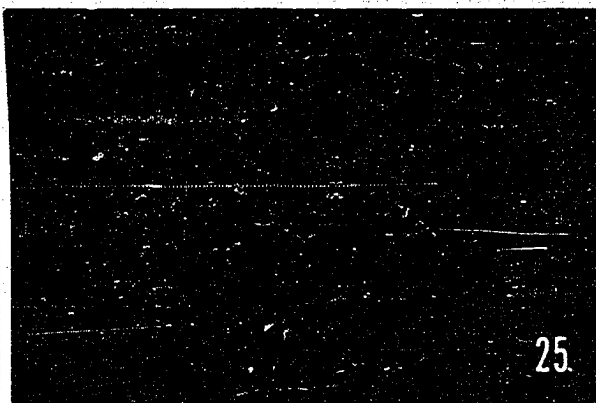
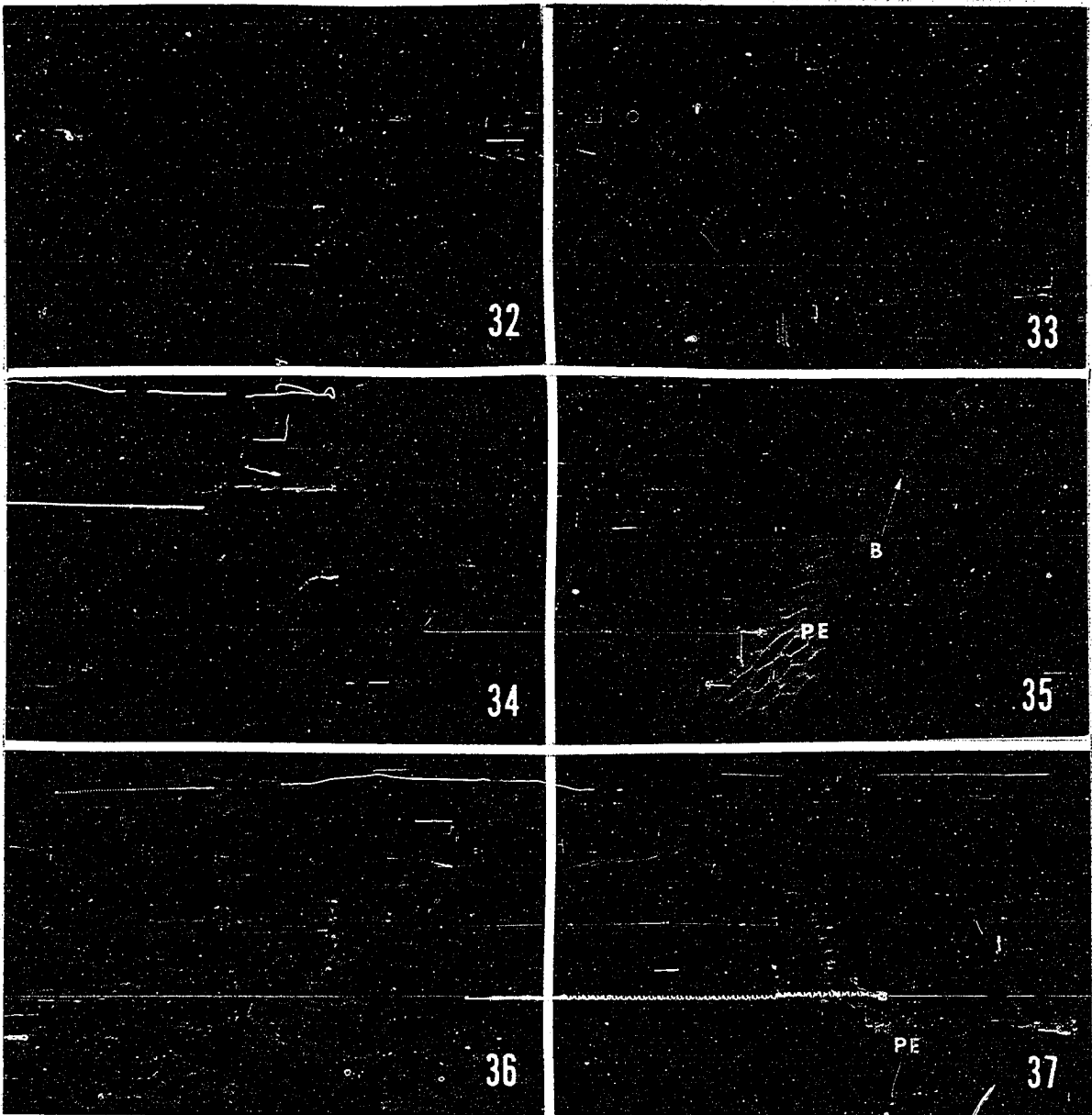


Fig. 24-31. Various stages of branch and bladder development; stained with Fast-green and observed under dark-field optics.

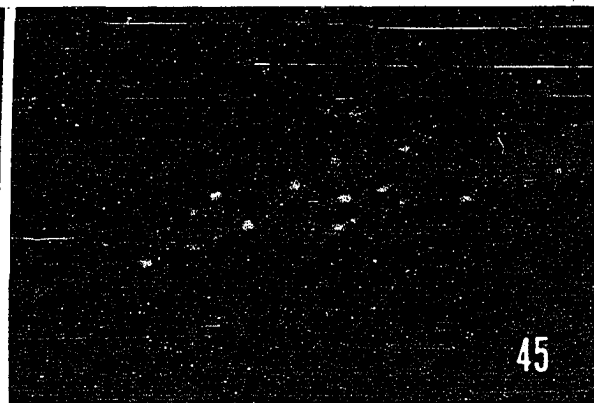
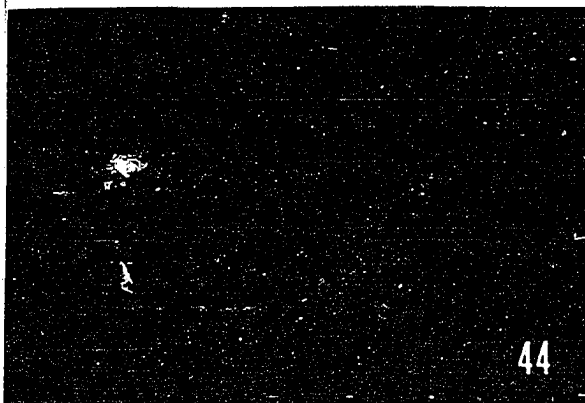
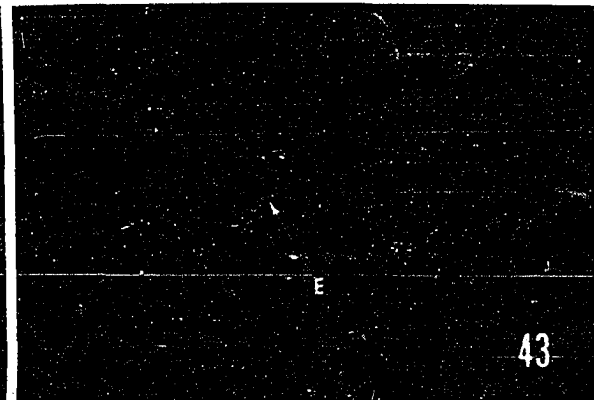
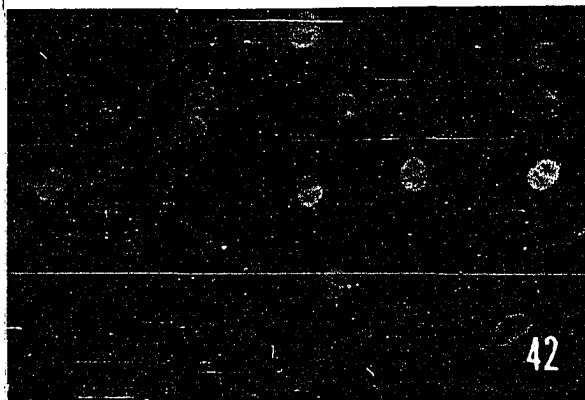
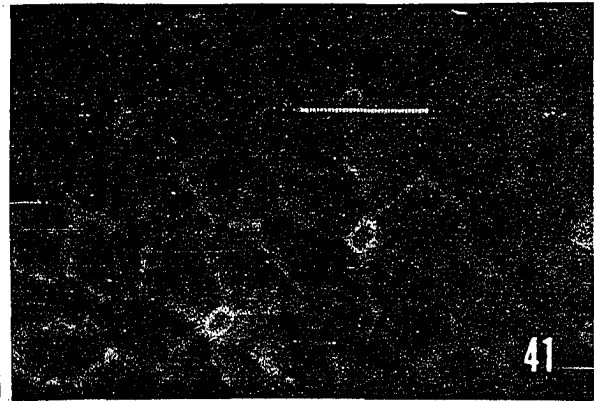
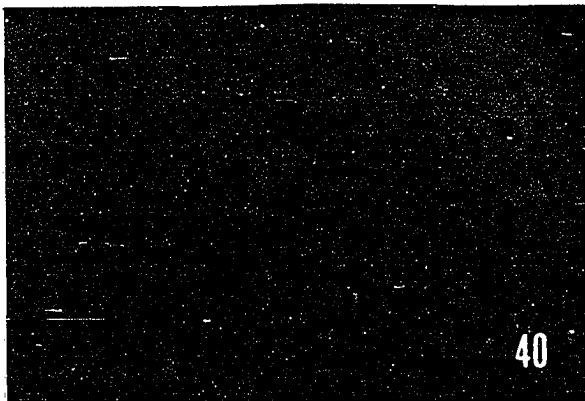
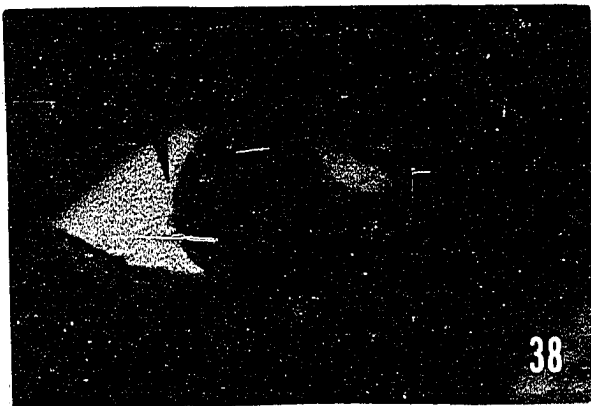
24. Coiled shoot tip from winter bud with large amounts of starch in shoot and adjacent branches. X 99 (SILFEN)
25. Coiled shoot tip from summer apical bud. Little or no starch is present in shoot and branches. X 99 (SILFEN)
26. Section showing starch in shoot and branches of winter bud. Young developing bladder shows no starch. X 99 (SILFEN)
27. Section showing starch concentrated in inner branches of winter bud, less starch in outer branches and no starch in the developing bladders. Cuticle on epidermal surfaces of bladders appears yellow. X 99 (SILFEN)
28. Developing bladder showing branching vascular bundle (arrow) traversing layer of cells beneath pavement epithelium. X 99 (SILFEN)
29. Developing bladder showing initiation of aperture trichomes (arrow). X 99 (SILFEN)
30. Developing bladder showing initiation of door-stop cells (arrow). X 99 (SILFEN)
31. Developing bladder showing development of inner epidermal surface trichomes, bifids and quad-rifids (arrow). X 99 (SILFEN)



- Fig. 32. Young developing bladders stained with acridine orange and viewed with UV to show relative amounts of RNA in young bladders as compared to adjacent branches. X 99 (SPRING)
- Fig. 33. Same as Fig. 32 but bladder is at a later stage of development. X 99 (SPRING)
- Fig. 34. Mature bladder stained with acridine orange and viewed with UV. Note yellow autofluorescence of the endodermoid walls of the pavement epithelium and green autofluorescence of the velum. Bifids show secondary fluorescence with acridine orange indicating greatest amount of cytoplasm is in basal portion of bifid terminal cells. X 99 (SPRING)
- Fig. 35. Mature bladder sectioned to show pavement epithelial central cells in cross section and bifids at back of pavement epithelium. Endodermoid walls of both pavement epithelial cells and bifids autofluoresce yellow when viewed with UV. X 391 (SPRING)
- Fig. 36. Pavement epithelium viewed under phase contrast optics. Central cells appear darker. X 391 (SPRING)
- Fig. 37. Same as Fig. 36 but viewed under UV to demonstrate autofluorescence of endodermoid walls of pavement epithelium (arrow) and green autofluorescence of velum. X 391 (SPRING)



- Fig. 38. Young bladder unstained shown with phase contrast optics to demonstrate presence of lenticular trichomes (arrow) on surface. X 99 (SILFEN)
- Fig. 39. Same bladder as in Fig. 38 under UV and stained with acridine orange; no fluorescence of terminal cells of lenticular trichomes occurs. X 99 (SILFEN)
- Fig. 40. Mature unstained bladder viewed under UV to demonstrate autofluorescence of chlorophyll which is red and autofluorescence of endodermoid walls of spherical surface trichomes (arrow). X 242 (SILFEN)
- Fig. 41. Same region as Fig. 40 at a higher magnification to better demonstrate autofluorescence of endodermoid walls (arrow). X 391 (SILFEN)
- Fig. 42. Outer bladder surface spherical trichomes stained with aniline blue and viewed under UV to show positive fluorescence for callose in terminal cells (arrow). X 391 (SILFEN)
- Fig. 43. Outer bladder surface spherical trichomes unstained and viewed with UV to demonstrate autofluorescence of endodermoid walls and absence of yellow fluorescence observed in terminal cells when stained with aniline blue as in Fig. 42 (arrow). X 391 (SILFEN)
- Fig. 44. Unstained branch viewed with UV to demonstrate autofluorescence of chlorophyll and lack of fluorescence of outer surface trichomes. X 242 (SILFEN)
- Fig. 45. Branch stained with aniline blue and viewed with UV to demonstrate positive fluorescence for callose in terminal cell walls. X 241.5 (SILFEN)



- Fig. 46. Section through bladder walls stained with acridine orange showing early quadrifid development. X 241 (SILFEN)
- Fig. 47. Section through bladder walls stained with acridine orange showing quadrifids at later stage of development than Fig. 46. X 241 (SILFEN)
- Fig. 48. Section through bladder walls stained with acridine orange showing stage of quadrifid development when central cell anticlinal walls become impregnated with endodermoid material. X 241 (SILFEN)
- Fig. 49. Mature bifids stained with acridine orange showing endodermoid walls of central cell. X 241 (SILFEN)
- Fig. 50. Mature bifids under phase contrast optics. X 390 (3-CORN)
- Fig. 51. Same as Fig. 50 but stained with acridine orange and viewed under U.V. to show endodermoid central cell walls. X 390 (3-CORN)
- Fig. 52. Fresh specimen showing quadrifids under phase contrast optics. X 390 (SILFEN)
- Fig. 53. Same as Fig. 52 but viewed with polarization optics to show terminal cell vacuolar crystals (arrow--CR). X 390 (SILFEN)

46

47

48

49

50

51

52

53

Fig. 54. Typical plant of U. macrorhiza collected at Jemmerson Slough shown actual size.

Fig. 55. Typical plant of U. macrorhiza collected at Spring Lake shown actual size with arrow pointing to winter bud.

Fig. 56. Typical plant of U. macrorhiza collected at Three Corners Pond shown actual size.

Fig. 57. Typical plant of U. macrorhiza collected at Silver Lake Fen shown actual size.

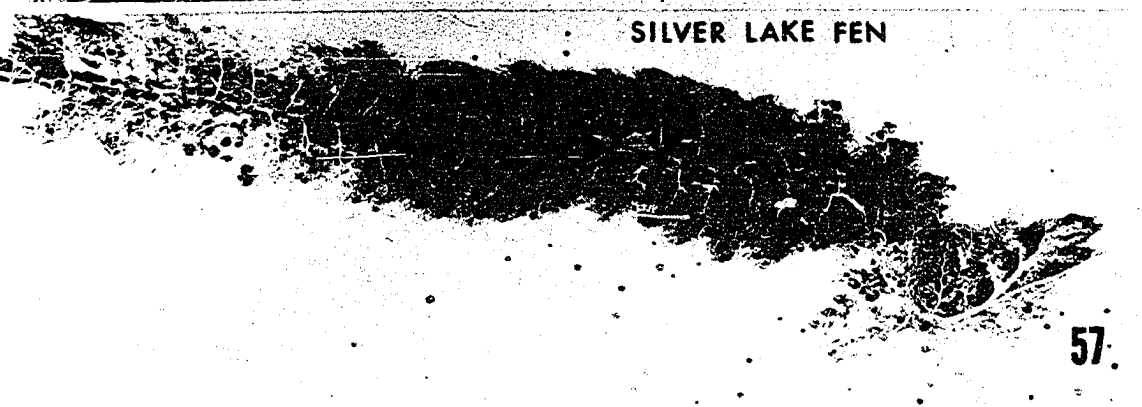
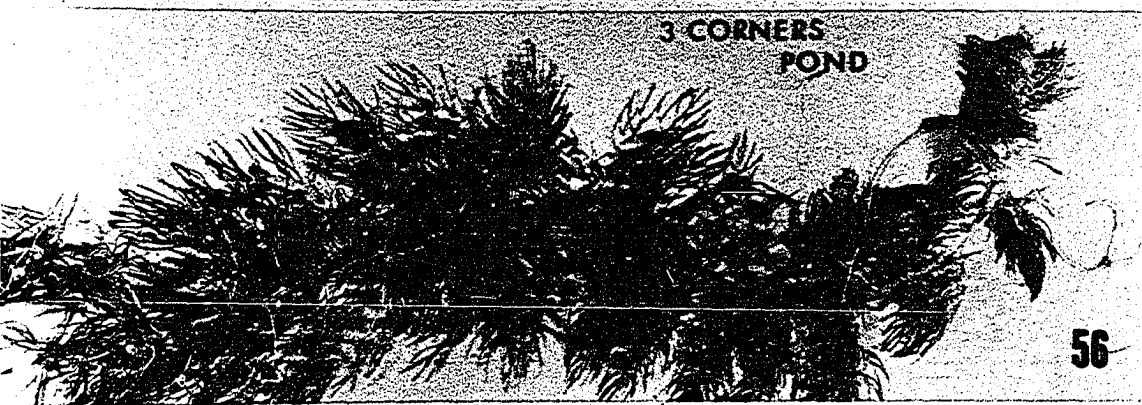
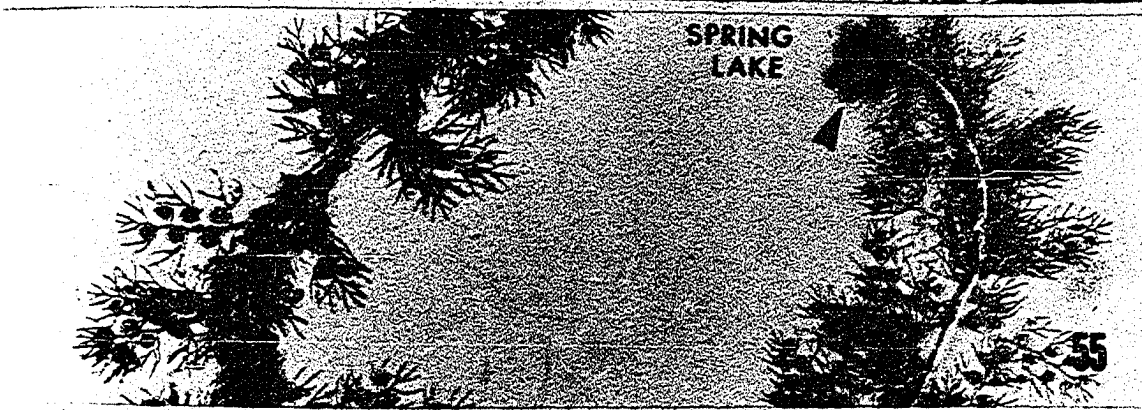


Fig. 58. Diagram of floating transplant container with screen cylinder and green styrofoam rectangular float anchored by rope and brick (not shown)

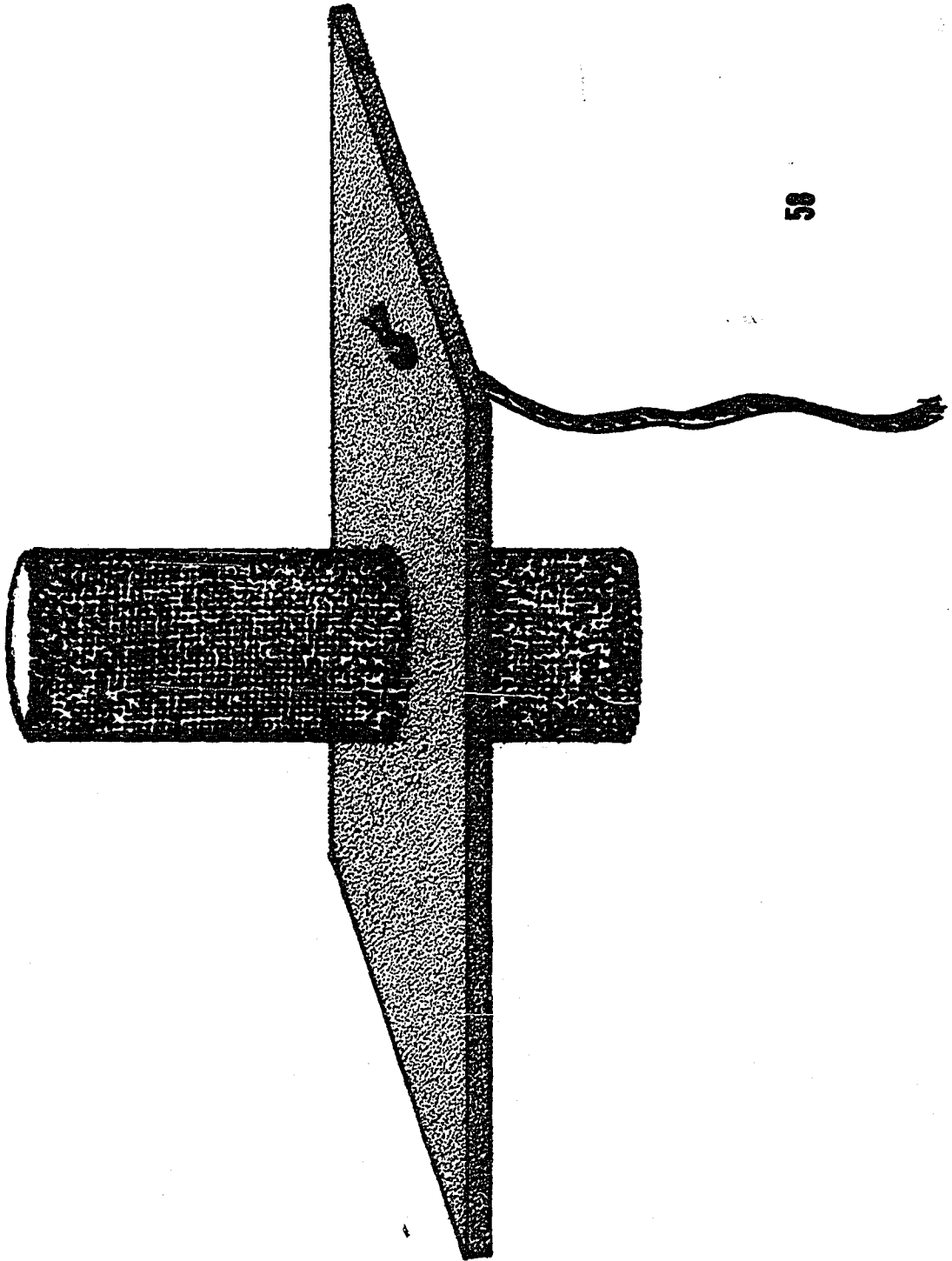
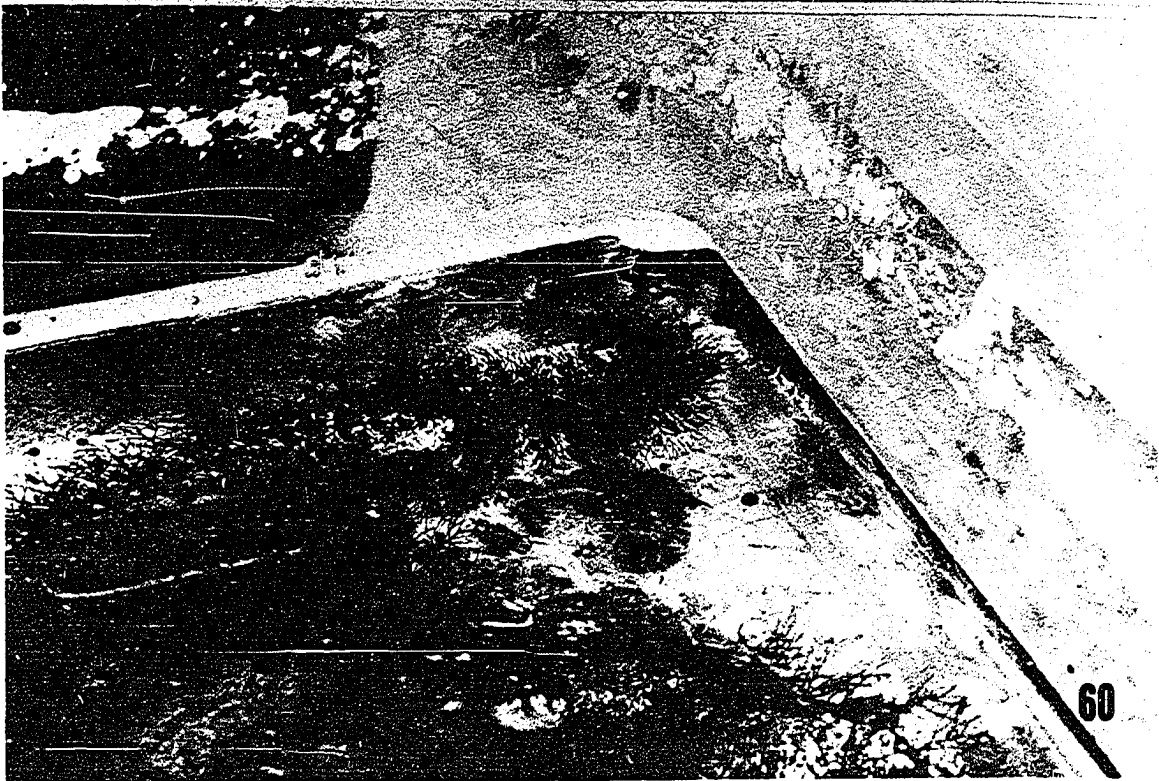
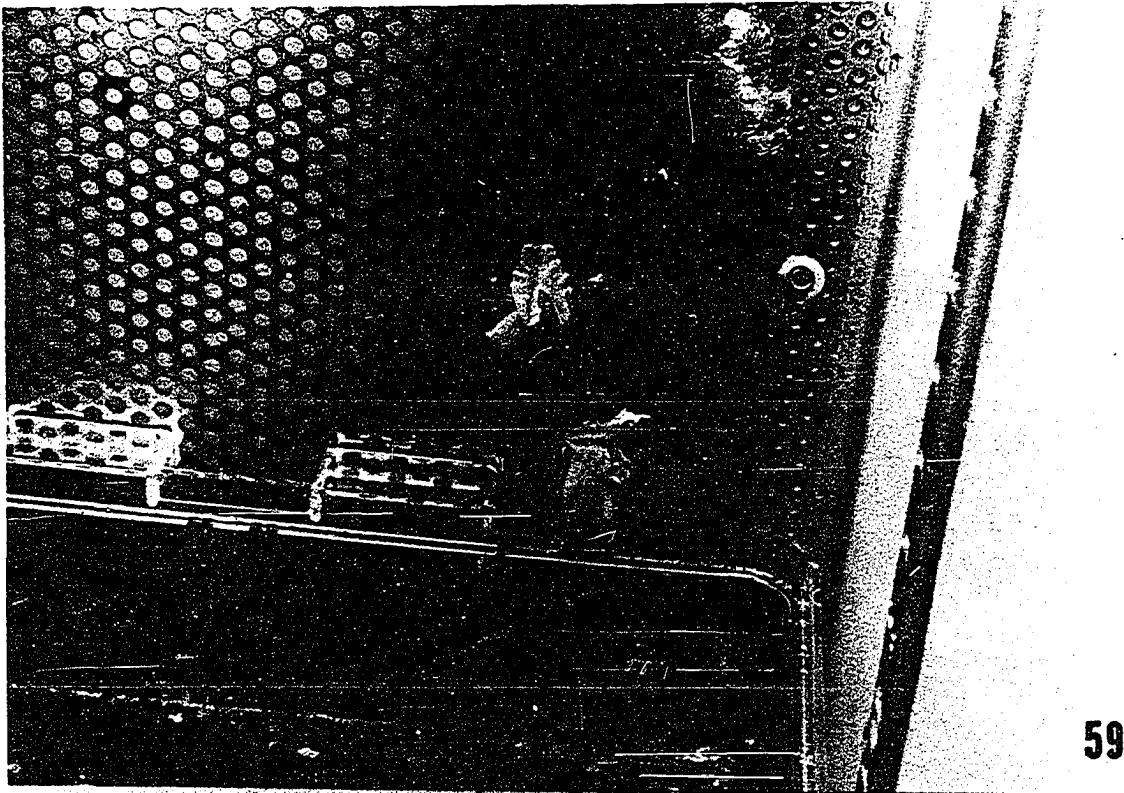
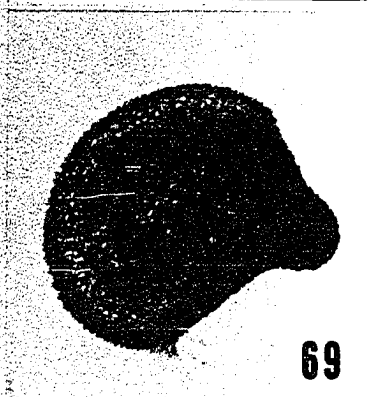
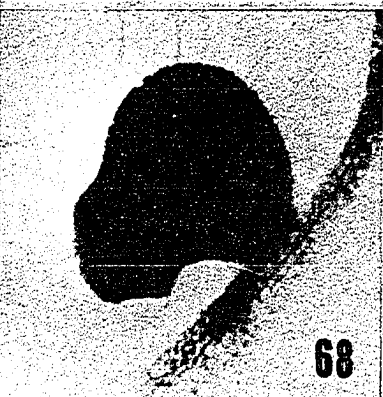
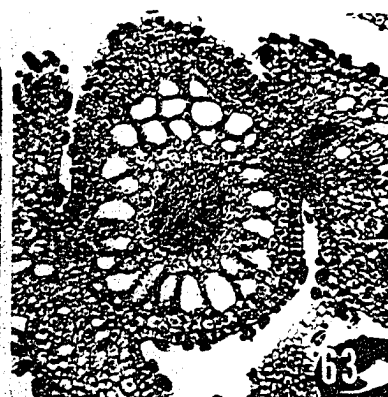


Fig. 59. Plants of U. macrorhiza collected from Silver Lake Fen and grown in growth chamber where flowers were produced.

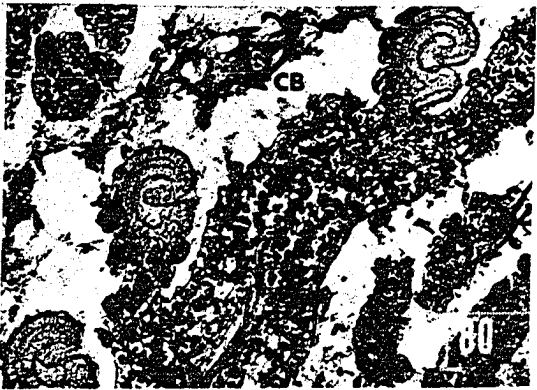
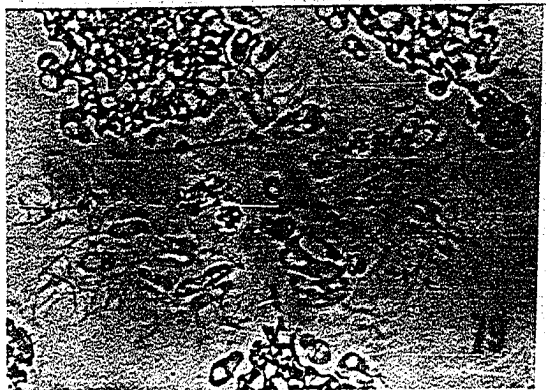
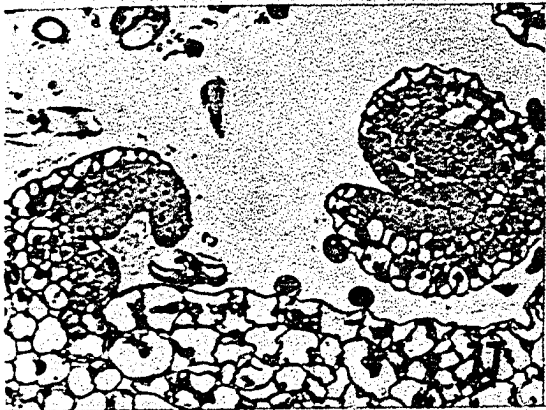
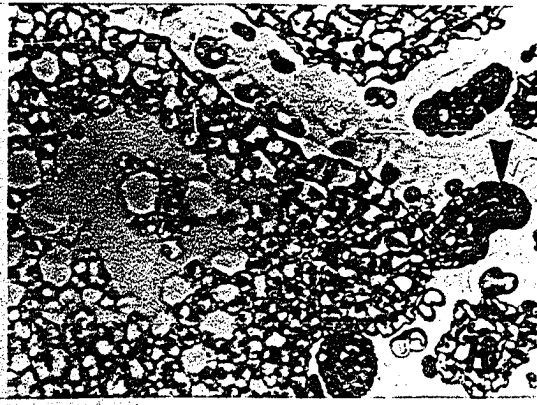
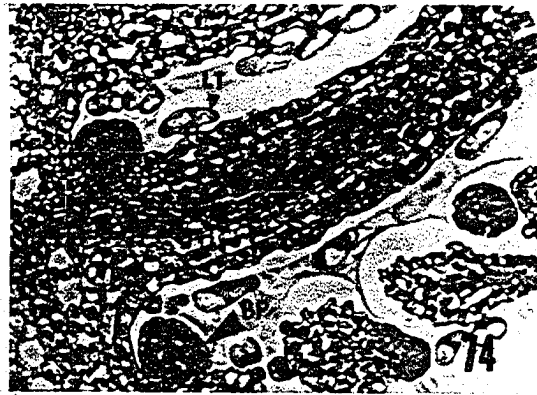
Fig. 60. Plants of U. macrorhiza from Silver Lake Fen in growth chamber prior to flowering.



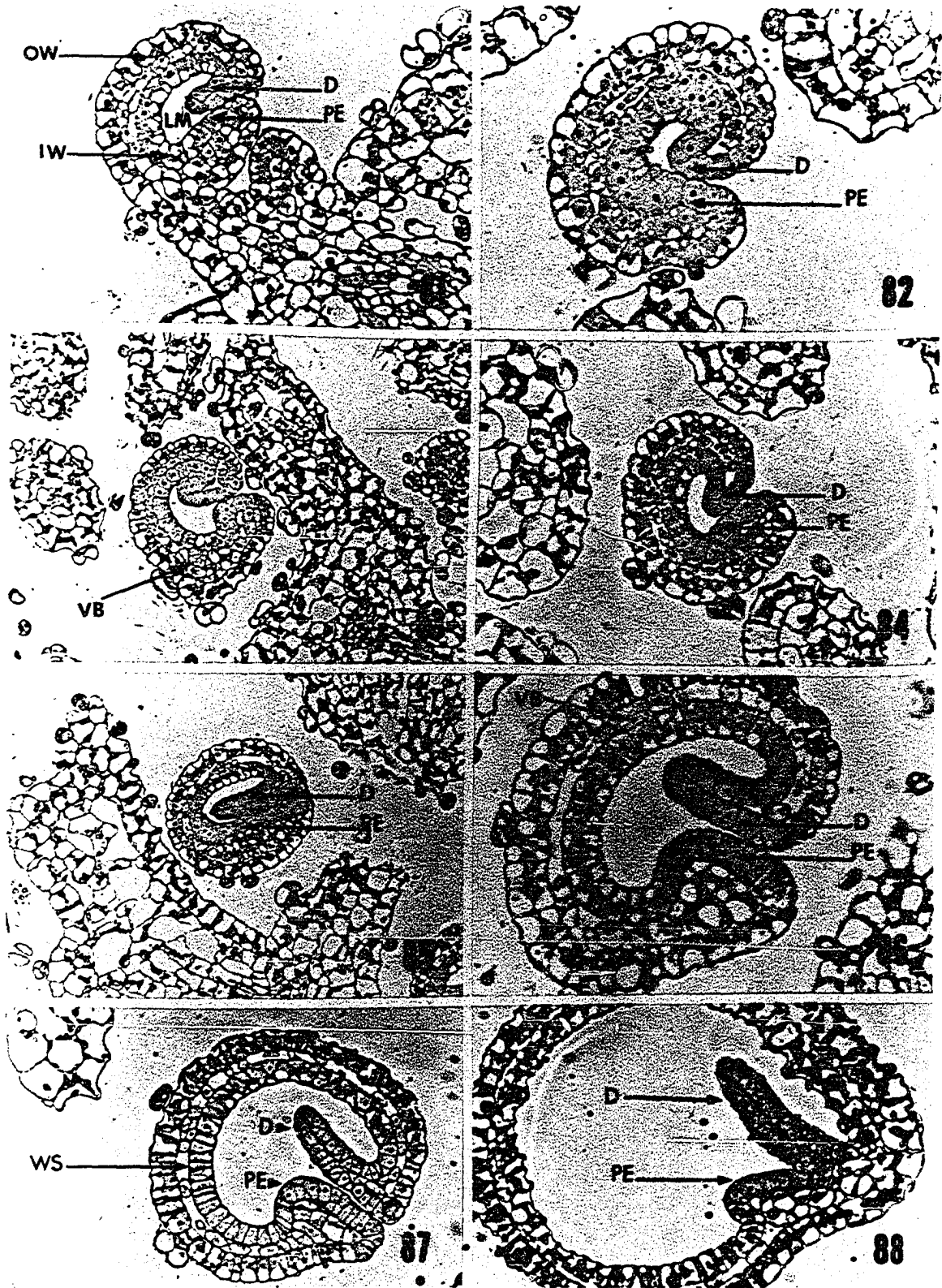
- Fig. 61. Tip of vegetative propagule. X 126 (SLOUGH)
- Fig. 62. Longitudinal section through tip of winter bud.
X 210 (3-CORN)
- Fig. 63. Cross section through main plant shoot. X 84
(3-CORN)
- Fig. 64. Shoot tip with lenticular trichomes. X 420 (SILFEN)
- Fig. 65. Small branches from winter bud. X 20 (SLOUGH)
- Fig. 66. Branch with young developing bladders.
X 20 (SLOUGH)
- Fig. 67. Clearing of branch with young bladders. X 126
(SILFEN)
- Fig. 68. Immature bladder oriented to show shape of anterior
future door region. X 20 (SLOUGH)
- Fig. 69. Immature bladder oriented to show shape from side.
X 20 (SLOUGH)
- Fig. 70. Immature bladder, cleared to show two types of tri-
chomes on surface, spherical trichomes and lenticu-
lar trichomes. X 84 (SLOUGH)
- Fig. 71. Immature bladder oriented to show developing door
region. X 20 (SLOUGH)
- Fig. 72. Mature bladder with trapped organisms inside. X 20
(SLOUGH)



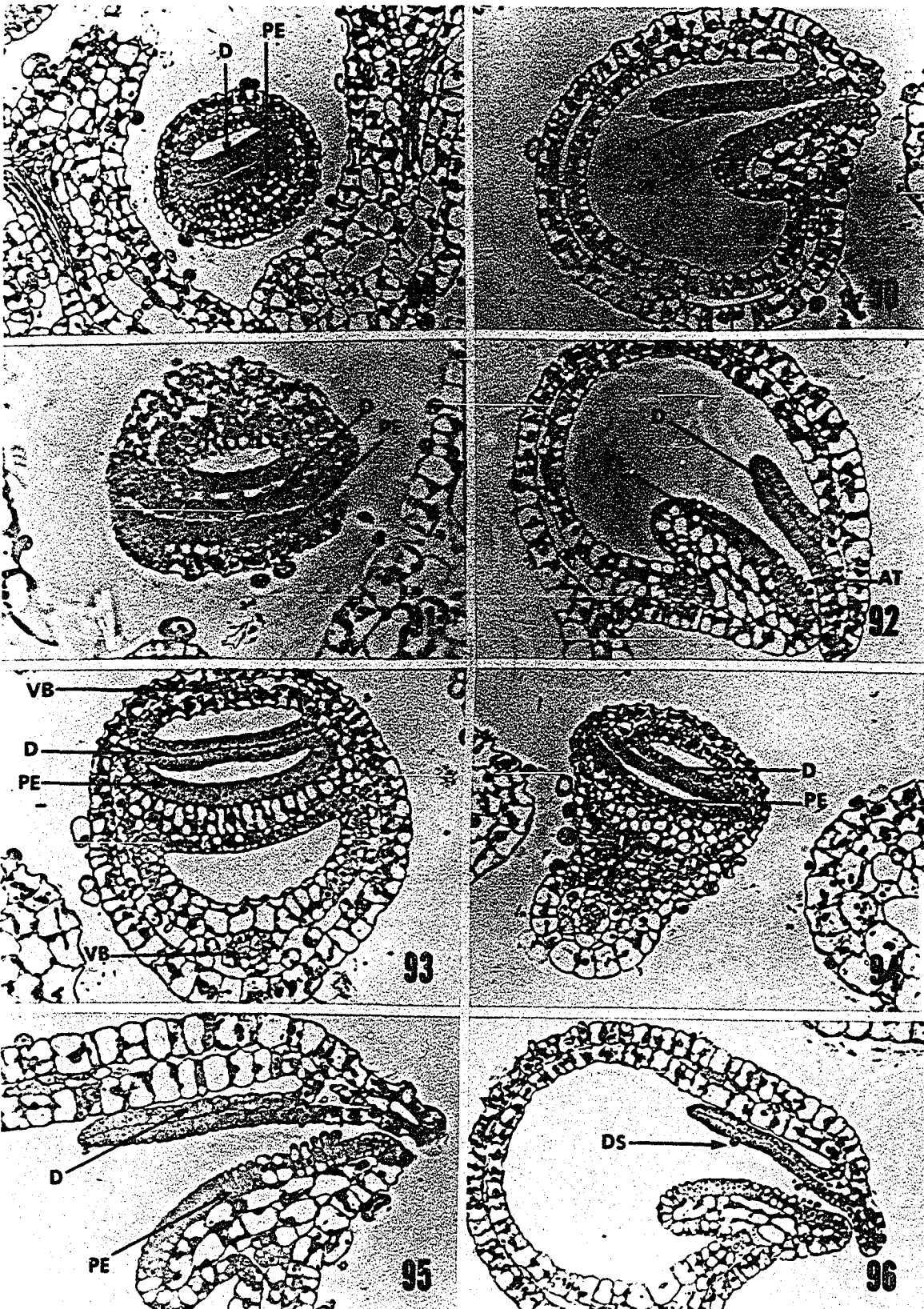
- Fig. 73. Section through developing bladder primordia.
X 210 (SLOUGH)
- Fig. 74. Section through winter bud branch to show bladder primordia and lenticular trichomes on branch surface. X 210 (SLOUGH)
- Fig. 75. Section through winter bud to show bladder primordia and carbohydrate secretory product on surface of the lenticular trichomes. X 210 (SLOUGH)
- Fig. 76. Section through winter bud to show elongation and unequal division of bladder primordium (arrow).
X 210 (SLOUGH)
- Fig. 77. Section through bladder primordium with apparent invagination of inner bladder wall. X 315 (SPRING)
- Fig. 78. Section through older bladder primordium showing three layers of cells; an outer wall layer, middle wall layer, and inner wall layer. X 315 (SPRING)
- Fig. 79. Section through lenticular trichomes to demonstrate that each is comprised of two terminal cells and is surrounded by secretory product. X 210 (SLOUGH)
- Fig. 80. Section through bladder primordia to demonstrate presence of carbohydrate product which protects the young branches and bladders during winter.
X 168 (SILFEN)



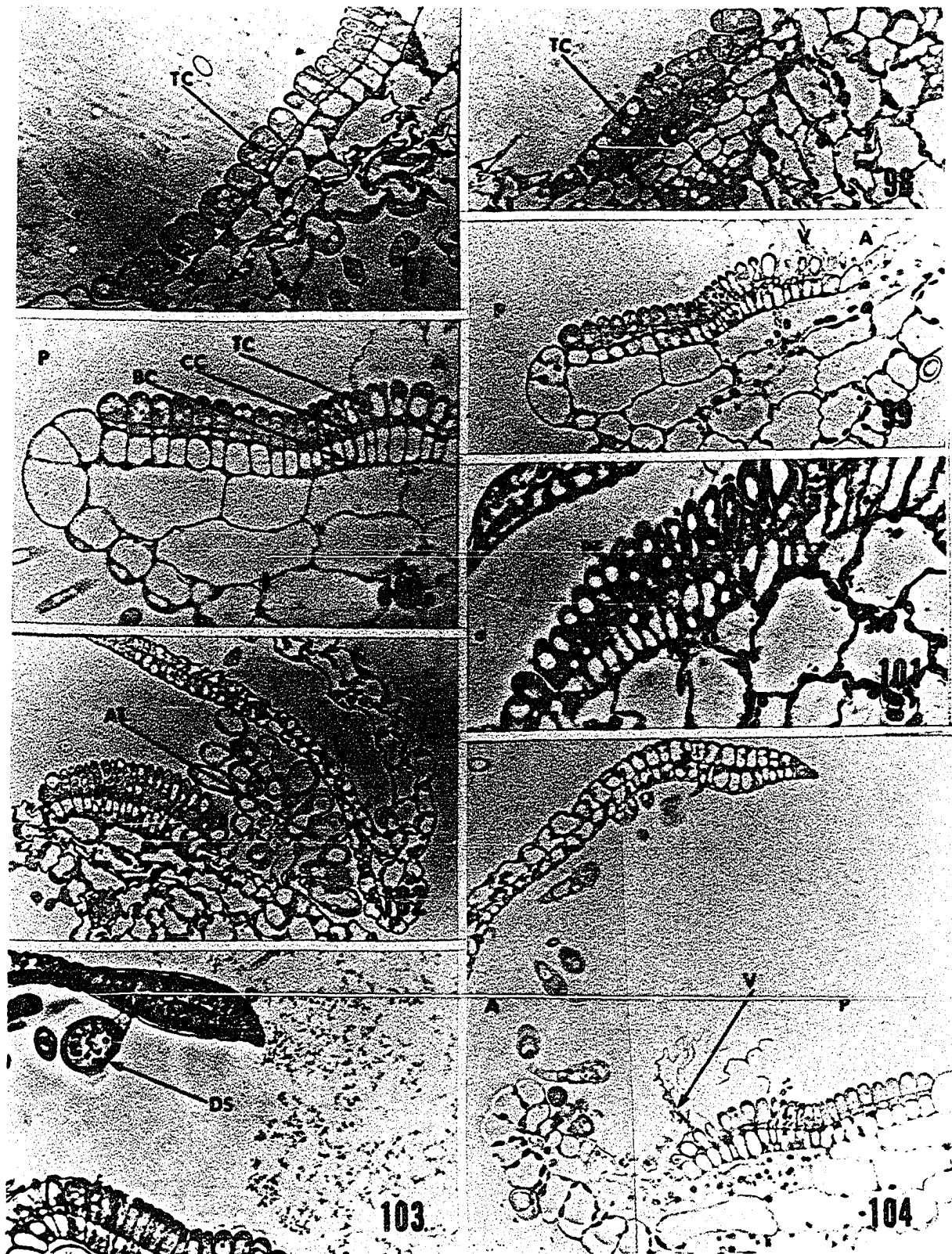
- Fig. 81. Longitudinal section through immature bladder showing outer wall, inner wall, lumen, door, and pavement epithelial regions (arrows). X 340 (SPRING)
- Fig. 82. Longitudinal section through immature bladder showing door and pavement epithelial regions (arrows). X 425 (SPRING)
- Fig. 83. Longitudinal section through immature bladder showing location of vascular bundle. X 210 (SPRING)
- Fig. 84. Longitudinal section through immature bladder showing door and pavement epithelial regions. X 340 (SPRING)
- Fig. 85. Section through immature bladder showing relationship between door and pavement epithelial regions.
- Fig. 86. Longitudinal section through immature bladder showing vascular bundle. X 425 (SPRING)
- Fig. 87. Longitudinal section through immature bladder showing spaces between cells of middle wall region. X 315 (SPRING)
- Fig. 88. Longitudinal section through immature bladder showing later development of door and pavement epithelial regions than shown in previous figures. X 210 (SPRING)



- Fig. 89. Cross section through anterior bladder region showing relationship between door and pavement epithelium. X 210 (SPRING)
- Fig. 90. Longitudinal section through young bladder showing door and pavement epithelium prior to development of trichomes. X 315 (SPRING)
- Fig. 91. Cross section through anterior bladder region showing relationship between door and pavement epithelium. X 210 (SPRING)
- Fig. 92. Longitudinal section through young bladder with developing aperture trichomes. X 315 (SPRING)
- Fig. 93. Cross section through anterior bladder region showing relationship between door and pavement epithelium and location of vascular bundles. X 210 (SPRING)
- Fig. 94. Cross section through young bladder more anterior than Fig. 93 showing relationship between door and pavement epithelium. X 210 (SPRING)
- Fig. 95. Longitudinal section through anterior bladder region showing door, pavement epithelium, and aperture trichome initiation anterior to the pavement epithelium. X 340 (SPRING)
- Fig. 96. Longitudinal section through developing bladder showing initiation of door stop trichomes. X 210 (SPRING)



- Fig. 97. Tangential section through pavement epithelium showing trichomes with two terminal cells. X 425 (SPRING)
- Fig. 98. Oblique cross section through pavement epithelium showing trichomes with two terminal cells. X 425 (SPRING)
- Fig. 99. Longitudinal section through pavement epithelium and velum. X 315 (SPRING)
- Fig. 100. Longitudinal section through pavement epithelium showing three cell types making up each trichome. X 425 (SPRING)
- Fig. 101. Longitudinal section through pavement epithelium. X 510 (SPRING)
- Fig. 102. Longitudinal section through anterior bladder region showing pavement epithelium, aperture trichomes, and door. X 315 (3-CORN)
- Fig. 103. Longitudinal section through pavement epithelium, door, and a door-stop cell. X 420 (SPRING)
- Fig. 104. Longitudinal section through anterior bladder region showing door, pavement epithelium, aperture trichomes, and velum. X 315 (SPRING)



- Fig. 105. Oblique longitudinal section through aperture trichomes. X 340 (SPRING)
- Fig. 106. Cross section through aperture trichomes showing carbohydrate secretory product surrounding each trichome. X 340 (SPRING)
- Fig. 107. Longitudinal section through anterior aperture region showing location of aperture trichomes. X 315 (SPRING)
- Fig. 108. Fresh preparation of door showing short spherical trichomes (arrow) on surface. X 340 (SLOUGH)
- Fig. 109. Clearing of aperture region showing aperture trichomes. X 340 (SPRING)
- Fig. 110. Clearing of aperture trichomes showing three cell types making up each trichome. X 340 (SPRING)
- Fig. 111. Clearing showing anterior bladder region with location of door and aperture trichomes. X 315 (SPRING)
- Fig. 112. Fresh preparation showing branched antennae of anterior bladder region. X 84 (SPRING)

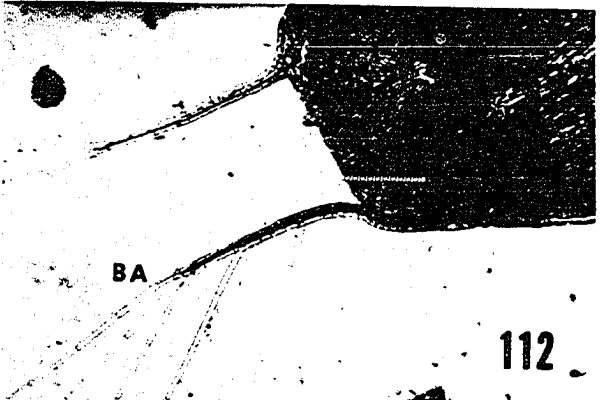
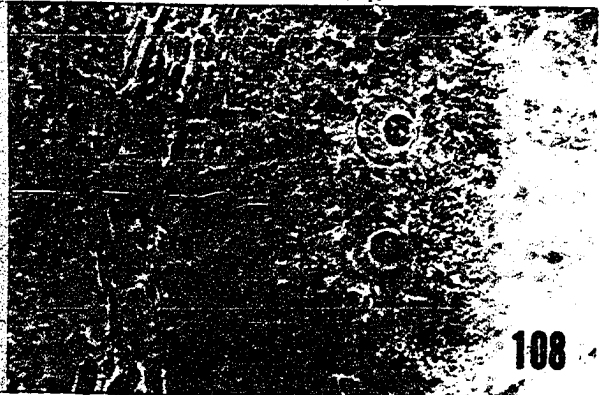
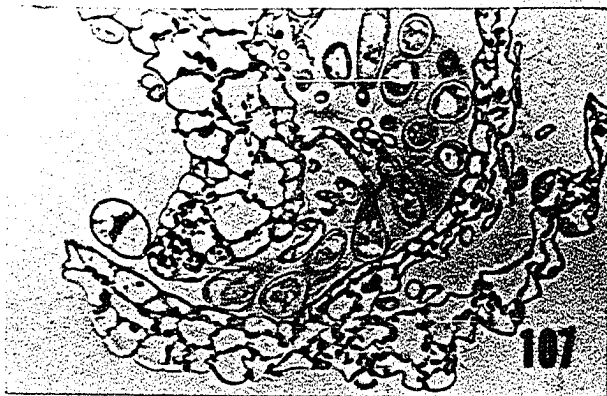
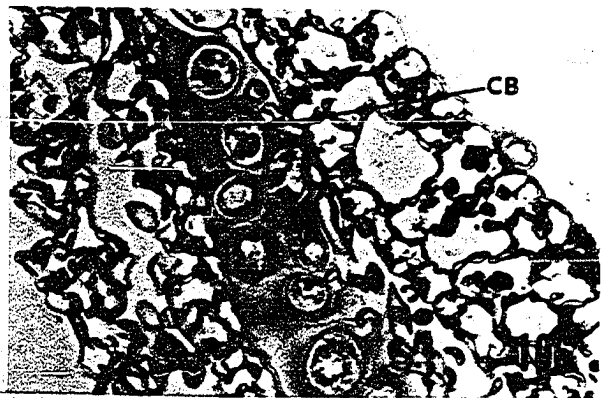


Fig. 113. SEM of mature bladder showing trichomes and antennae surrounding bladder aperture. X 60 (SLOUGH)

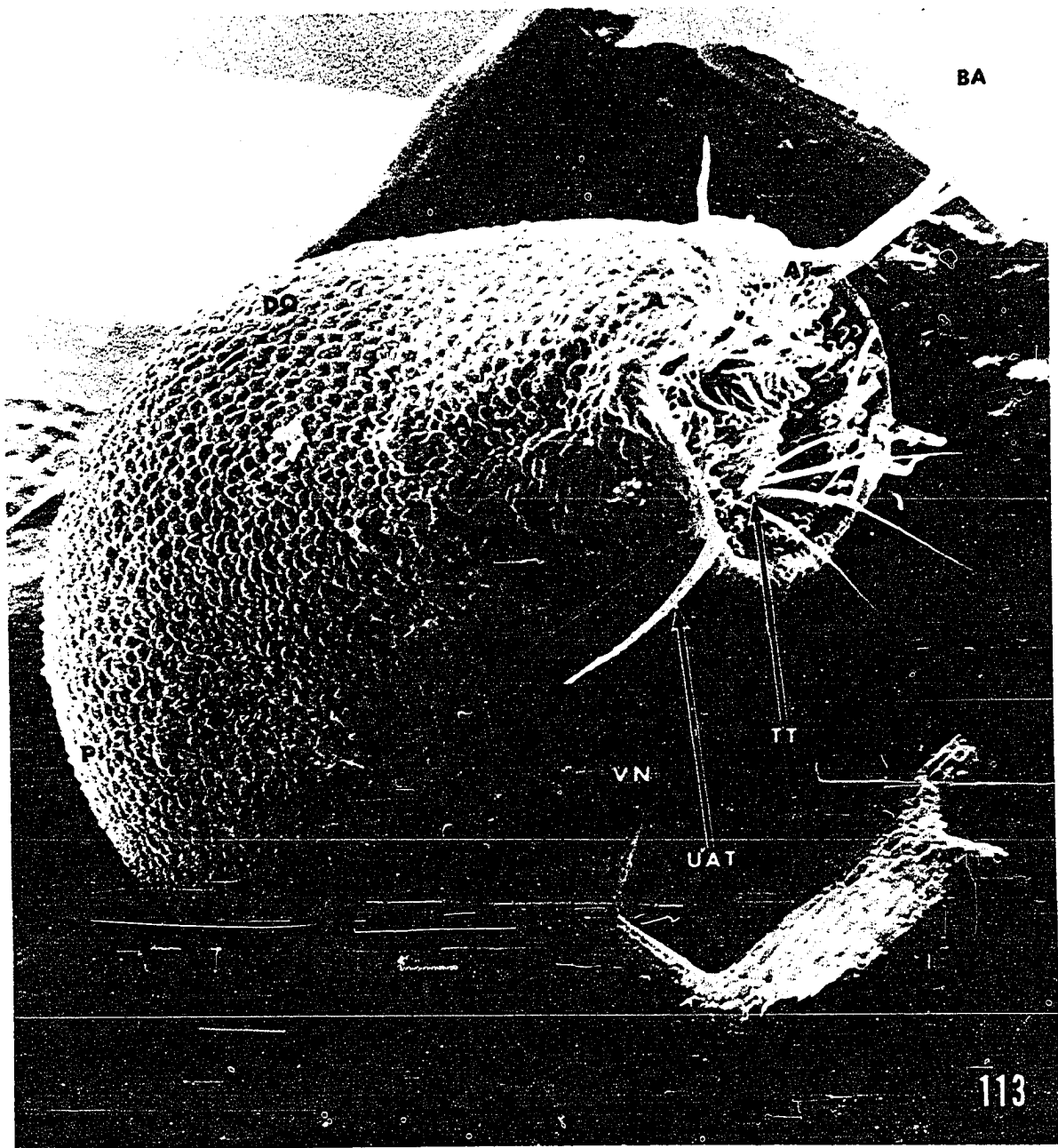


Fig. 114. SEM of base of a multicellular branched antenna.
X 600 (SLOUGH)

Fig. 115. SEM of branched antenna, spherical trichomes,
and base of trigger trichomes. X 600 (SLOUGH)

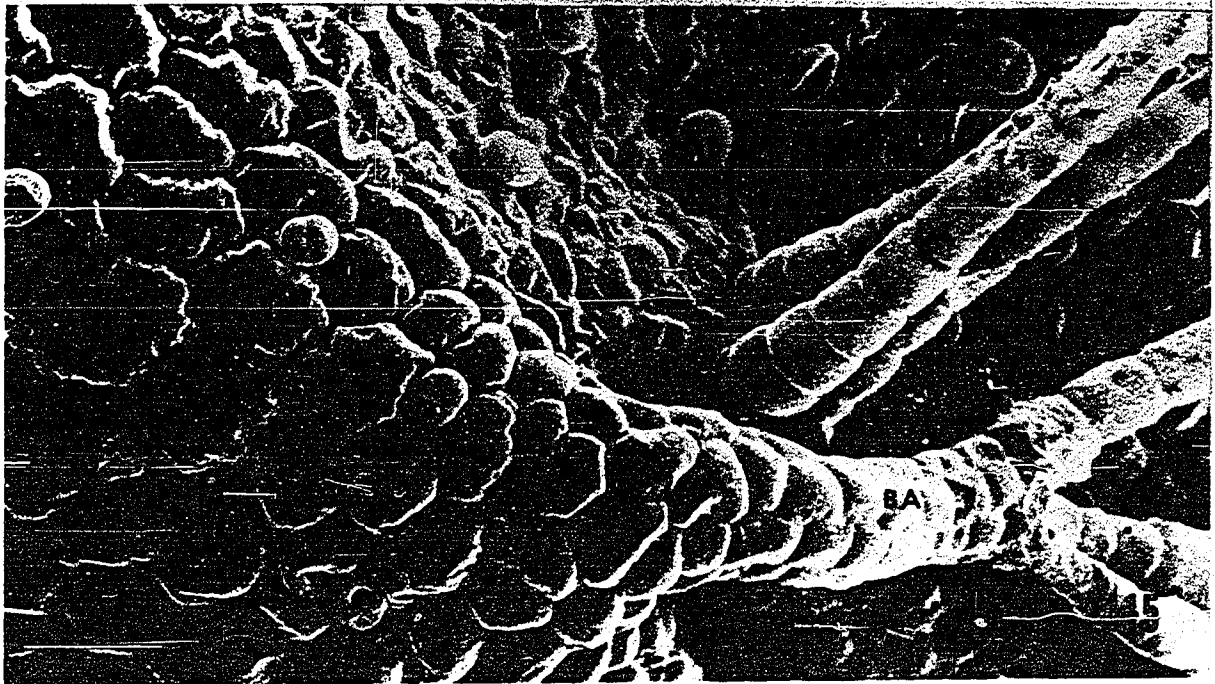
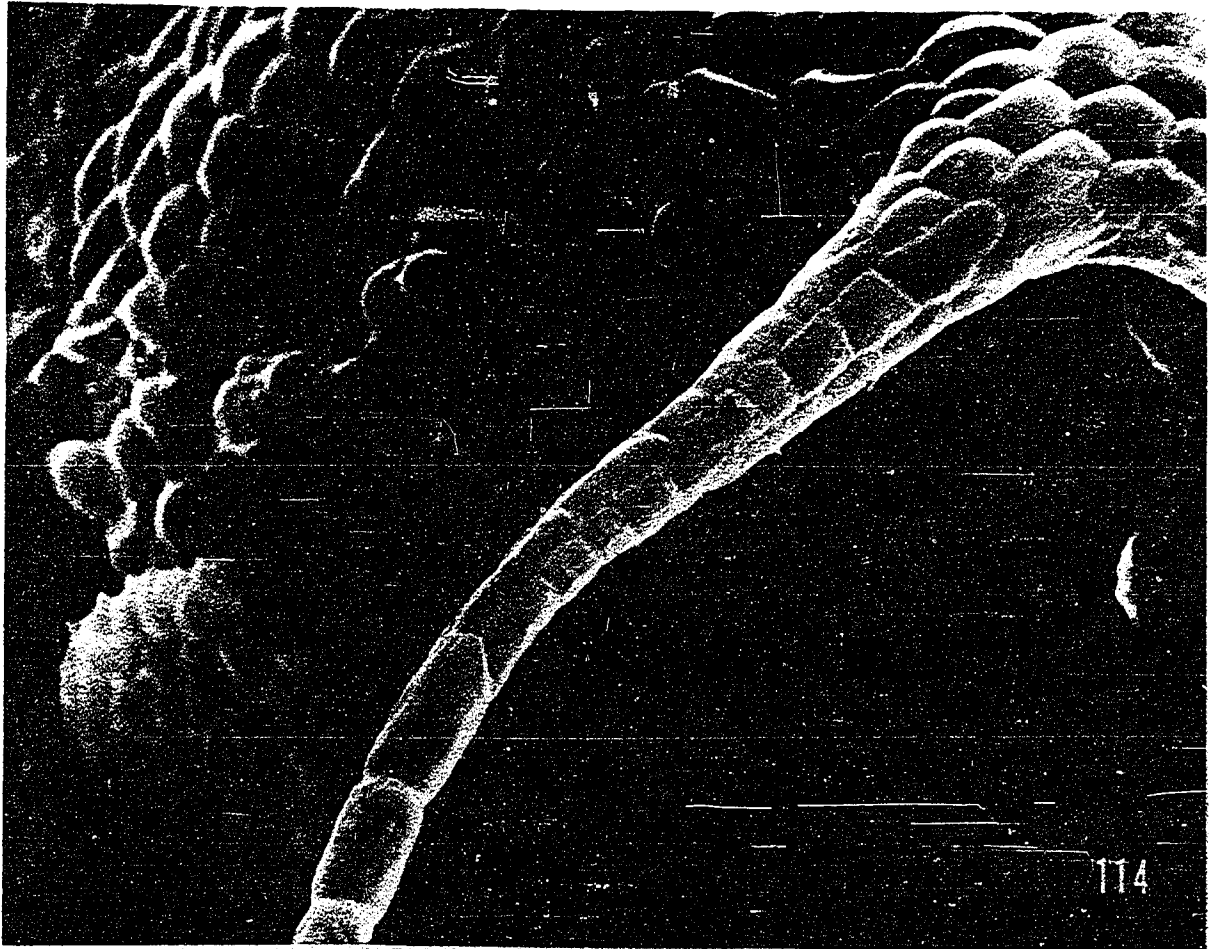
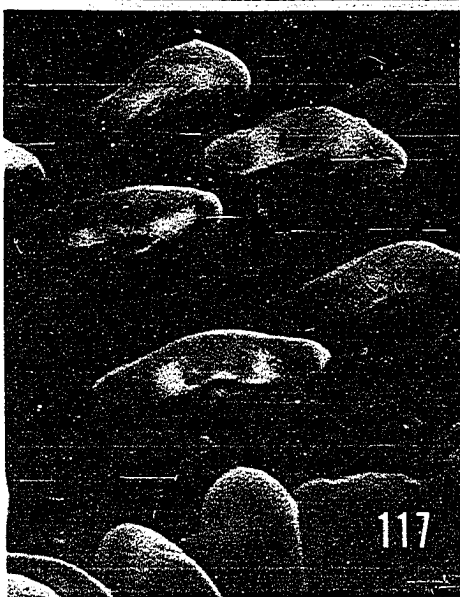
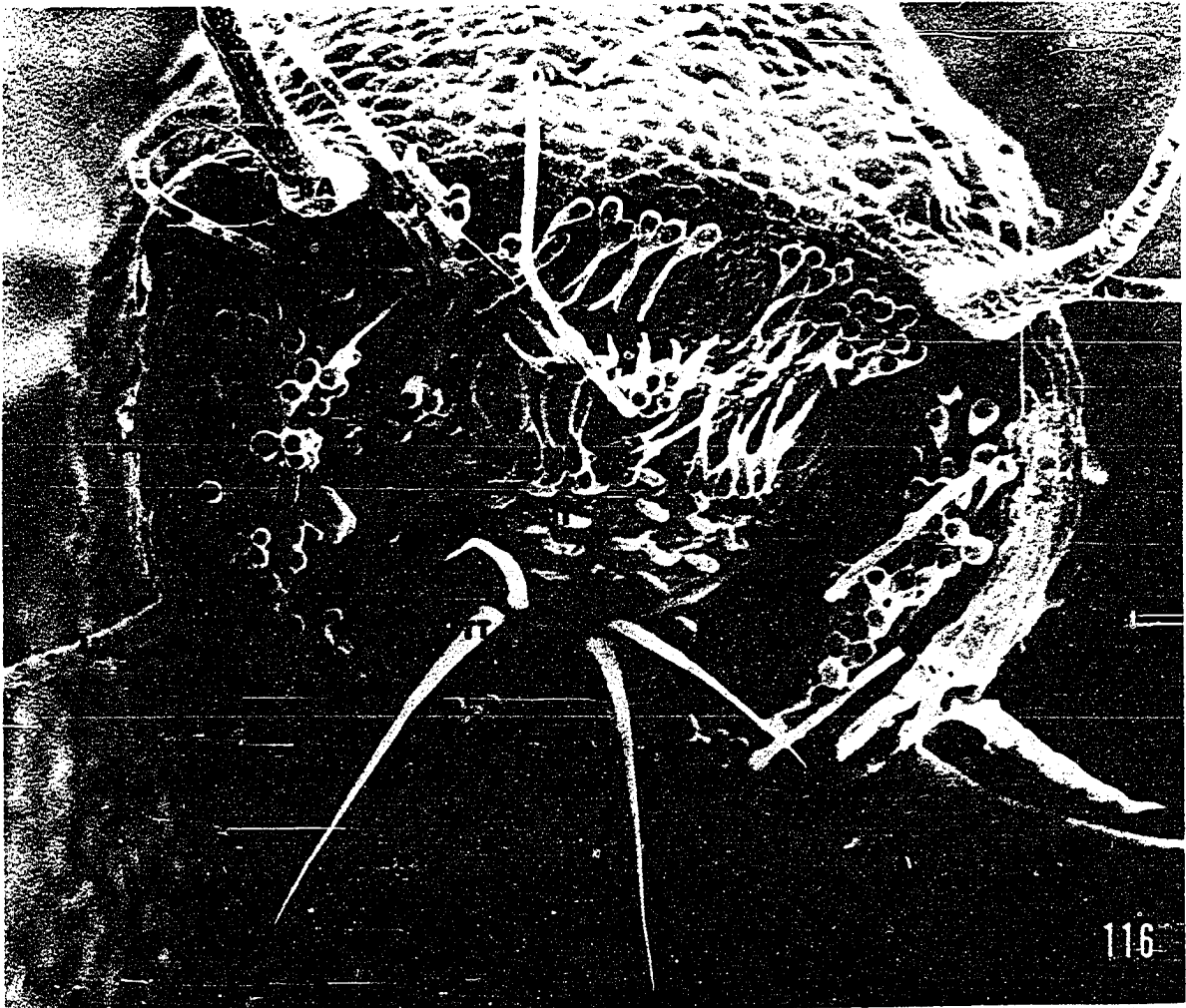


Fig. 116. SEM of aperture region showing closed door with associated trichomes, aperture trichomes, and both branched and unbranched antennae. X 103 (SLOUGH)

Fig. 117. SEM of hammer-head trichomes on door surface. X 500 (SLOUGH)

Fig. 118. SEM of aperture trichomes showing carbohydrate secretory product on surface and three cell types making up each trichome. X 920 (SLOUGH)



- Fig. 119. Longitudinal section through door showing scalloped walls of anterior door cells (arrow) and relationship between trigger trichomes and door-stop trichomes. X 210 (SPRING)
- Fig. 120. Trigger trichomes viewed with polarization optics. X 210 (SILFEN)
- Fig. 121. Fresh preparation of door with trigger trichomes and other surface trichomes. X 210 (SILFEN)
- Fig. 122. Fresh preparation of aperture region with protruding trigger trichomes. X 210 (SILFEN)
- Fig. 123. SEM of aperture region showing door, door-stop trichomes, trigger trichomes, velum, and bifids. X 230 (SPRING)
- Fig. 124. SEM of aperture region with door closed showing trigger trichomes and aperture trichomes. X 230 (SPRING)

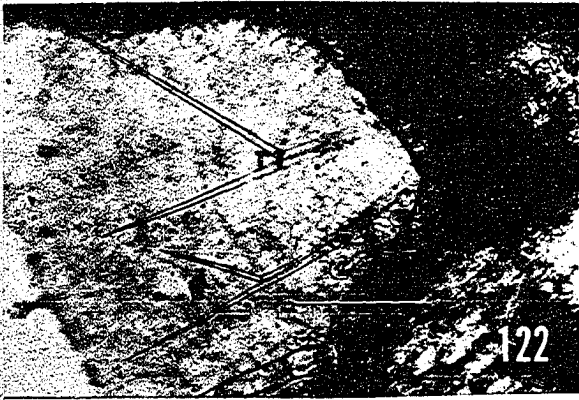
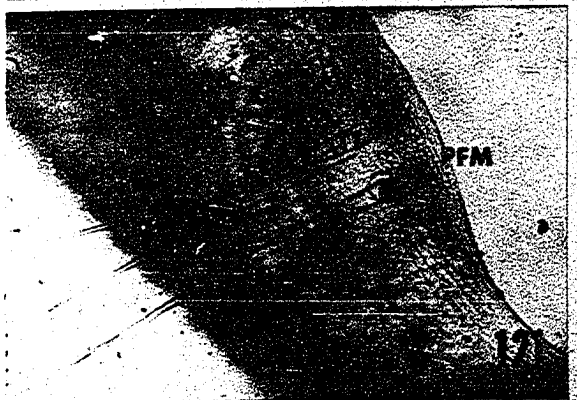
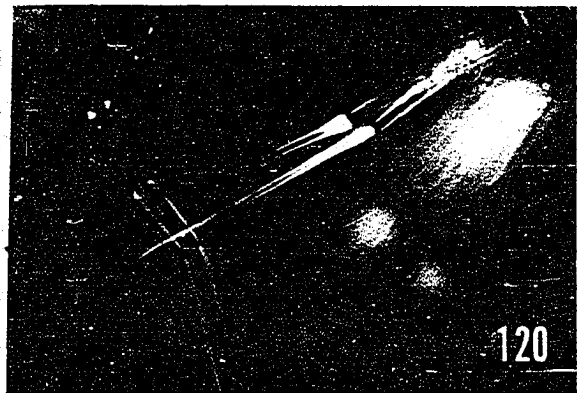
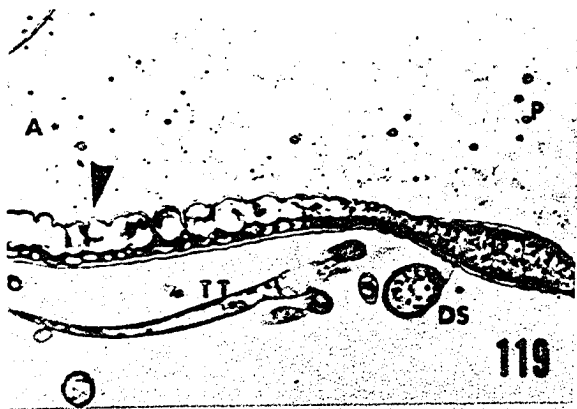


Fig. 125-126. Longitudinal section through portions of
young developing trigger trichomes.
X 5,872 (SPRING)

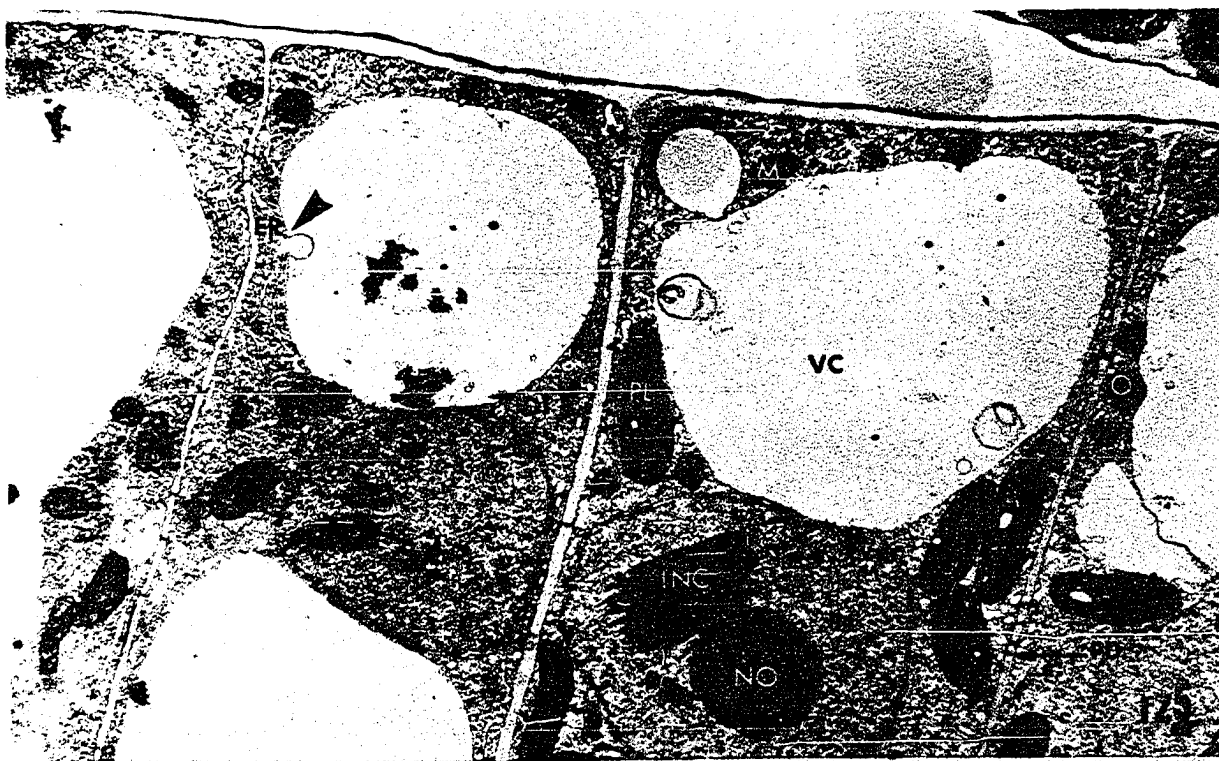


Fig. 127. Longitudinal section through aperture trichome.
X 4,370 (SPRING)

Fig. 128-129. Higher magnification of Fig. 127 through terminal cell of trichome showing abundance of RER and the exfoliation of cuticle. X 11,000 (SPRING)

Fig. 130. Higher magnification of Fig. 127 through central cell region showing endodermoid wall. X 11,000 (SPRING)

Fig. 131. Higher magnification of Fig. 127 through lower central cell and upper basal cell regions.
X 11,000 (SPRING)



Fig. 132. Longitudinal section through terminal cell of aperture trichome showing exfoliated cuticle, carbohydrate secretory product, and cutin cystolith wall. X 7,340 (SPRING)

Fig. 133. Longitudinal section through central cell of aperture trichome. X 7,340 (SPRING)

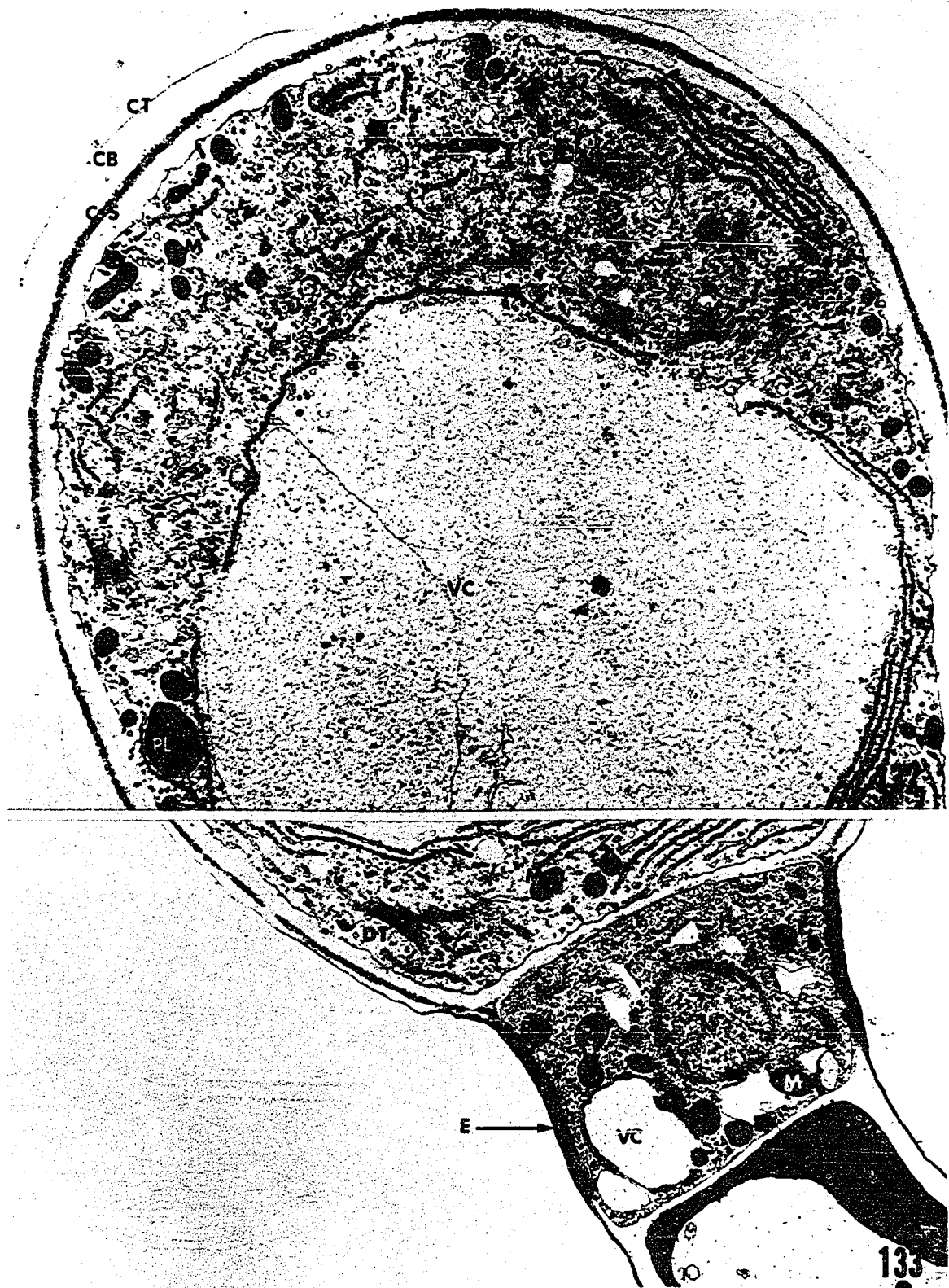


Fig. 134-135. Cross section through bladder vascular bundle showing relationship among phloem parenchyma, sieve tube elements, and companion cells. X 7,340 (SPRING)

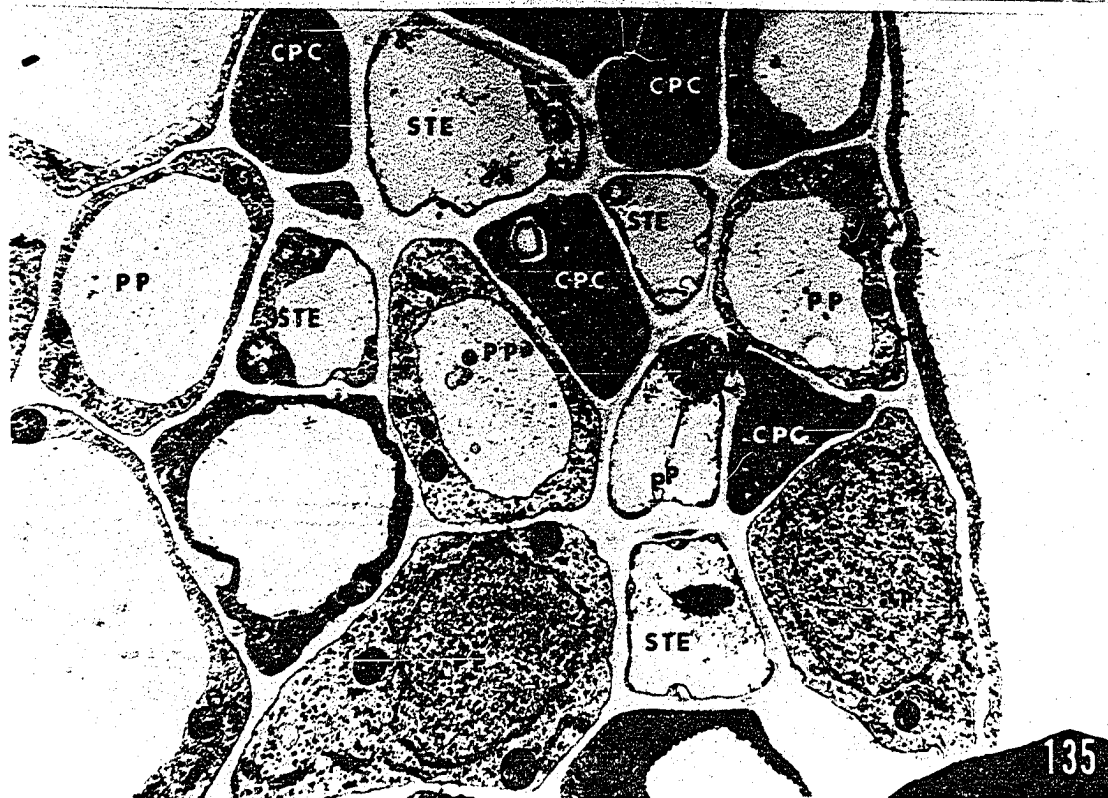
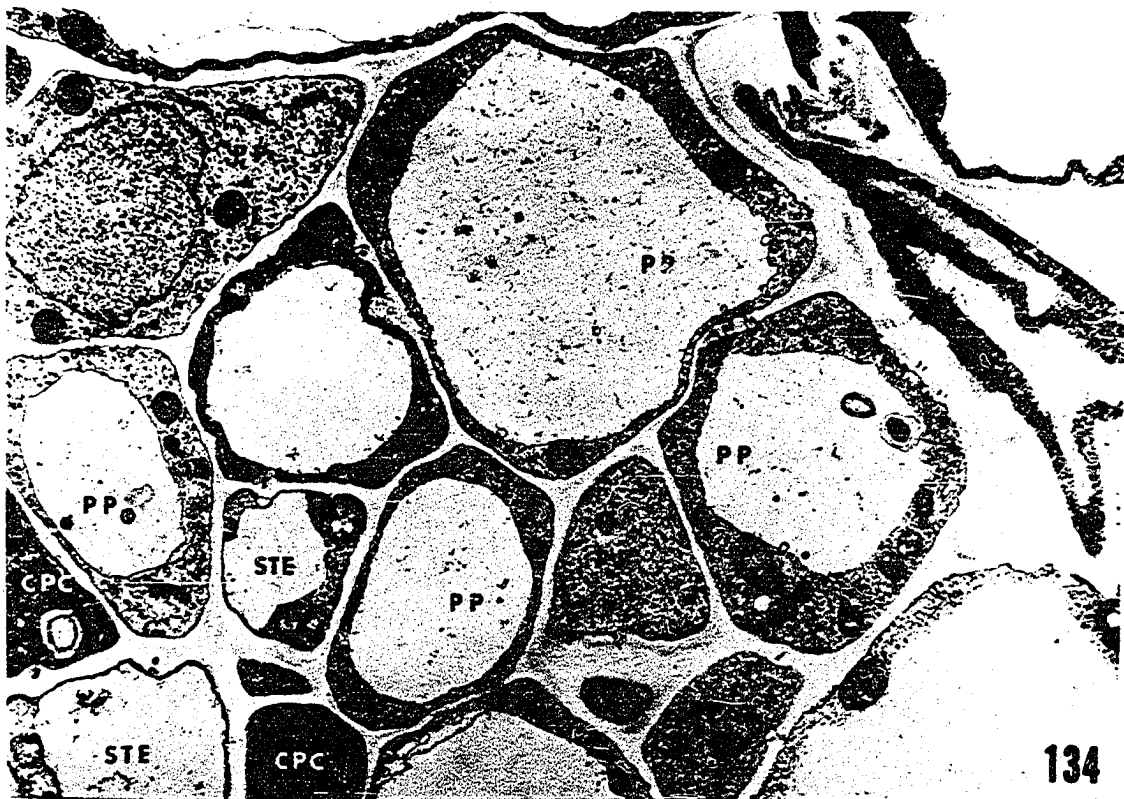


Fig. 136. Longitudinal section through chloroplast in bladder wall cell. X 42,200 (3-CORN)

Fig. 137. Longitudinal and cross section through chloroplasts in bladder wall cells. X 17,200 (3-CORN)



136



Fig. 138. SEM of inner door surface showing four regions: posterior free margin, lateral door, anterior door, and central door. X 380 (SPRING)

Fig. 139. SEM of posterior free margin of closed door and bifids on posterior pavement epithelium. X 380 (SPRING)

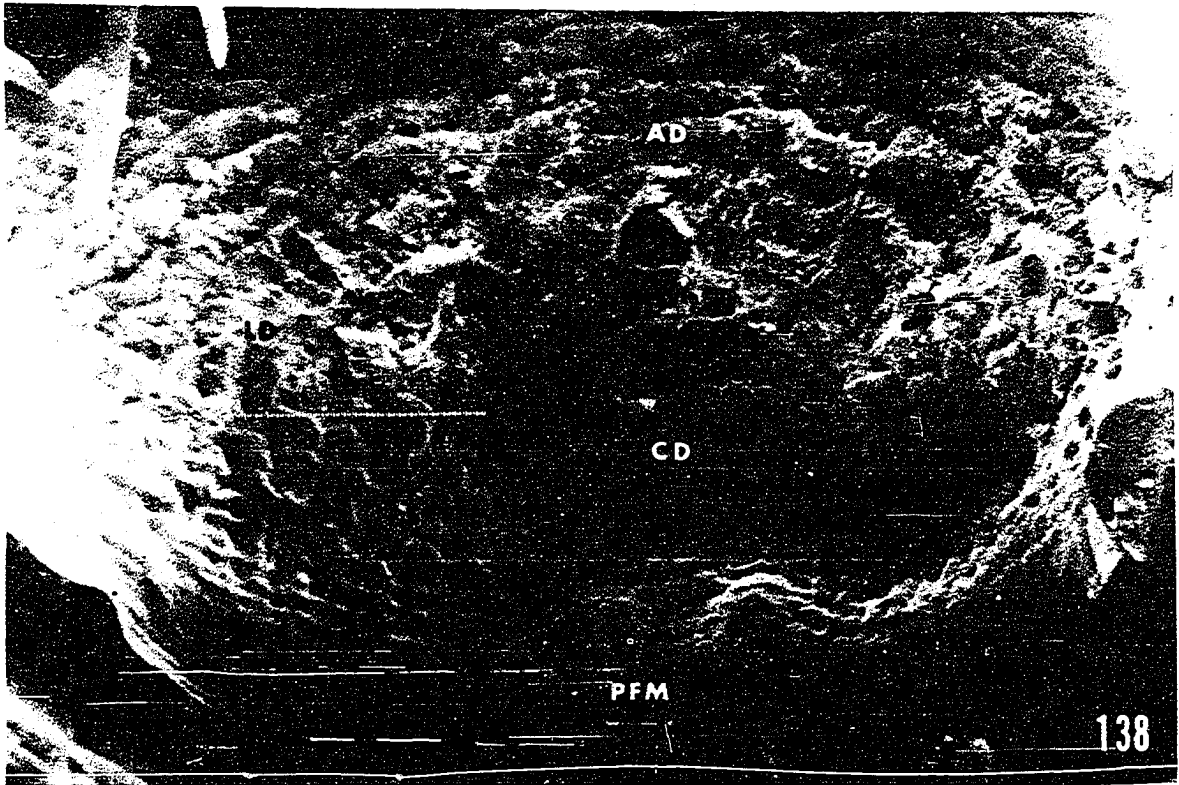


Fig. 140-141. Longitudinal sections through posterior free margin region of door. X 11,200 (SPRING)

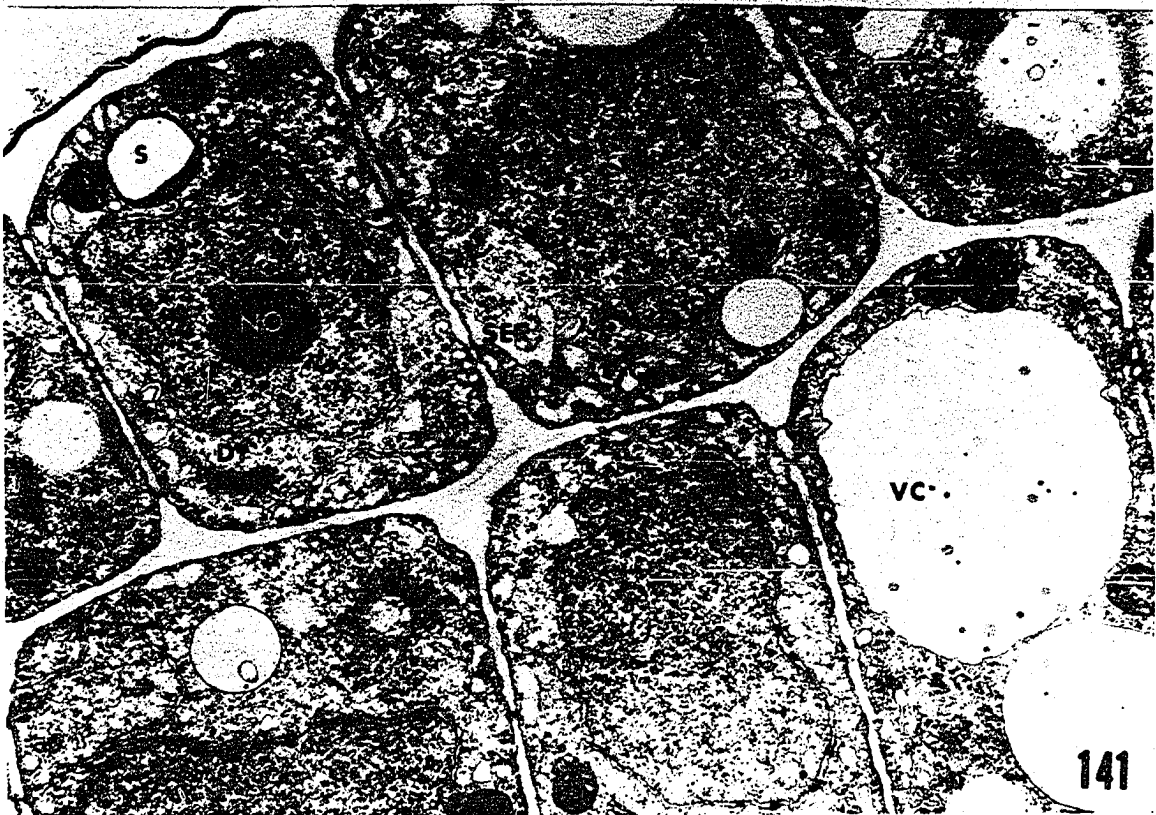
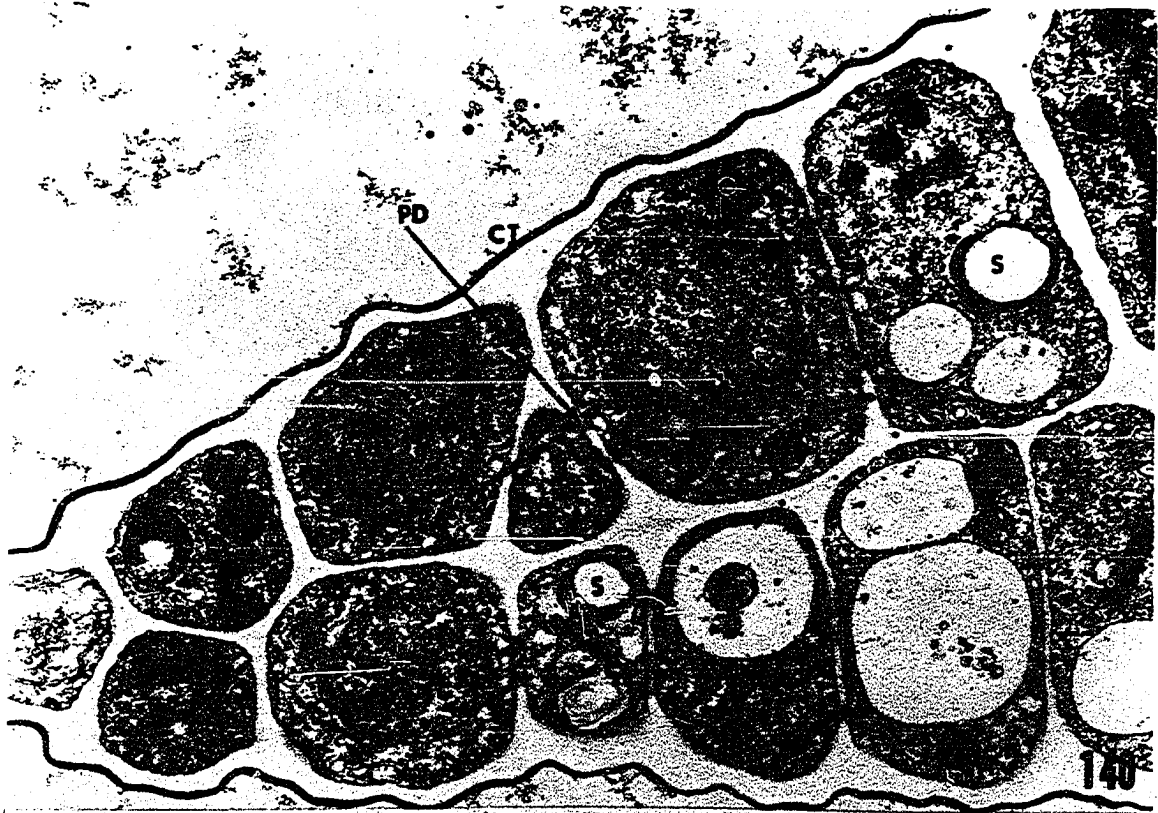


Fig. 142-144. Longitudinal sections through lateral region
of door showing small wall thickenings.
X 7,340 (SPRING)



Fig. 145-146. Longitudinal sections through central door
region showing large wall thickenings.
X 7,340 (SPRING)



Fig. 147. Longitudinal section through door showing door surface trichome. X 7,340 (SPRING)



Fig. 148. Longitudinal section through immature pavement
epithelium. X 9,370 (SLOUGH)

Fig. 149. Longitudinal section through immature pavement
epithelium. X 8,400 (SLOUGH)

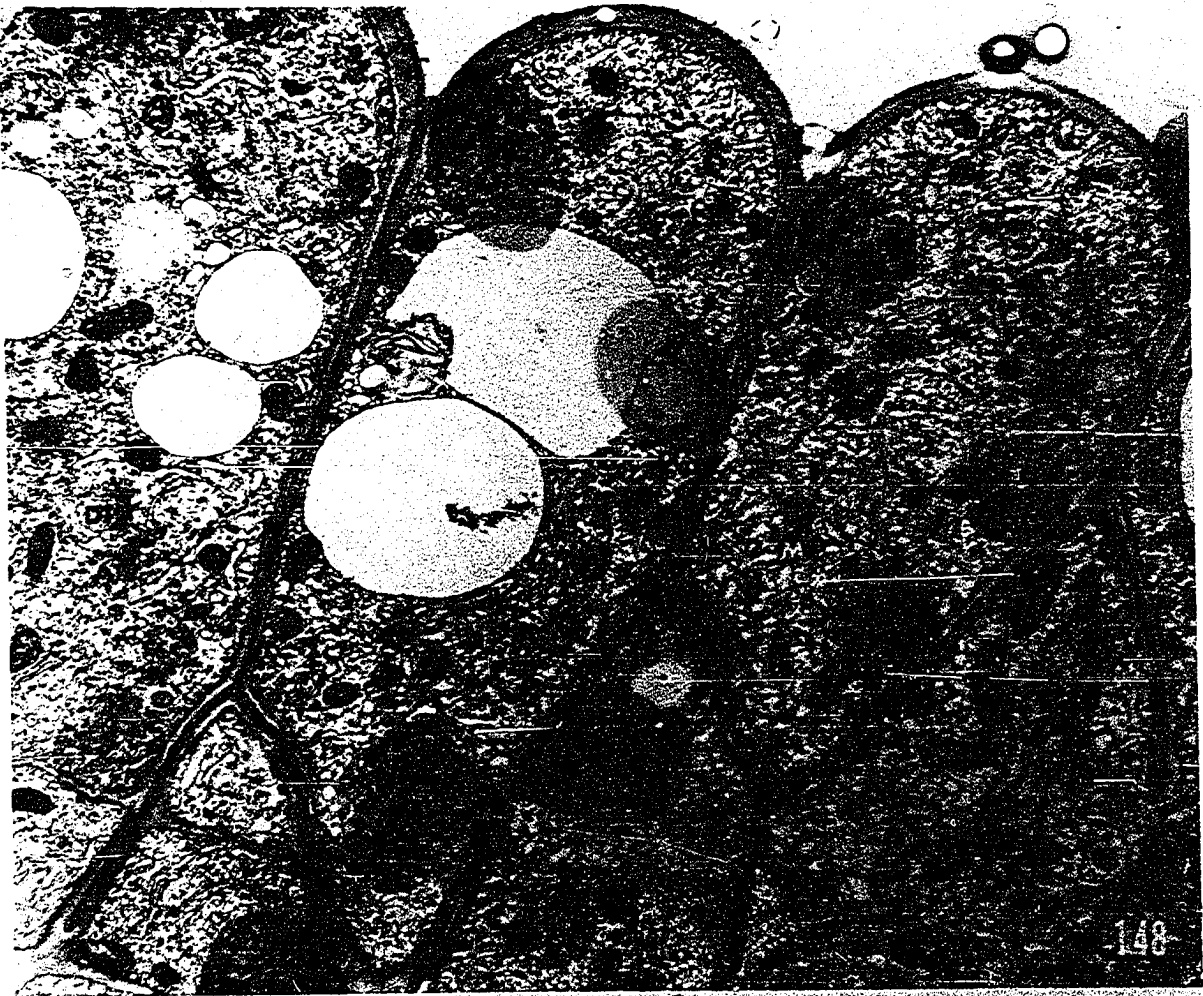


Fig. 150. Longitudinal section through pavement epithelial trichome showing three cell types making up trichome. X 5,500 (SPRING)

Fig. 151. Longitudinal section through proximal region of terminal cell of pavement epithelial trichome showing thick wall and associated cuticle. X 22,200 (SPRING)

Fig. 152. Longitudinal section through distal periclinal central cell wall showing plasmodesmata. X 22,200 (SPRING)

Fig. 153. Longitudinal section through endodermoid wall of central cell in pavement epithelial trichome. X 22,200 (SPRING)

Fig. 154. Longitudinal section through wall in basal cell showing plasmodesmata. X 7,340

Fig. 155. Longitudinal section through basal cell proximal periclinal wall showing absence of plasmodesmata. X 7,340 (SPRING)

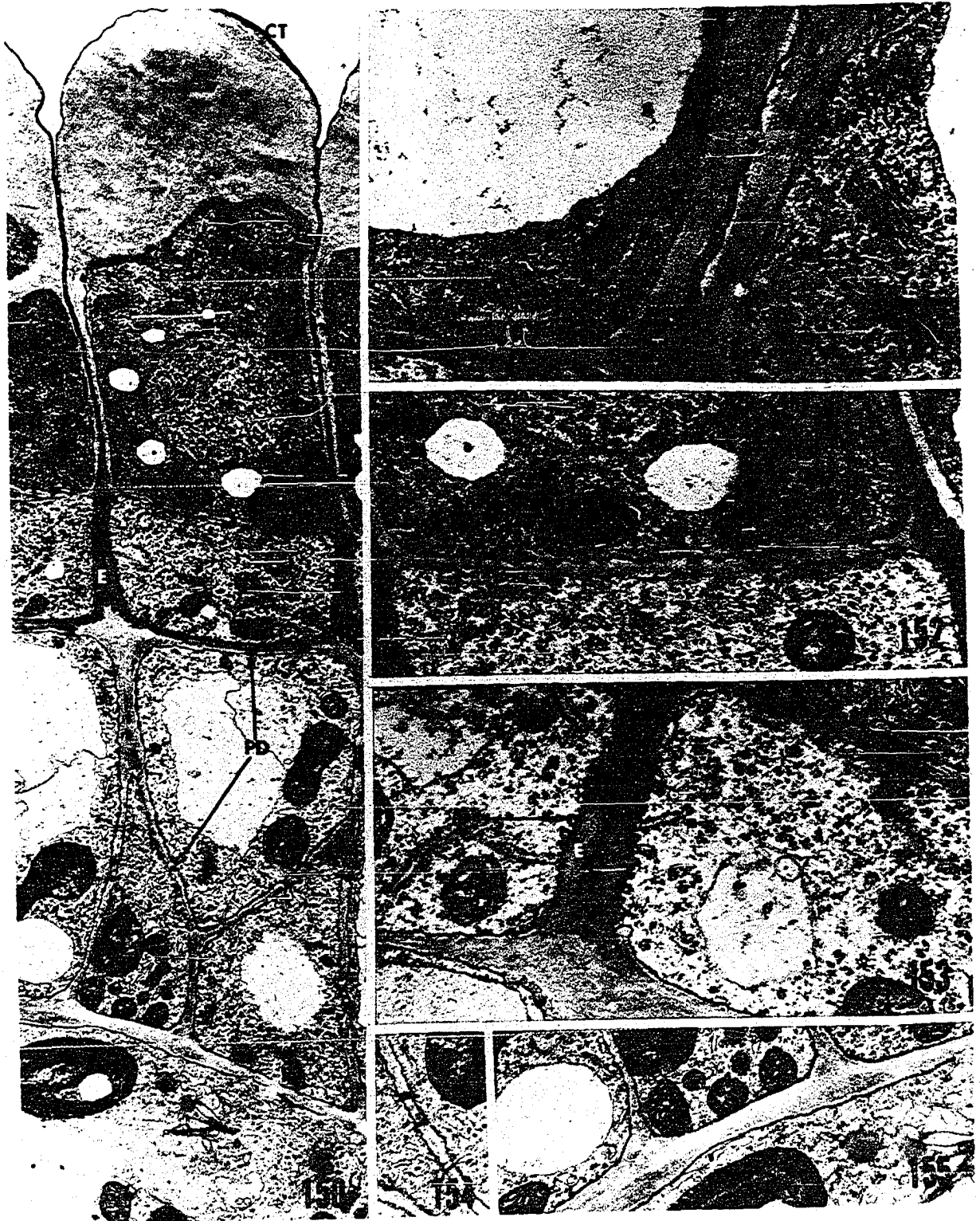


Fig. 156. Longitudinal section through middle region of
pavement epithelium. X 9,175 (SPRING)



Fig. 157. Longitudinal section through anterior pavement epithelium showing formation of velum. X 9,175 (SPRING)

Fig. 158. Longitudinal section through central cell endodermoid wall showing irregular plasmalemma. X 56,000 (SPRING)

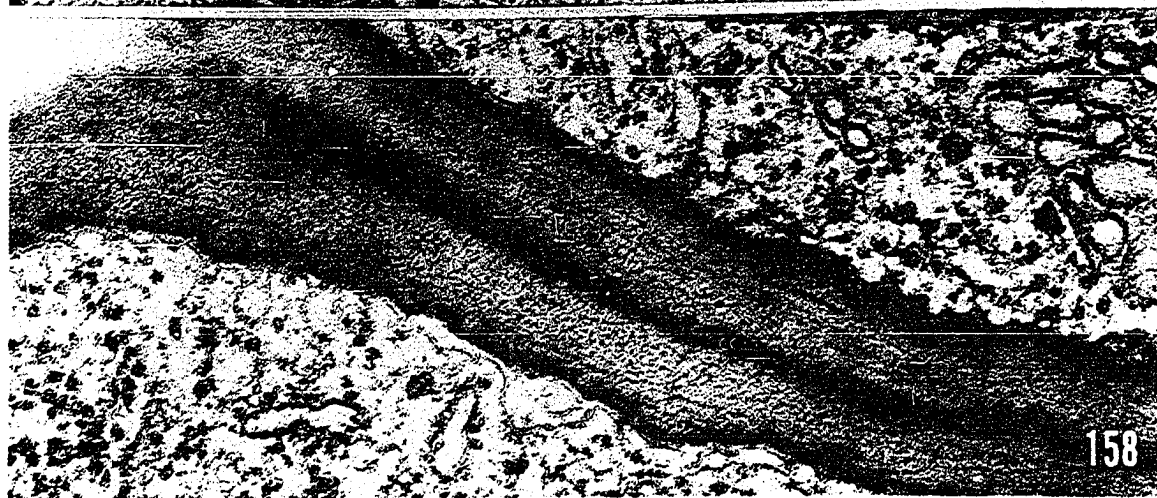
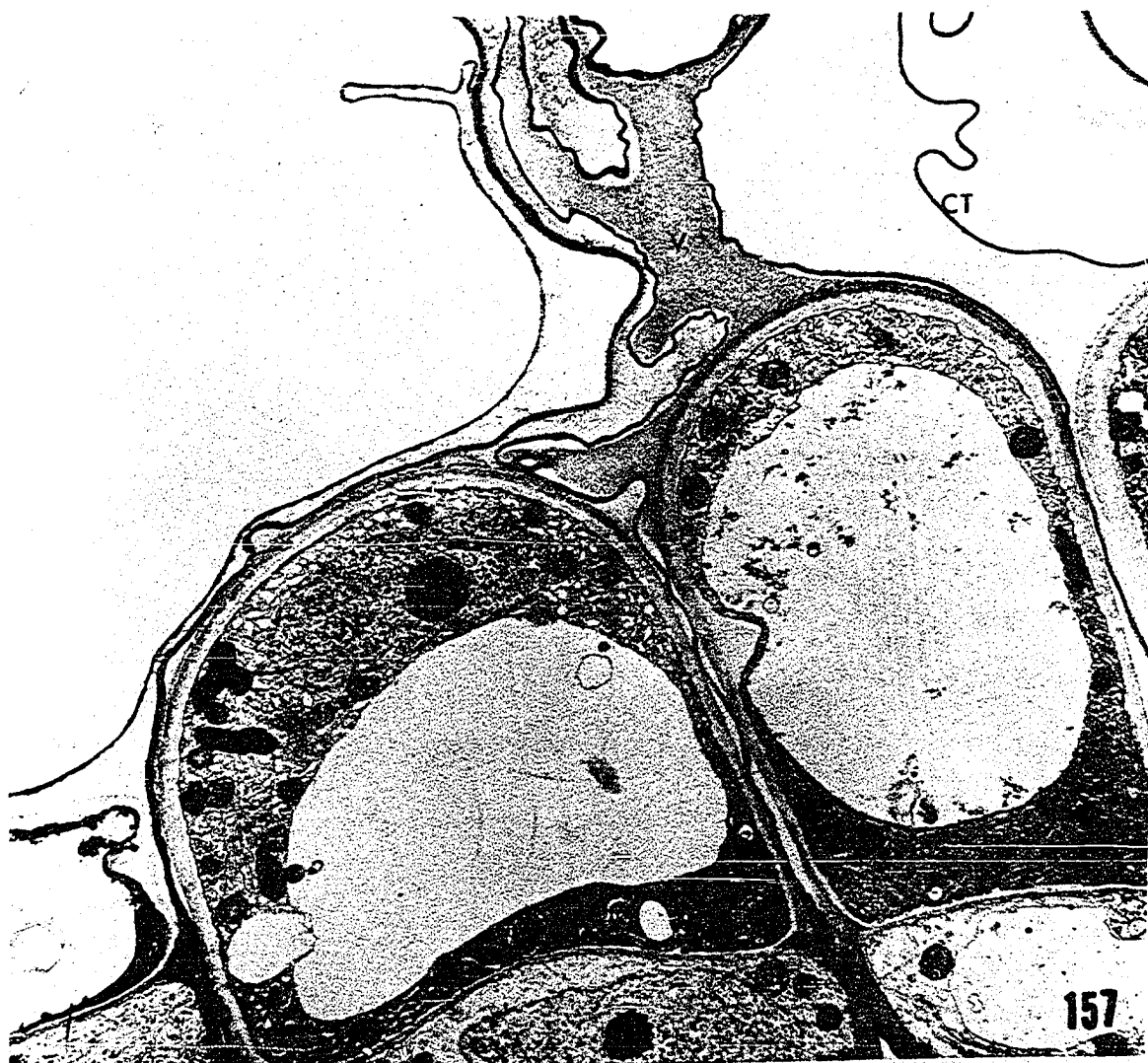


Fig. 159. Section through cell region beneath pavement epithelium showing arrangement of plasmodesmata in groups of threes. X 30,600 (SPRING)

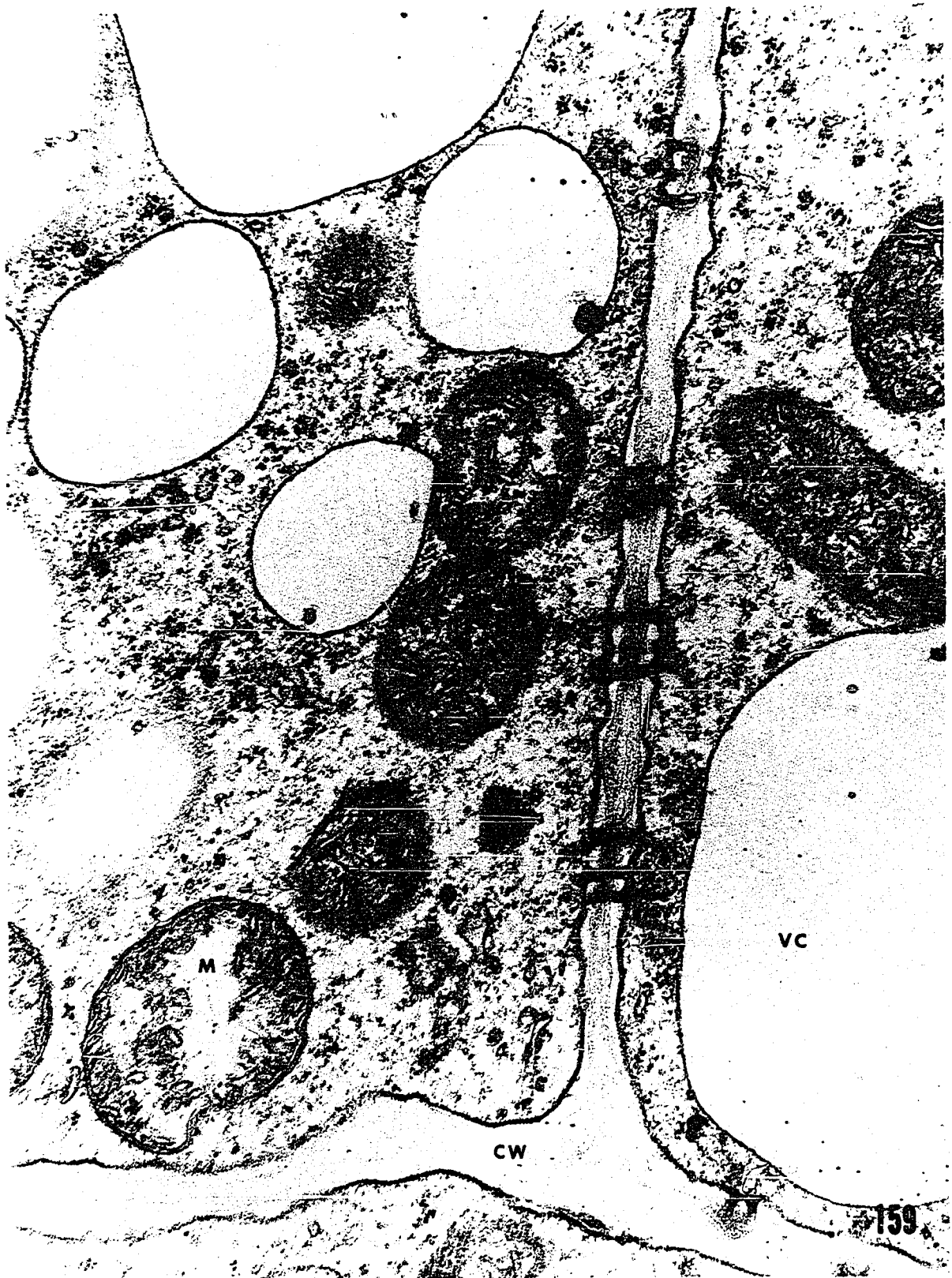


Fig. 160-195. Developmental sequence for quadrifid and bifid trichomes. Bacteria present on surface of young trichomes in Fig. 165 (arrow). Fig. 185 and 186 show similarity of bifids to quadrifids. Fig. 187-195 show digestive products in lumen and quadrifid terminal cells.
X 510 (SPRING)

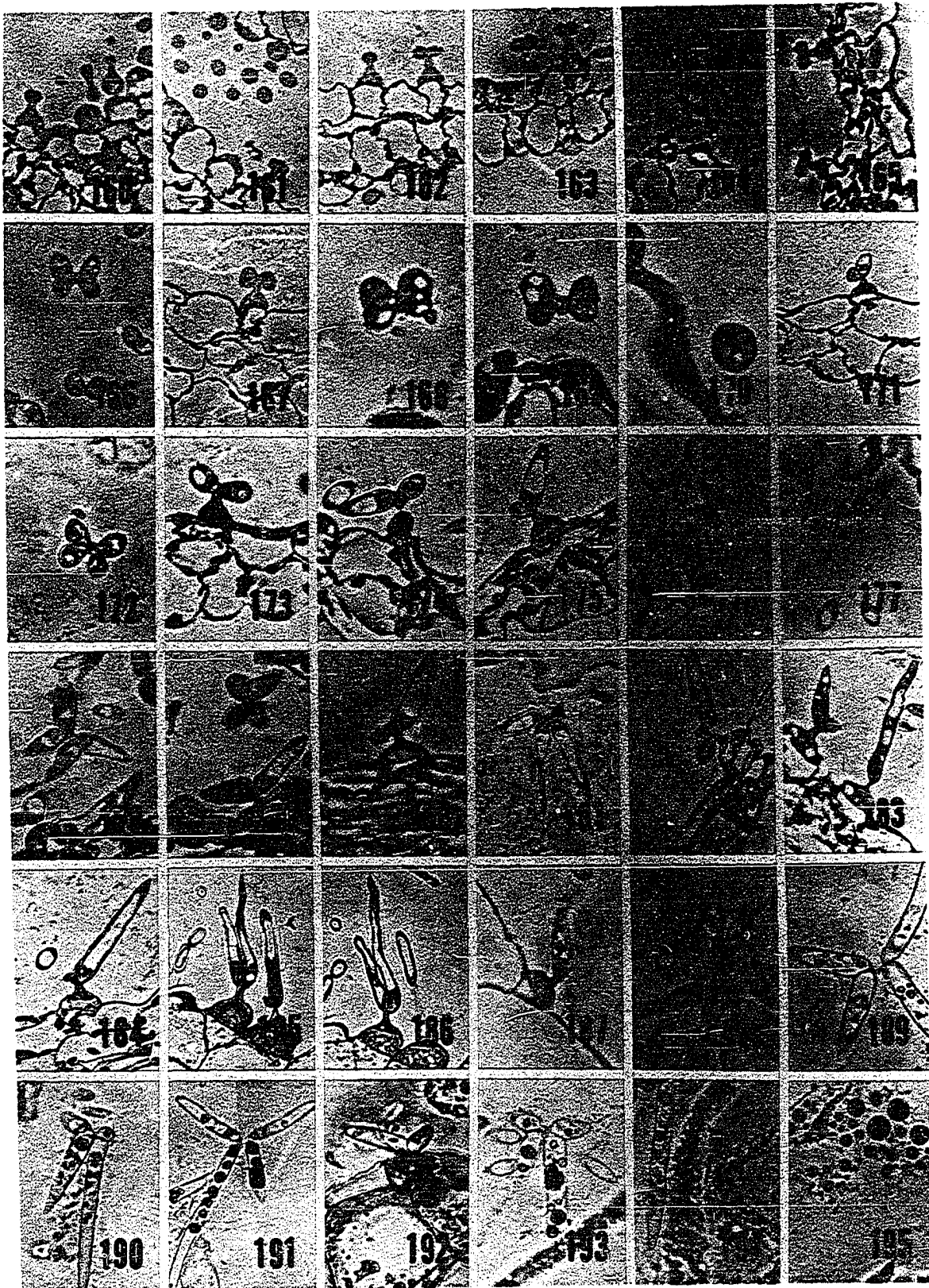


Fig. 196. Longitudinal section through quadrifid initial
X 22,400 (SPRING)

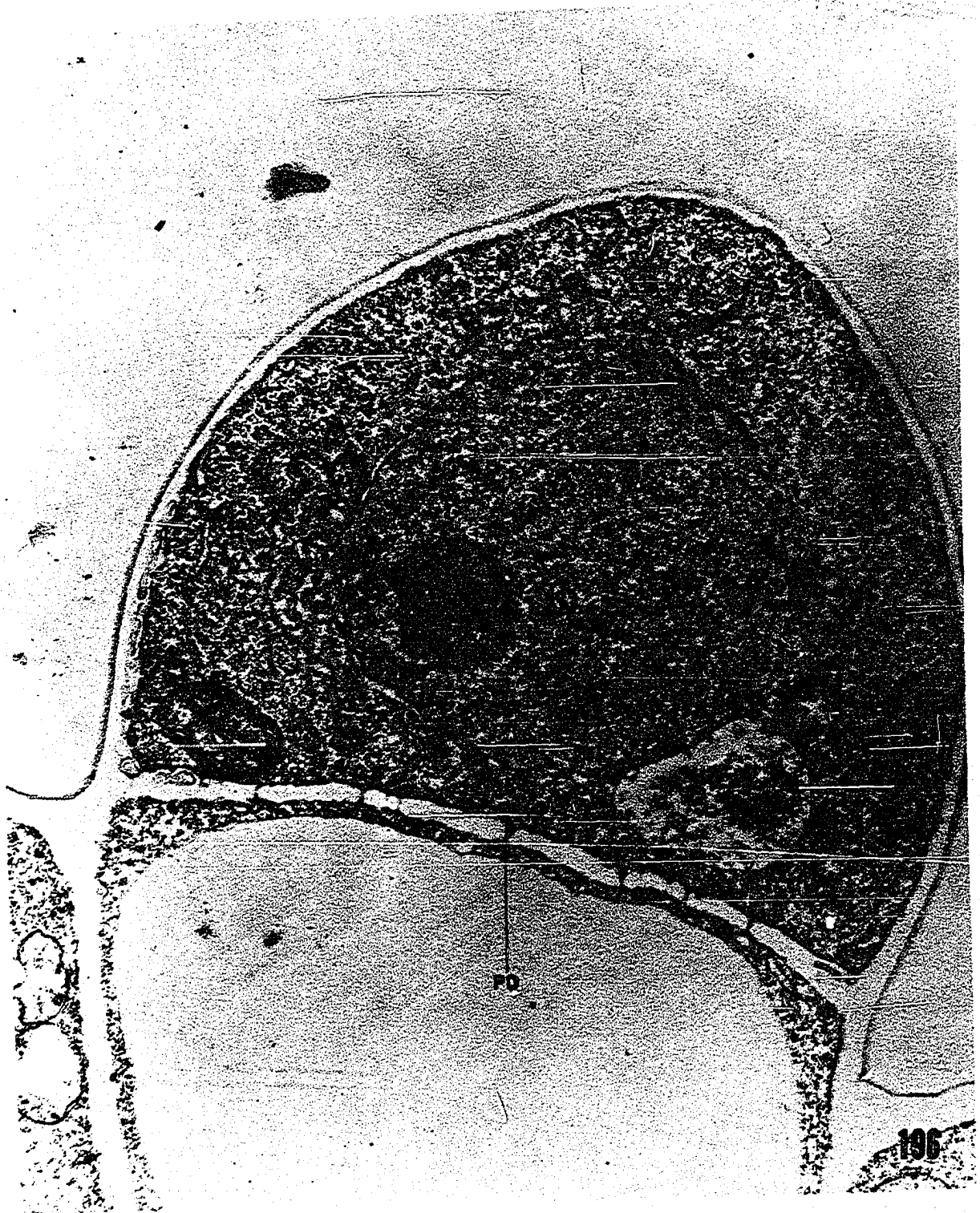


Fig. 197. Longitudinal section through quadrifid during early development when trichome is bowling pin shaped. X 28,000 (SPRING)

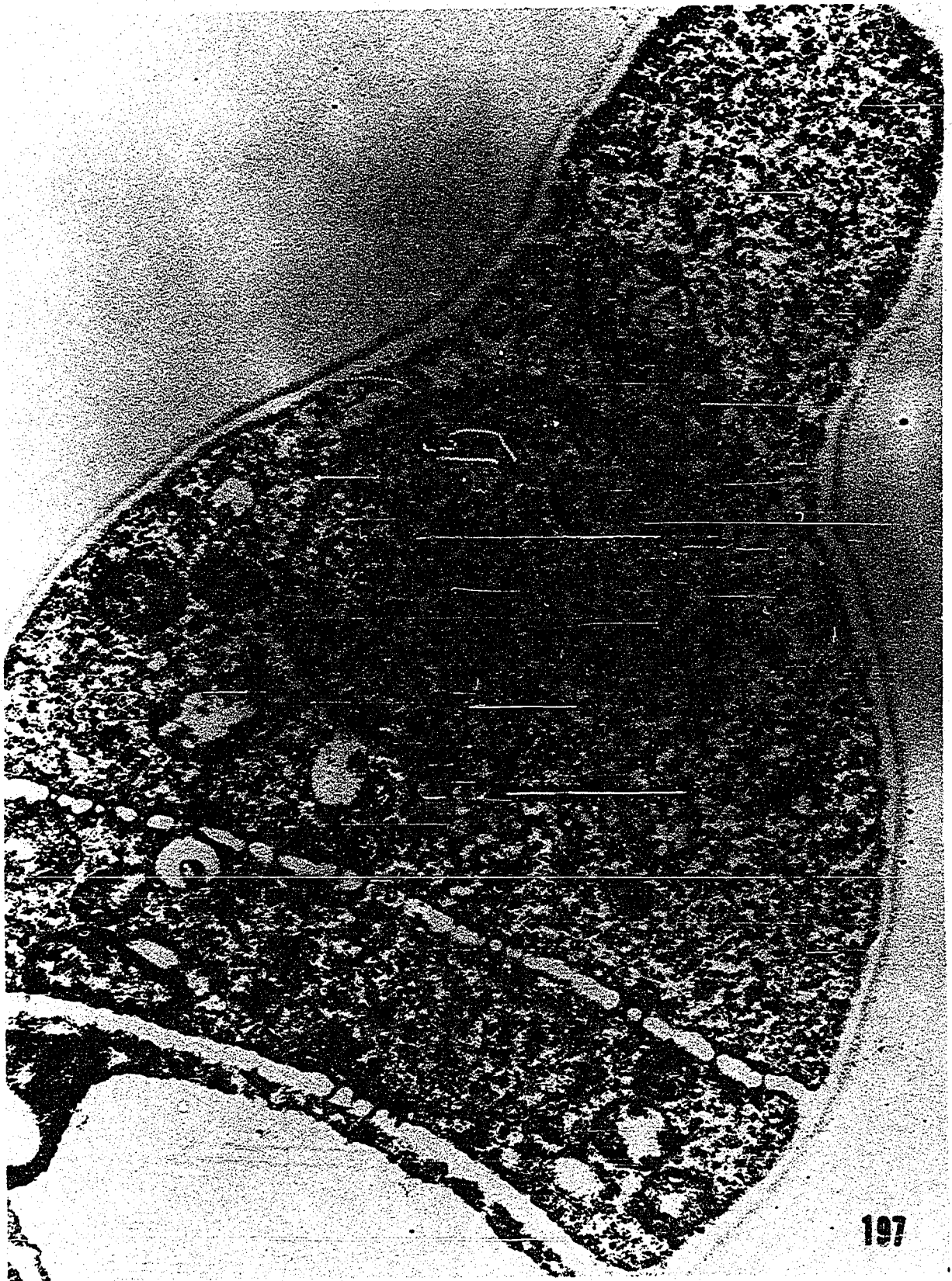
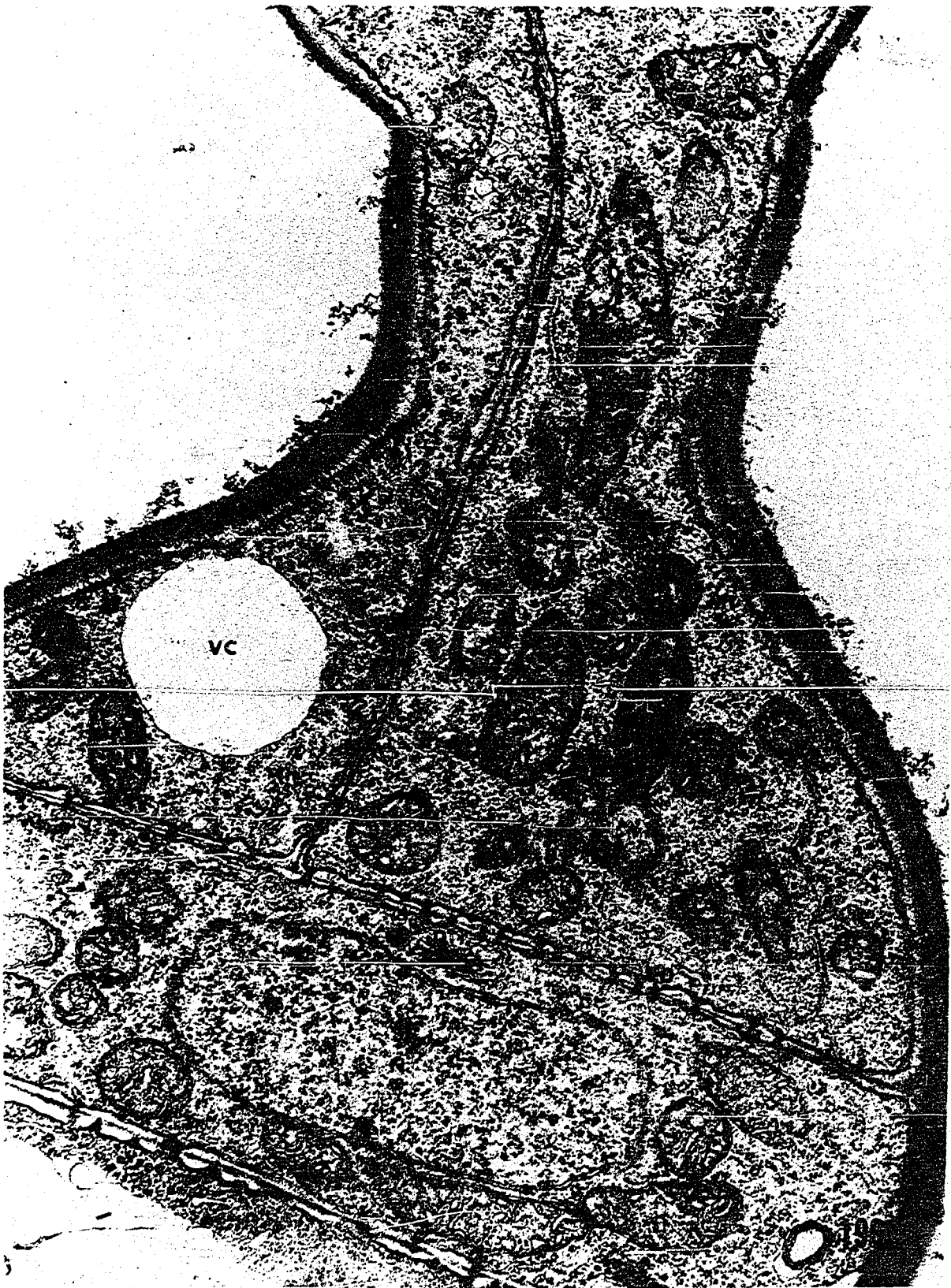


Fig. 198. Quadrifid in longitudinal section after terminal
cells have divided but not yet separated distally.
X 28,000 (SPRING)



- Fig. 199. SEM of developing bifid at bowling pin shaped stage of development. X 27,000 (SPRING)
- Fig. 200. SEM of developing bifids prior to elongation of terminal cell as in Fig. 199. X 24,000 (SPRING)
- Fig. 201. SEM of bifids after division of terminal cell but terminal cells only slightly elongated. Note presence of coccoid bacteria on bladder wall surface (arrow). X 16,700 (SPRING)
- Fig. 202. SEM of developing bifids slightly more elongated than in Fig. 201 but not at mature length. X 24,000 (SPRING)

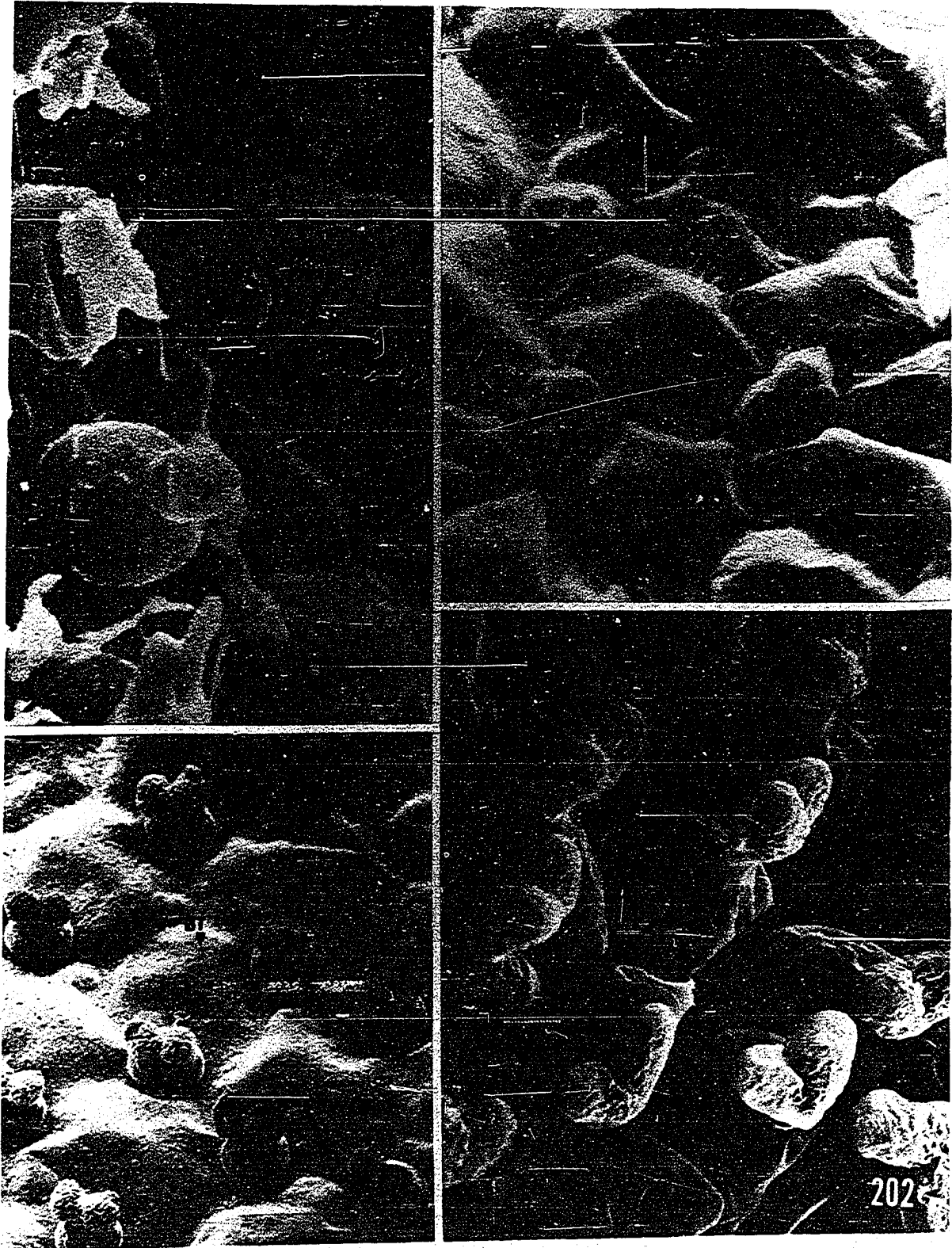


Fig. 203. SEM of bladder inner and outer walls with quadrifids developing on inner wall surface.
X 6,000 (SLOUGH)

Fig. 204. SEM of developing quadrifids showing unequal rate of elongation of pairs of terminal cells.
X 16,000 (SLOUGH)

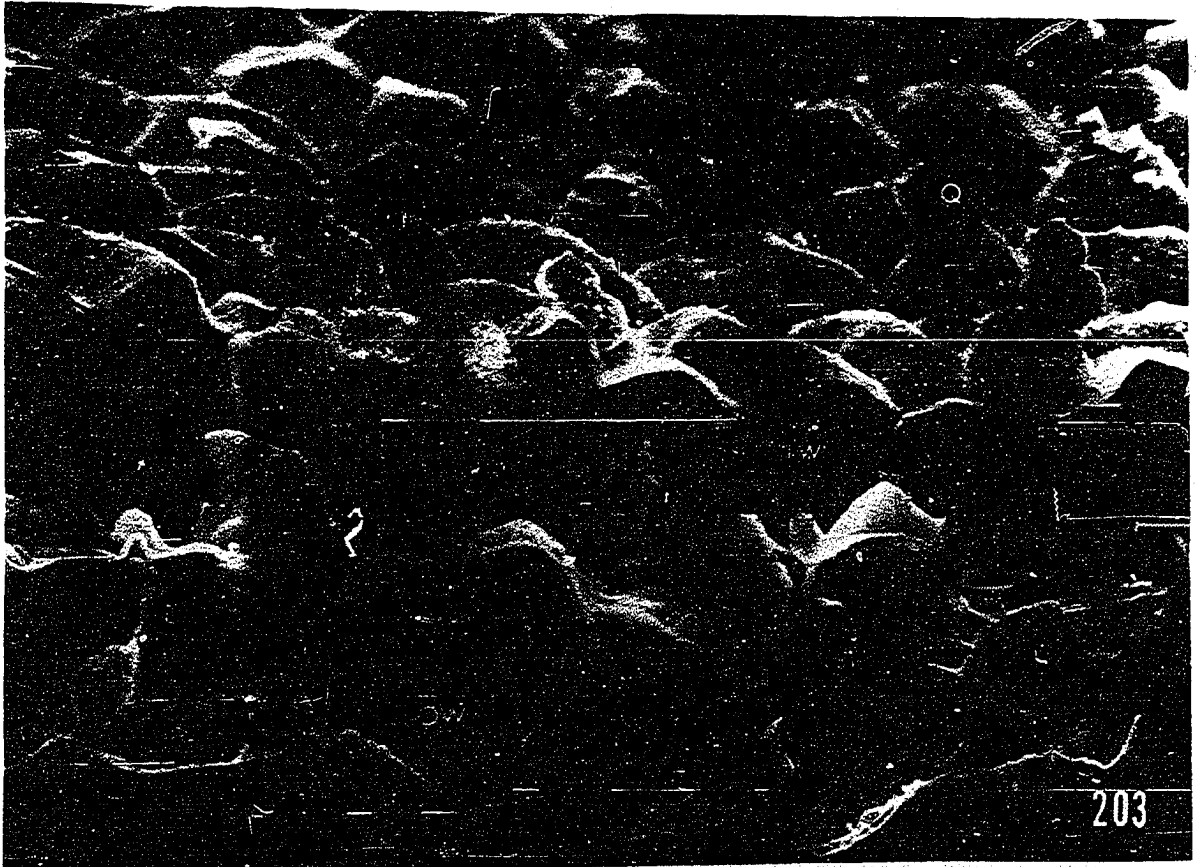


Fig. 205. SEM of mature quadrifid showing constriction where four terminal cells meet. X 20,000 (SLOUGH)

Fig. 206. SEM of mature quadrifid showing unequal length of pairs of terminal cells. X 6,200 (SLOUGH)

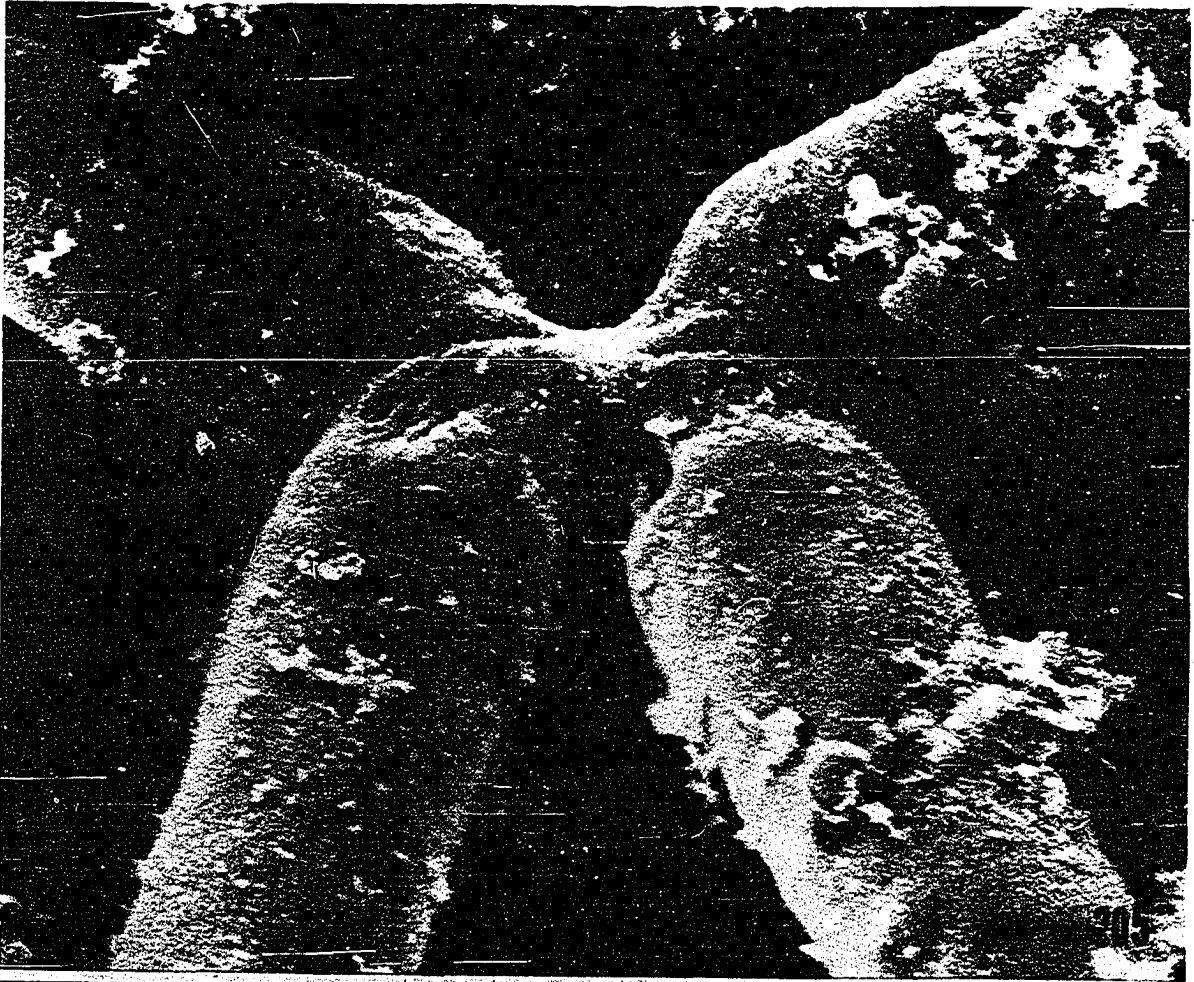


Fig. 207. SEM of field of quadrifids showing projection of long terminal cells at an angle into the bladder lumen with shorter terminal cells more parallel to bladder surface. X 450 (SLOUGH)

Fig. 208. SEM of two quadrifid terminal cells showing fibrous like webbs of material adhering to surface. X 19,000 (SLOUGH)



Fig. 209. SEM of mature quadrifids showing orientation of
terminal cells in relation to bladder surface.
X 15,000 (SILFEN)

Fig. 210. SEM of mature quadrifids with bacteria present on
inner surface. X 920 (SILFEN)

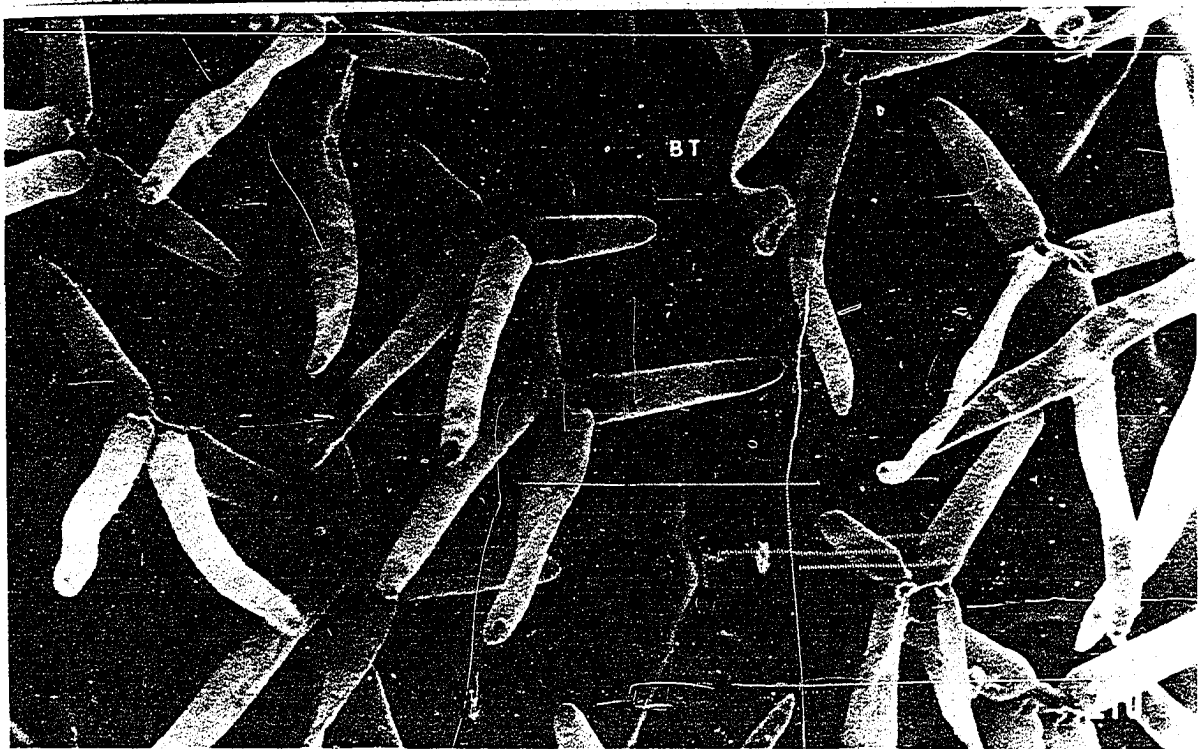


Fig. 211. Mature quadrifid viewed with SEM showing material of different nature from Fig. 208 and 213 adhering to surface of terminal cells and bladder wall cells. X 6,200 (SLOUGH)

Fig. 212. SEM of field of quadrifids showing surface material on terminal cells and orientation of terminal cells always in same direction, with longer cells always pointing toward posterior end of bladder. X 900 (SLOUGH)

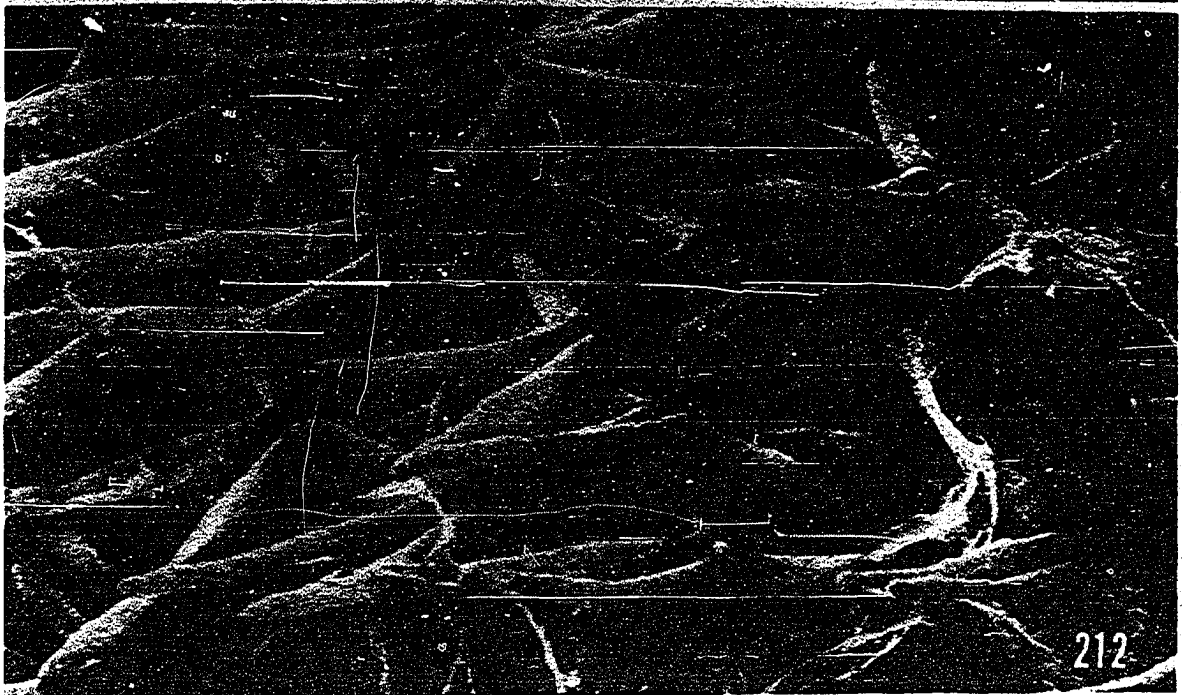


Fig. 213. SEM of mature quadrifid with different surface
adhering material than previous figures.
X 6,200 (3-CORN)

Fig. 214. SEM of inner bladder surface showing quadrifids
and trapped organisms. X 630 (SILFEN)

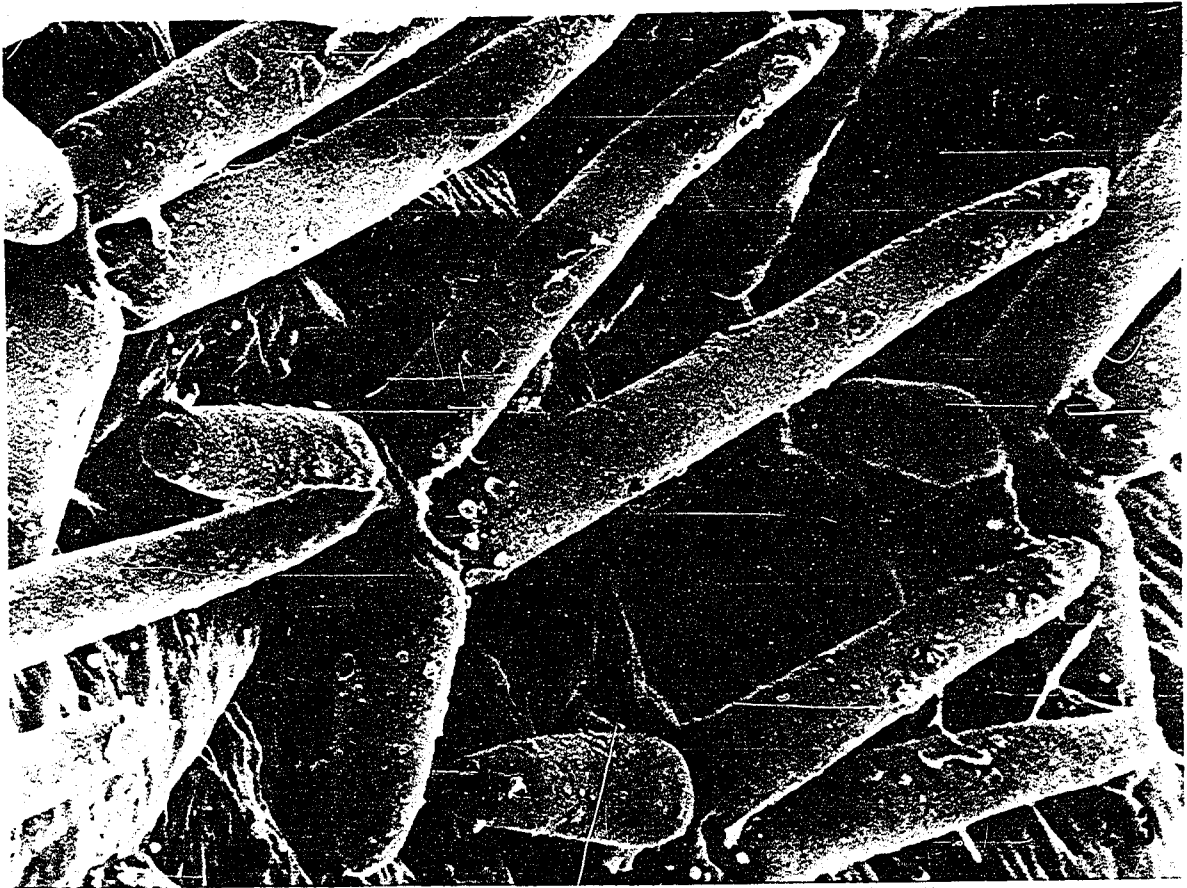


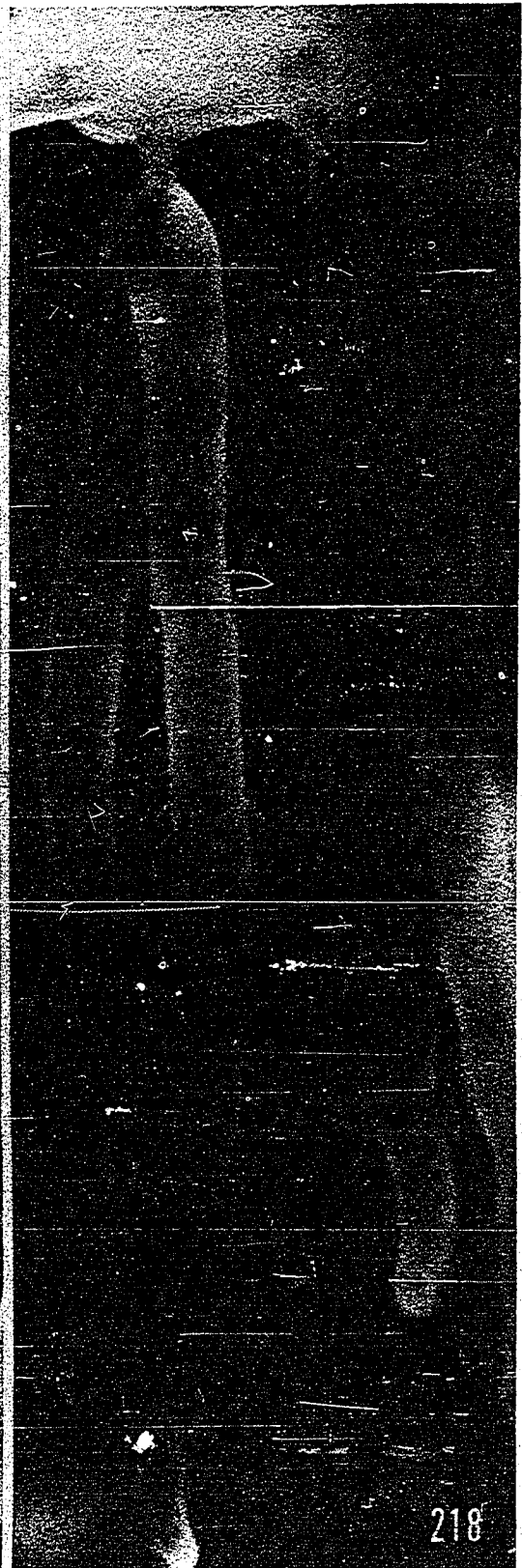
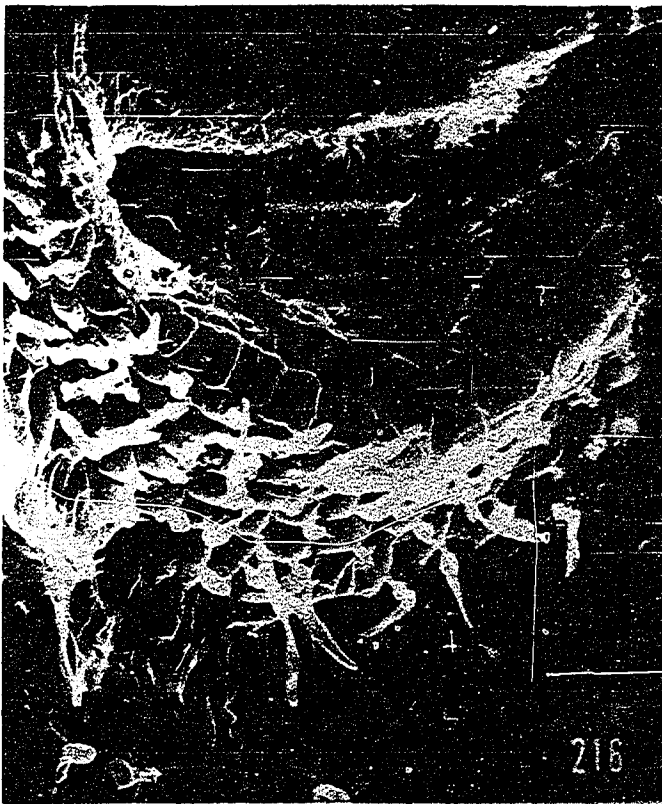
Fig. 215. SEM of mature quadrifid showing central cell with terminal cells removed. X 6,000 (SLOUGH)



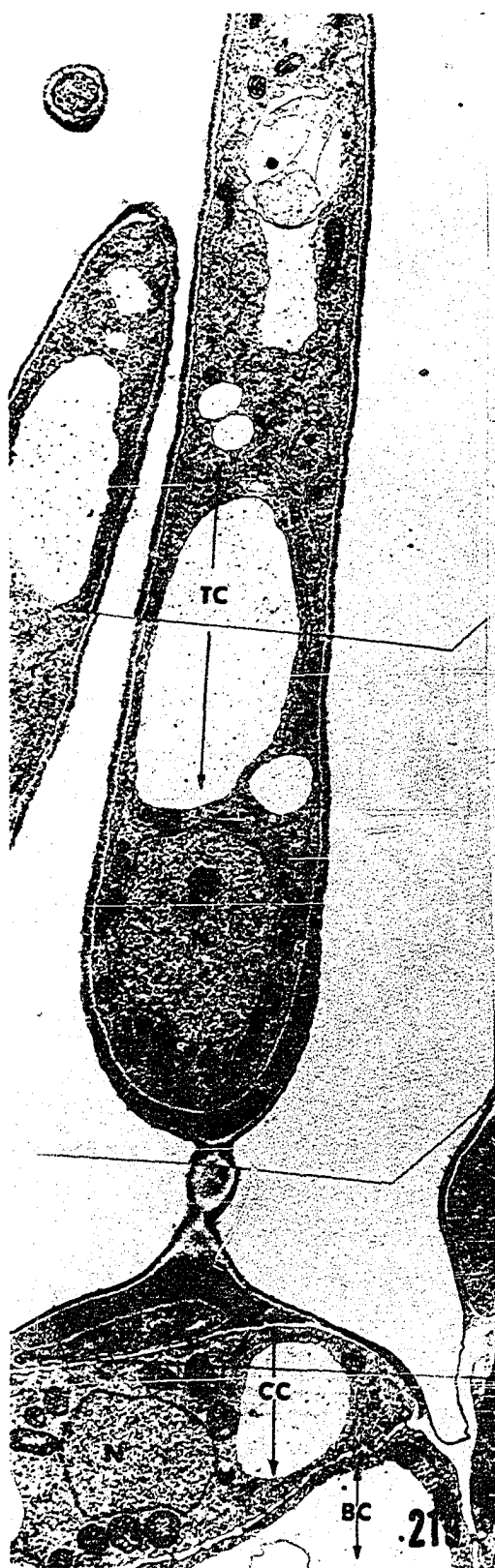
Fig. 216. SEM of posterior margin of pavement epithelium showing bifids in this region. X 300 (SLOUGH)

Fig. 217. SEM of mature bifids showing equal length of two terminal cells and constriction at base of terminal cells. X 7,200 (SLOUGH)

Fig. 218. SEM of mature bifids. X 20,000 (SLOUGH)



- Fig. 219. Longitudinal section through maturing bifid showing three cell types making up trichome. X 4,370 (3-CORN)
- Fig. 220. Distal most region of bifid terminal cell showing fibrous wall and lack of cuticle. X 22,200 (3-CORN)
- Fig. 221. More proximal region than Fig. 220 showing terminal cell wall where cuticle ends (arrow) and sponge-like wall becomes associated with fibrous wall. X 22,200 (3-CORN)
- Fig. 222. Constricted region of bifid terminal cell with absorbed product in channels of sponge-like wall. X 22,200 (3-CORN)
- Fig. 223. Wall region between central cell and basal cell showing plasmodesmata in periclinal wall and endodermoid central cell wall with cuticle continuous with epidermal cell cuticle. X 22,200 (3-CORN)
- Fig. 224. Wall region between terminal cell and central cell showing contrast between endodermoid wall of central cell and sponge-like wall of terminal cell. X 22,200 (3-CORN)



222



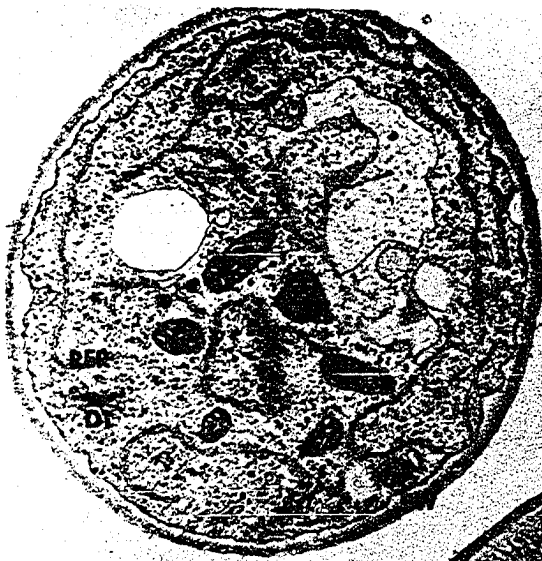
223



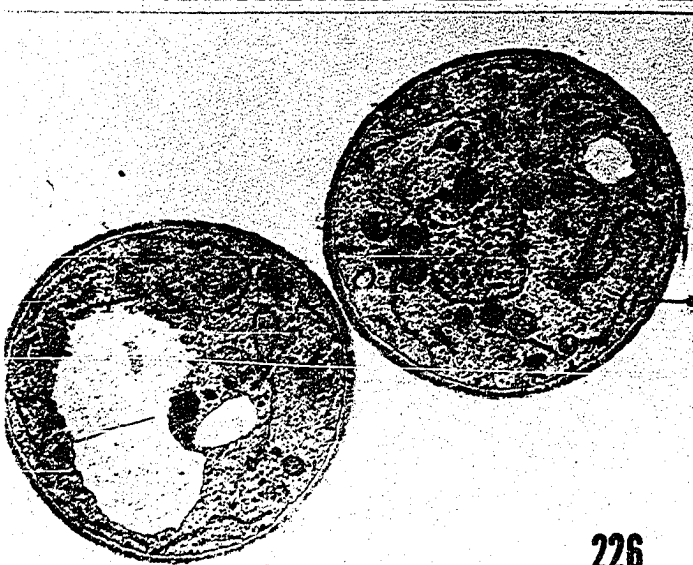
Fig. 225. Longitudinal section through lower quadrifid
and cross section through upper terminal cell.
X 7,340 (SPRING)

Fig. 226. Cross section through two terminal bifid cells
showing distal ends are separated. X 5,500
(SPRING)

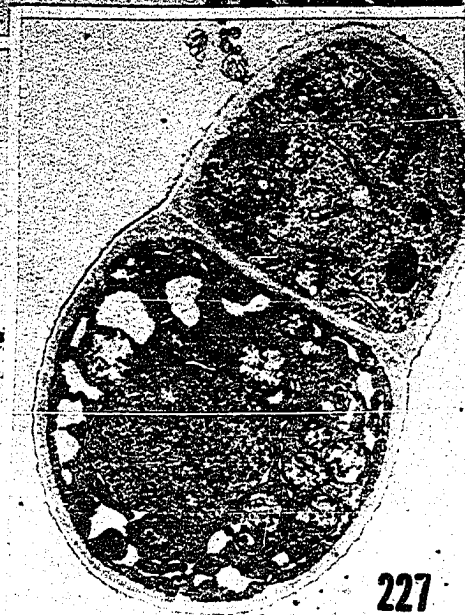
Fig. 227. Cross section through two terminal bifid cells
showing proximal ends are joined. X 5,500
(SPRING)



225



226



227

Fig. 228. Longitudinal section through bifid central cell and proximal terminal cell showing absorbed product in terminal cell sponge-like wall. X 12,900 (SPRING)

Fig. 229. Region between central cell and basal cell showing plasmodesmata. X 56,000 (3-CORN)

Fig. 230. Region between terminal cell and central cell showing plasmodesmata. X 56,000 (SPRING)

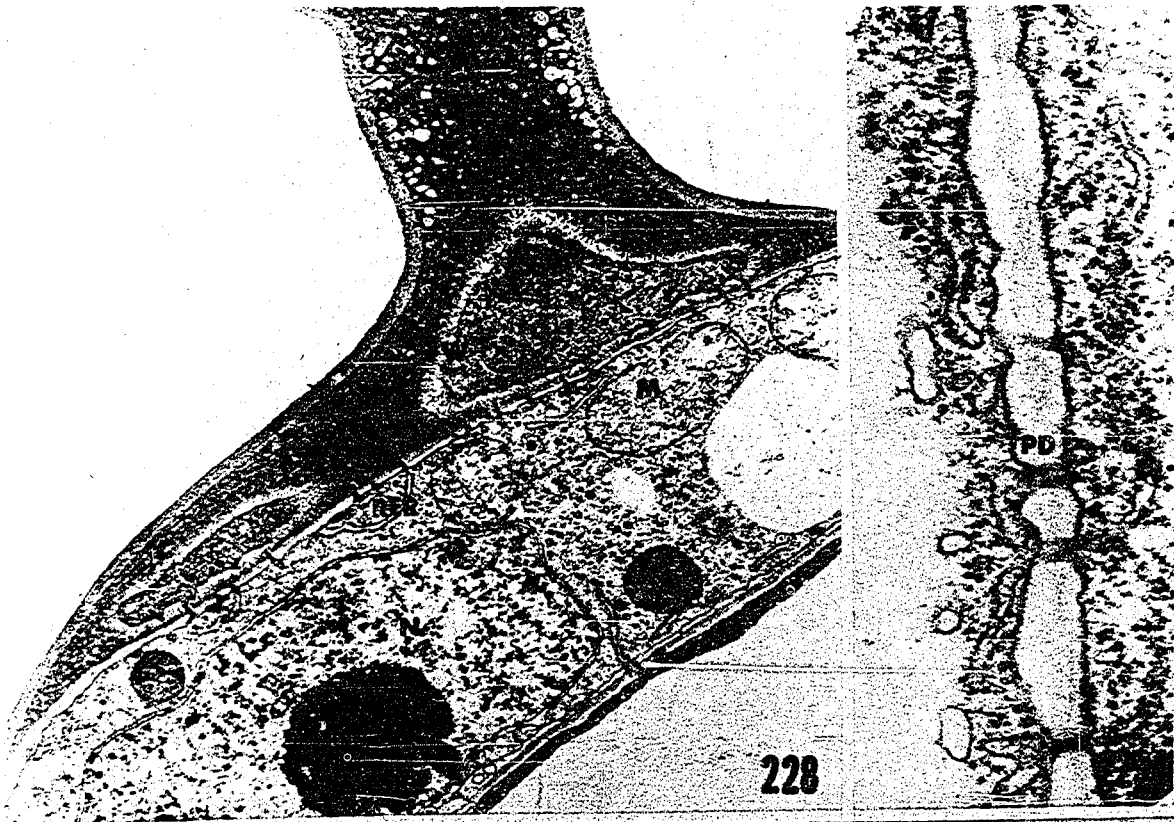


Fig. 231-232. Longitudinal sections through two quadrifids
showing additional central periclinal wall in
constricted region not seen in bifids.
X 7,500 (SPRING)

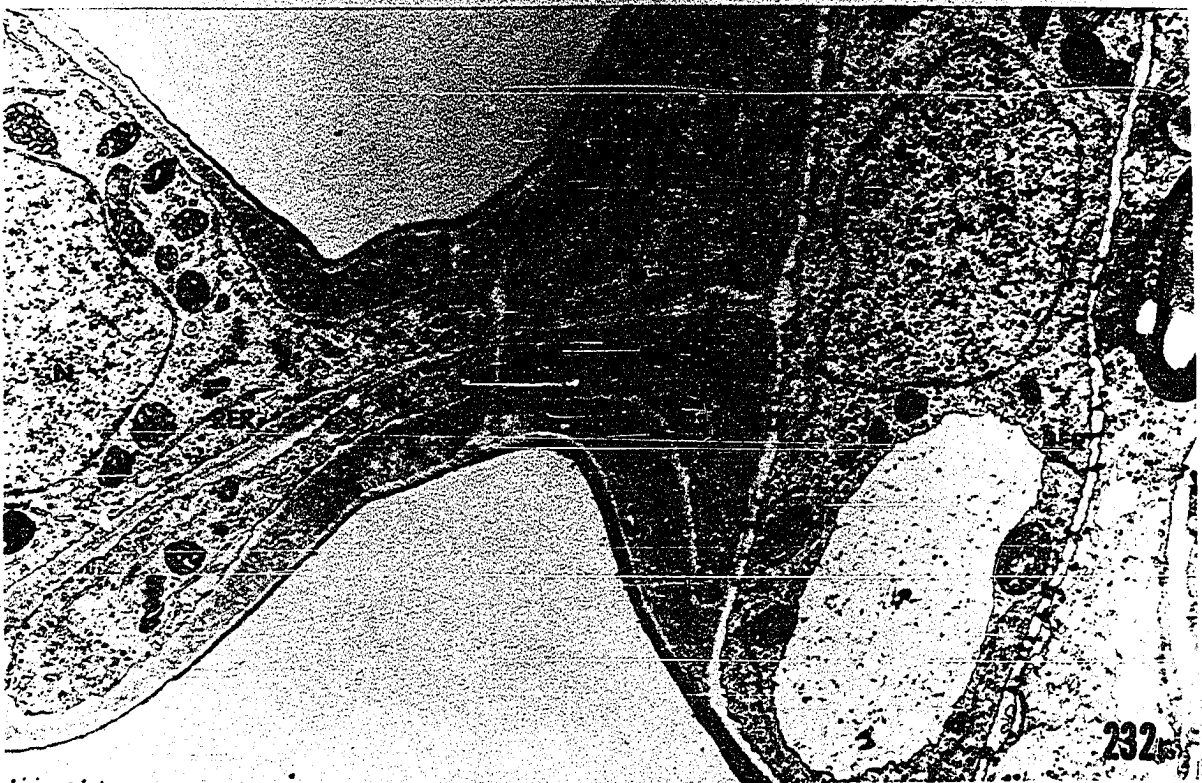
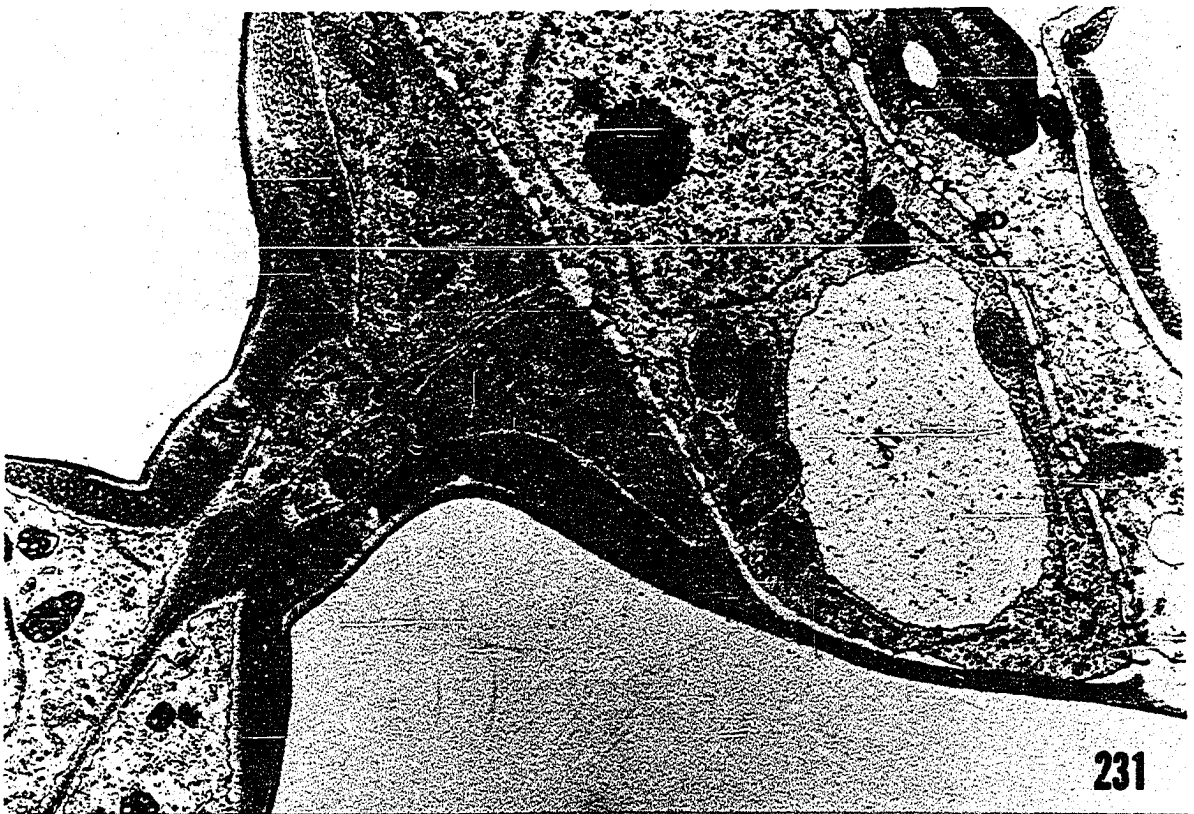


Fig. 233. Region between central cell endodermoid wall and basal cell wall. X 56,000 (SPRING)

Fig. 234. Section through proximal portion of quadrifid showing sponge-like wall and fibrous wall of terminal cells and plasmodesmata between terminal cells and central cell. X 22,000 (SPRING)



Fig. 235. Constricted region of quadrifid with sponge-like wall and associated absorbed product. X 84,000 (3-CORN)

Fig. 236. Constricted region of bifid showing similarity to quadrifid in Fig. 235. X 84,000 (3-CORN)

Fig. 237. Wall between terminal bifid cells and central cell with plasmodesmata. X 22,000 (SPRING)

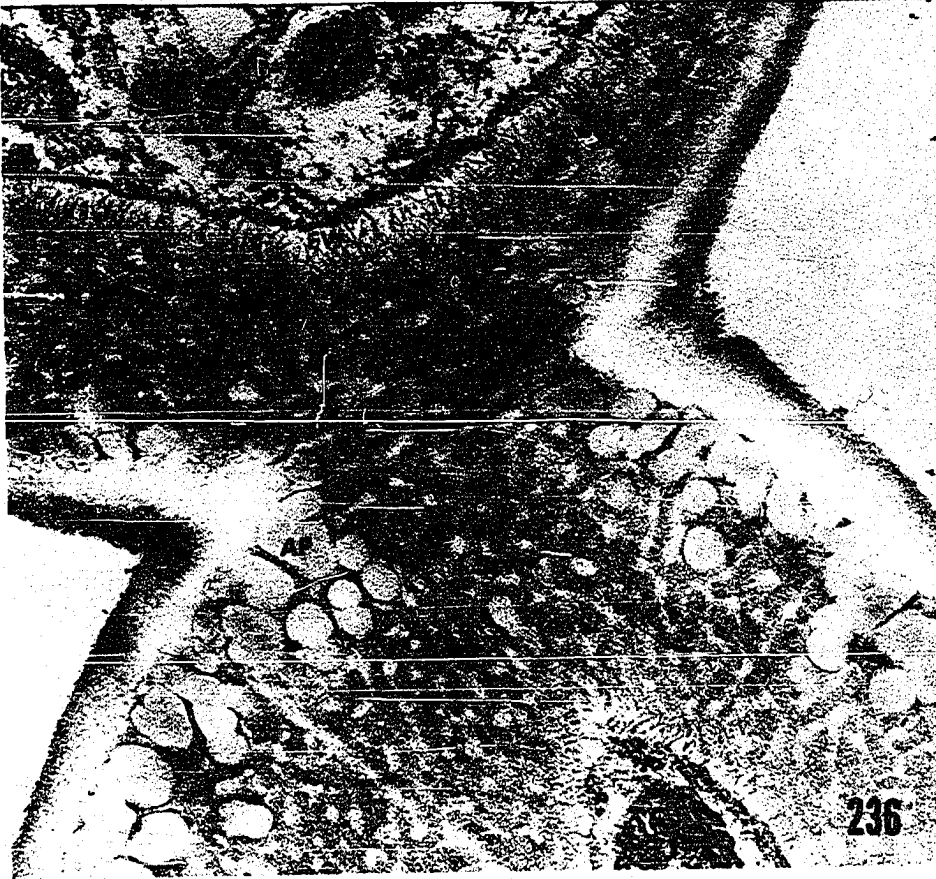
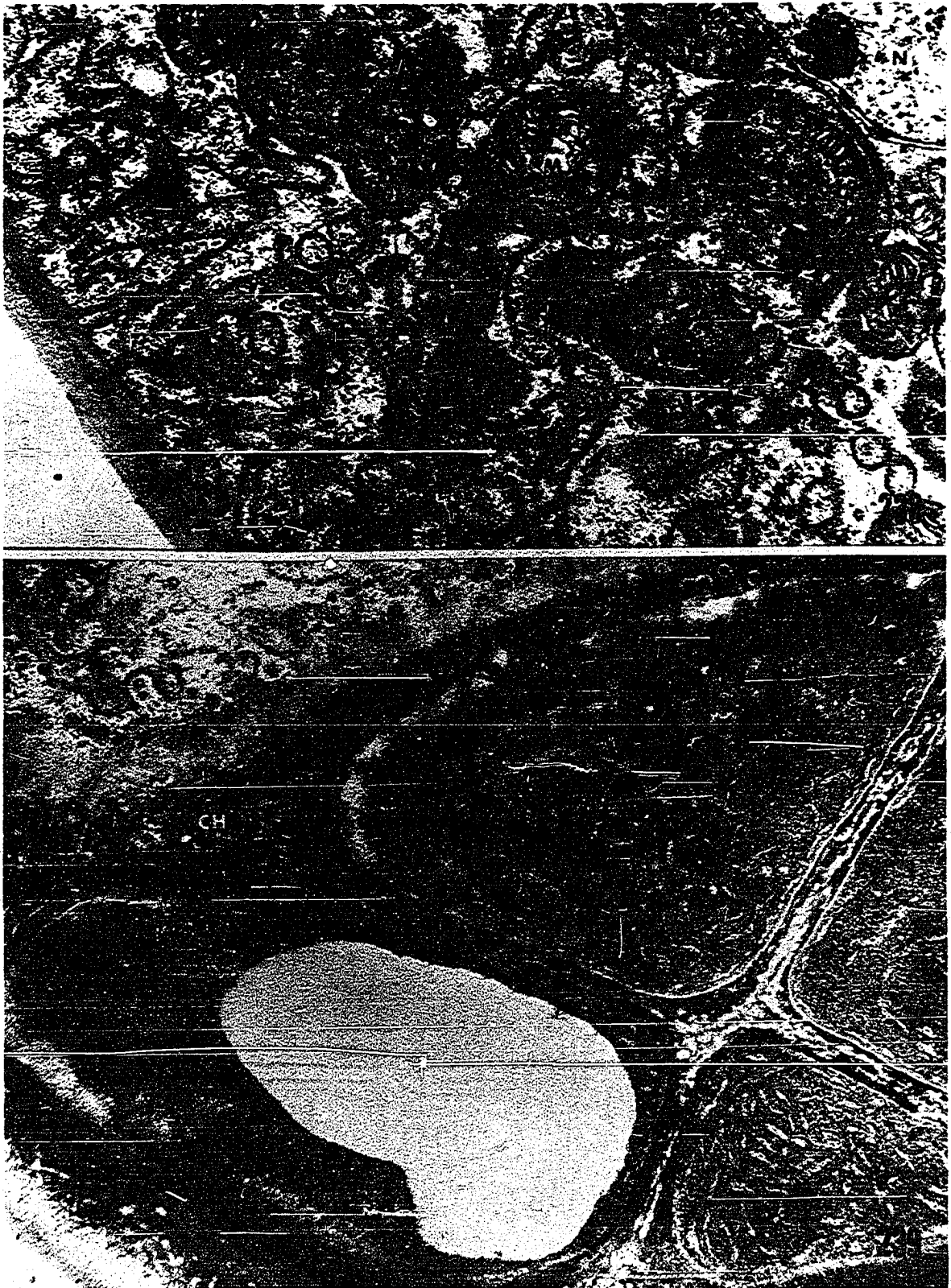


Fig. 238. Quadrifid central region showing invagination of plasmalemma. X 33,950 (SPRING)

Fig. 239. Cross section through terminal cells of quadrifid showing channels in sponge-like walls and abundance of plasmodesmata between terminal cells and central cell. X 19,400 (SPRING)



- Fig. 240. Longitudinal section through quadrifid terminal cell showing abundance of microtubules associated with fibrous wall. X 17,200 (SPRING)
- Fig. 241. Fibrous wall region of quadrifid terminal cell showing associated folded plasmalemma. X 84,000 (SPRING)
- Fig. 242. Central cell endodermoid wall showing associated microtubules. X 55,000 (SPRING)
- Fig. 243. Dictyosomes in a distal terminal quadrifid cell. X 56,000 (SPRING)

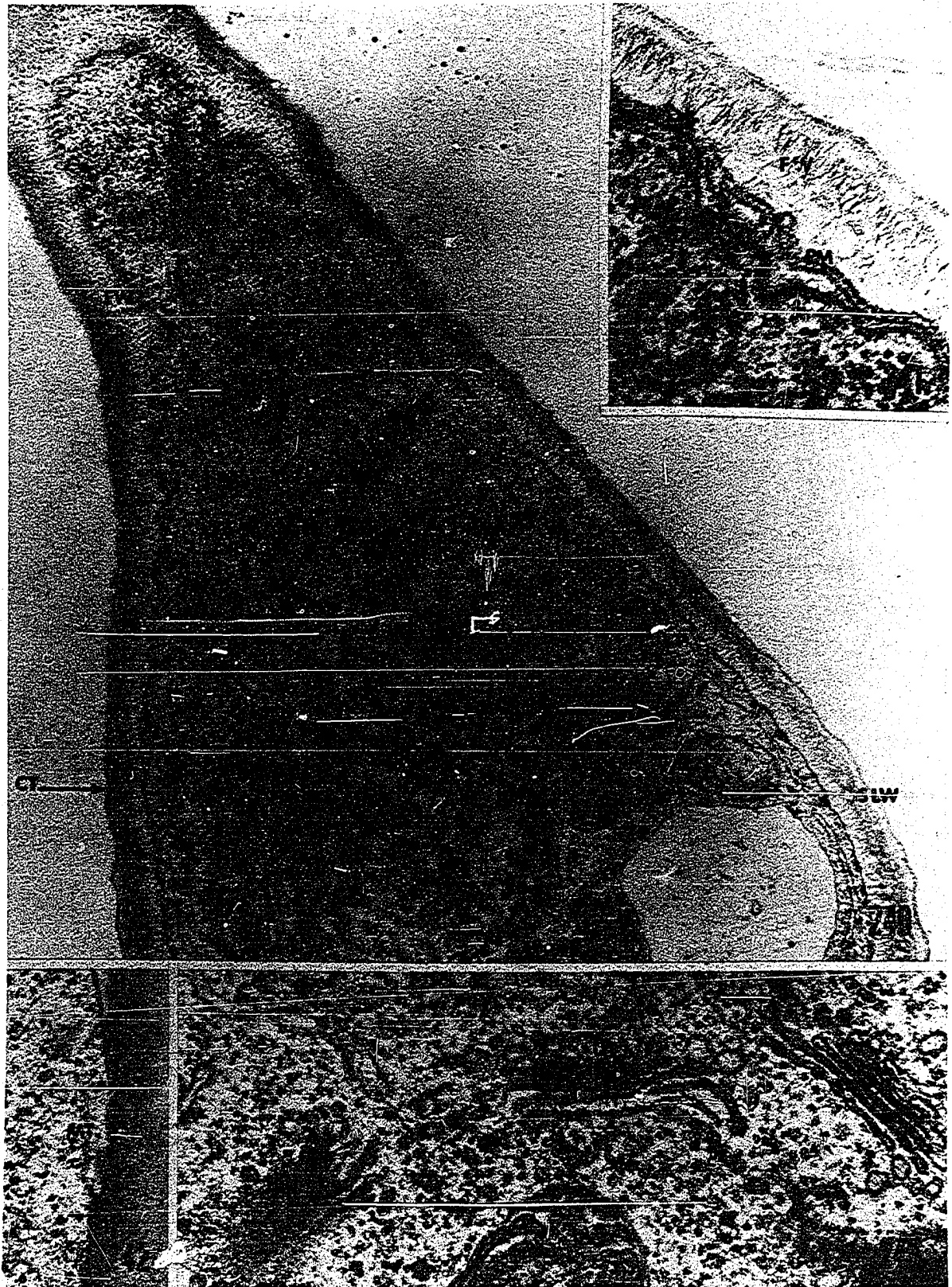


Fig. 244-245. Portion of distal cell of terminal quadrifids showing fibrous wall with associated microtubules and dictyosomes, folding of plasmalemma, and numerous vesicles. X 84,000 (SPRING)

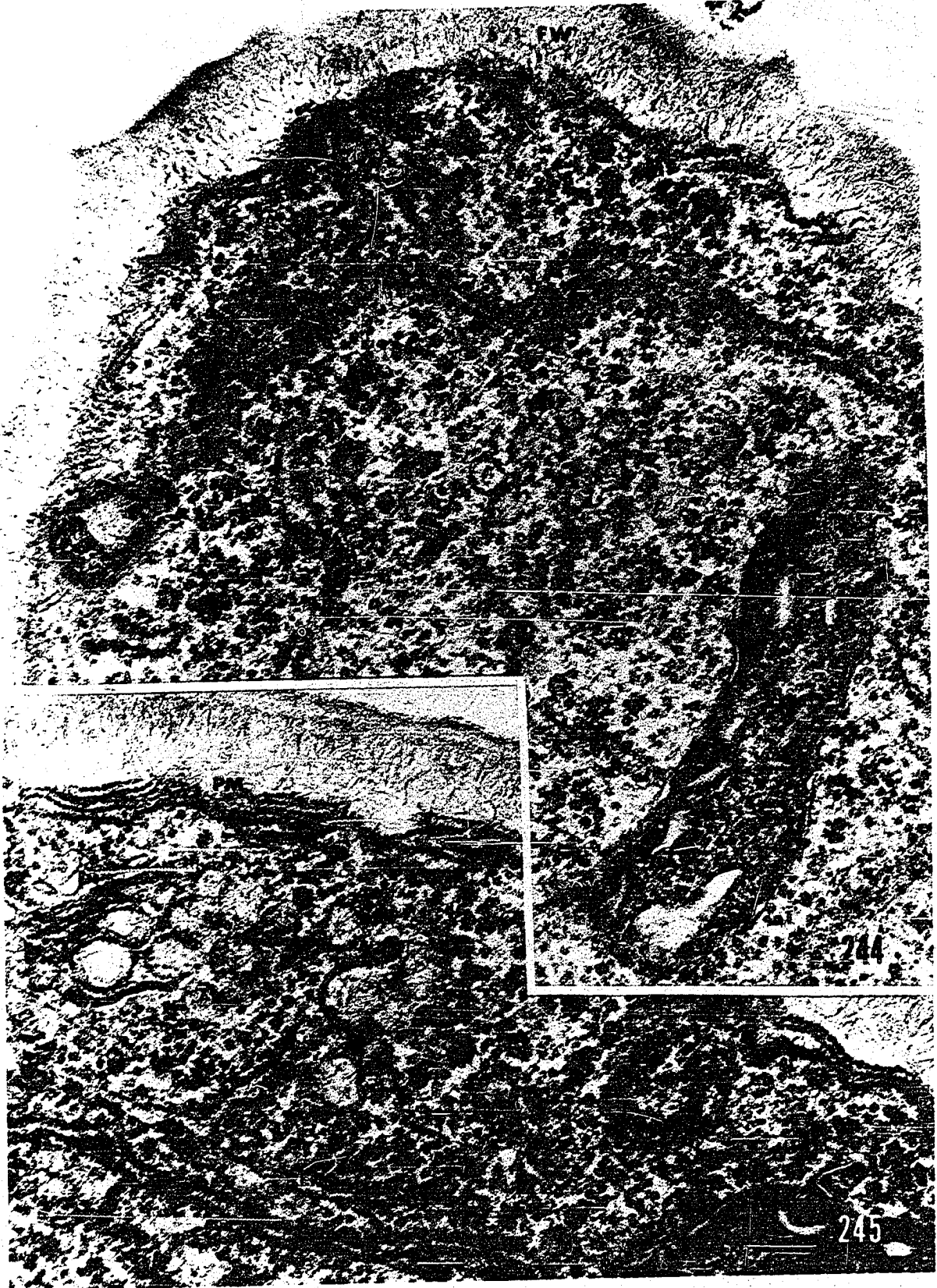


Fig. 246. Outer bladder wall surface showing lenticular and spherical trichomes. X 3,900 (SLOUGH)

Fig. 247. Same as Fig. 246 at lower magnification. X 1,000 (SLOUGH)

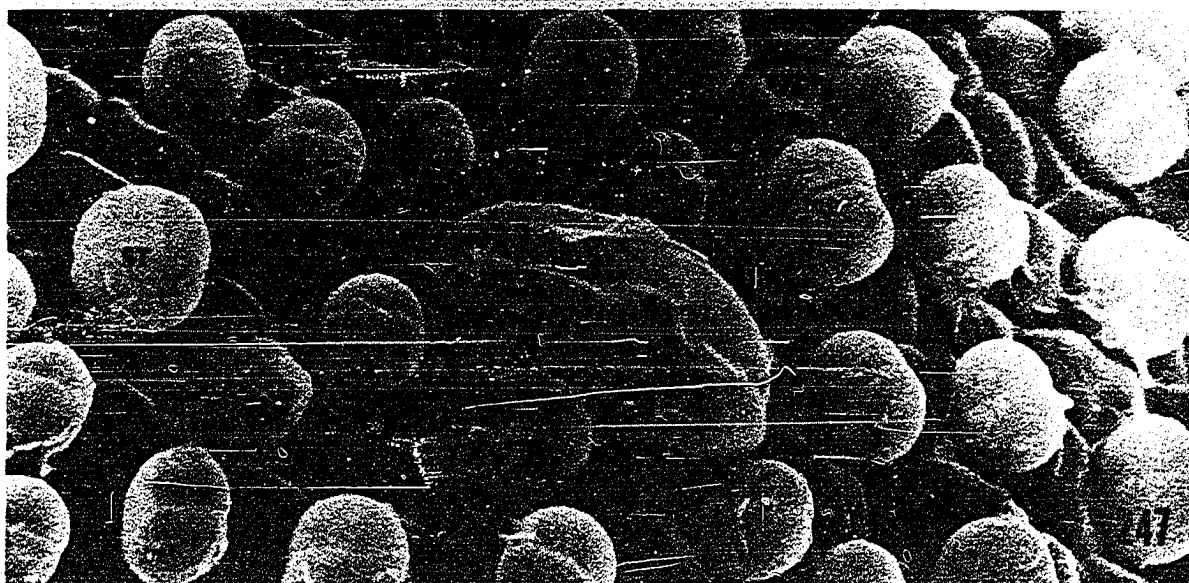


Fig. 248. Section through bladder primordium showing initiation of a surface trichome. X 9,200 (3-CORN)

Fig. 249. Section through spherical trichome of young bladder, with presence of a carbohydrate secretory product. X 9,200 (3-CORN)



Fig. 250. Spherical trichome on young bladder showing exfoliation of the cuticle and associated carbohydrate secretory product. X 18,300 (SLOUGH)

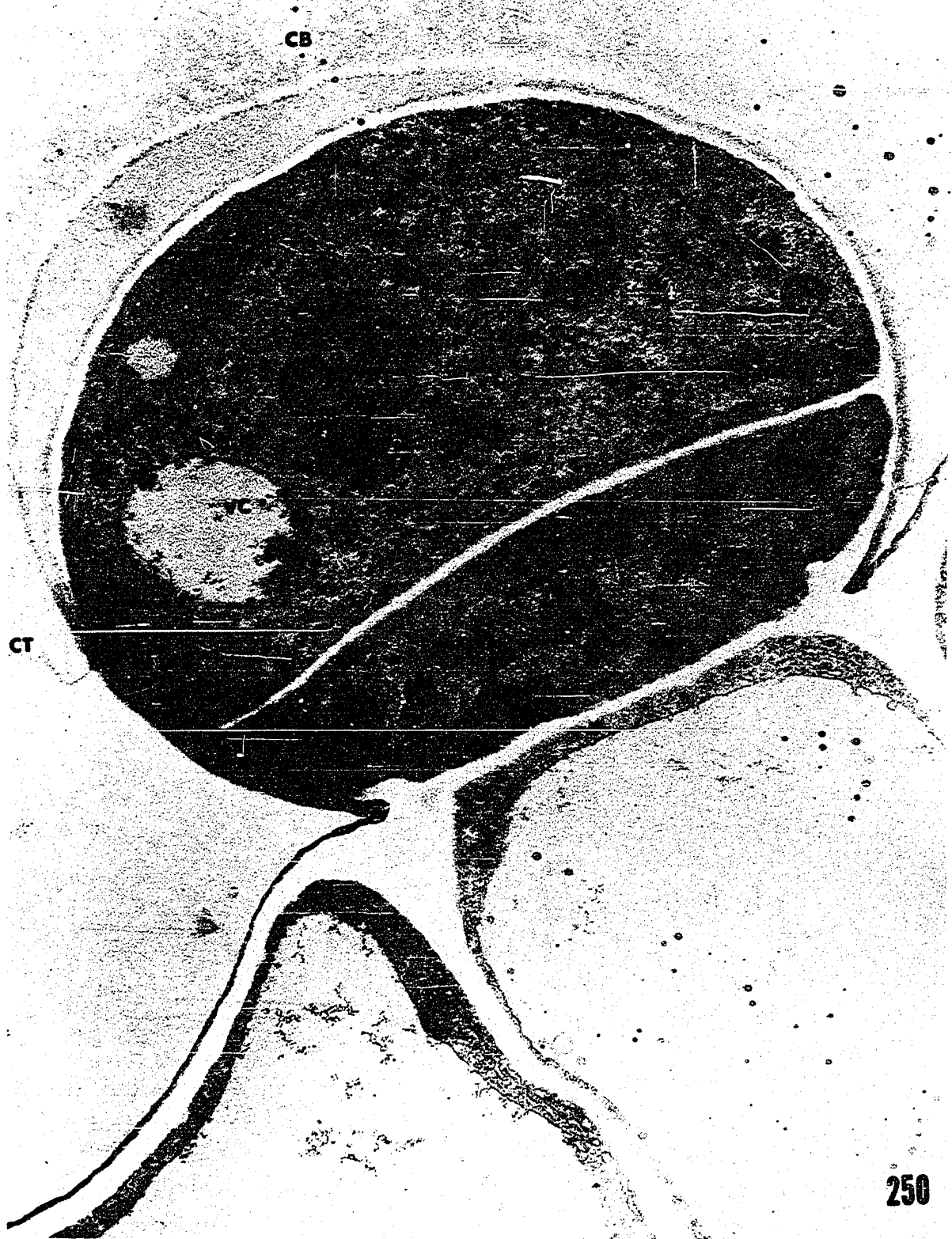


Fig. 251. Longitudinal section through lenticular trichome on immature bladder outer surface. X 9,200 (SLOUGH)

Fig. 252. Portion of lenticular terminal cell showing associated carbohydrate secretory product. X 7,340 (SLOUGH)

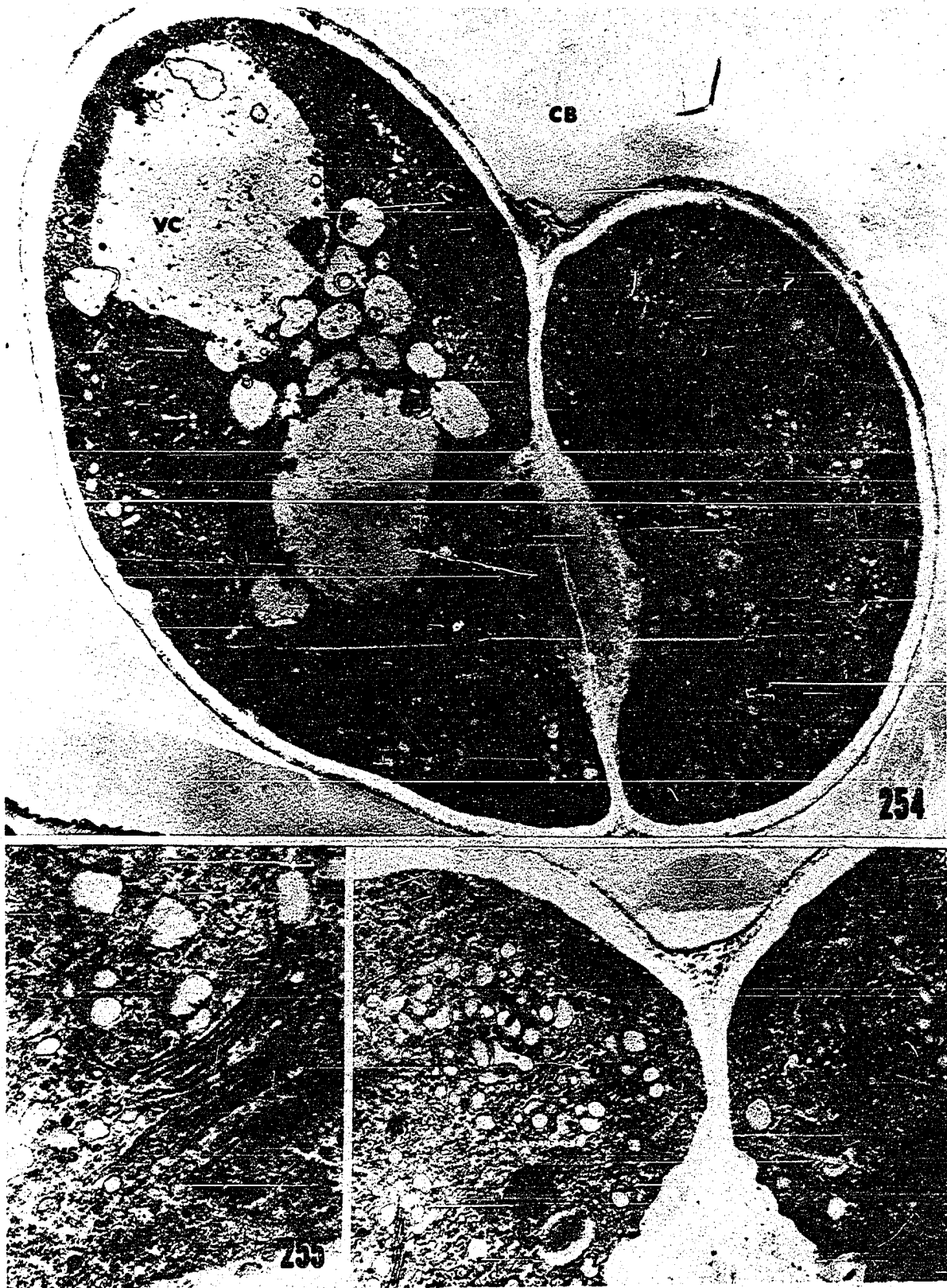
Fig. 253. Portion of lenticular trichome showing endodermoid wall of central cell. X 7,340 (SLOUGH)



Fig. 254. Cross section through two terminal cells of a lenticular trichome showing associated carbohydrate secretory product.

Fig. 255. Dictyosomes of lenticular terminal cell.
X 42,000 (SLOUGH)

Fig. 256. Terminal cells of lenticular trichome showing abundance of vesicles and peripherally located mitochondria. X 20,400 (SLOUGH)



- Fig. 257. SEM of mature outer bladder surface showing spherical trichomes with two terminal cells and an abundance of various types of bacteria. X 980 (SLOUGH)
- Fig. 258. SEM of outer spherical trichomes showing separation (arrow) of terminal cells due to poor fixation. X 1900 (SLOUGH)
- Fig. 259. SEM of mature outer bladder surface with spherical trichomes slightly recessed in epidermis. X 1200 (SPRING)
- Fig. 260. SEM of mature bladder surface with spherical trichomes and associated bacteria and algae. X 1200 (SPRING)



Fig. 261. Mature branch surface with spherical trichomes; occasional trichomes with only one terminal cell are present. X 490 (SLOUGH)

Fig. 262. Mature bladder surface with identical spherical trichomes to those found on branch surfaces. X 490 (SLOUGH)

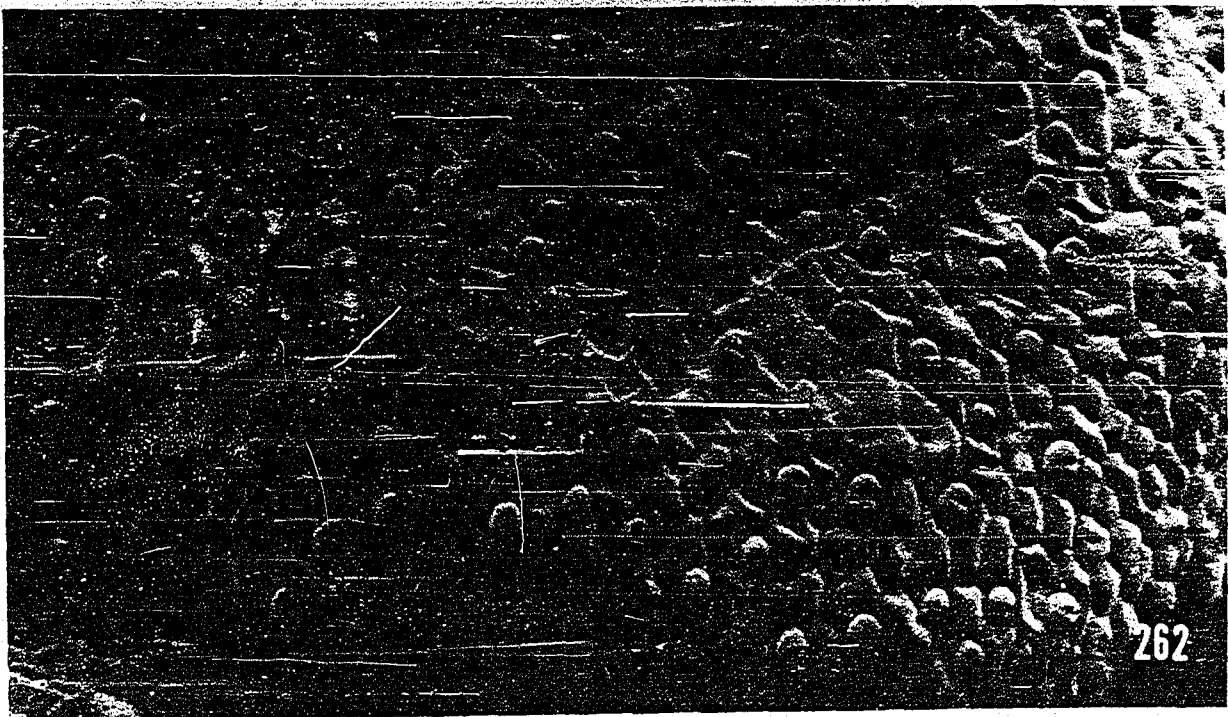
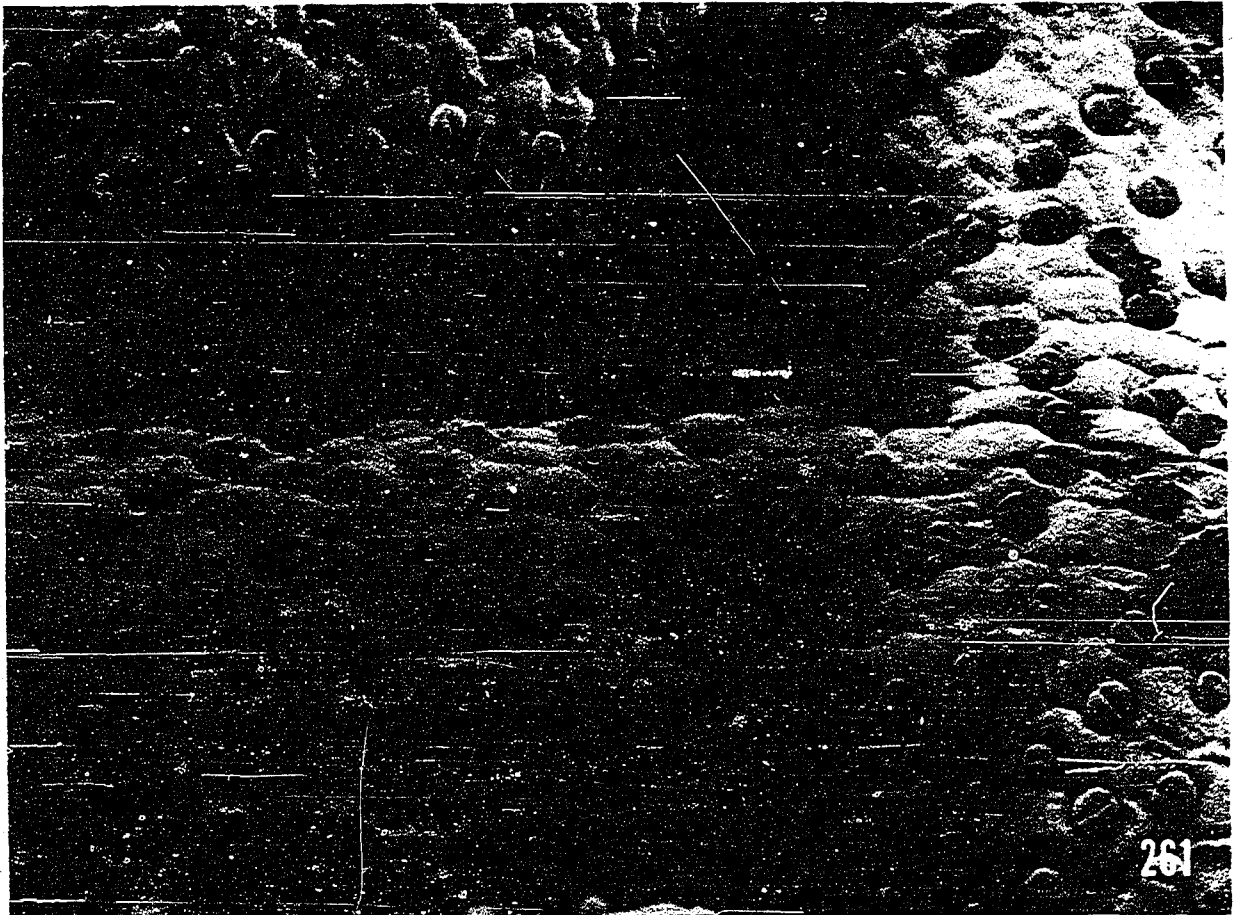


Fig. 263. Longitudinal section through immature spherical trichome prior to division of terminal cell.
X 19,600 (SPRING)

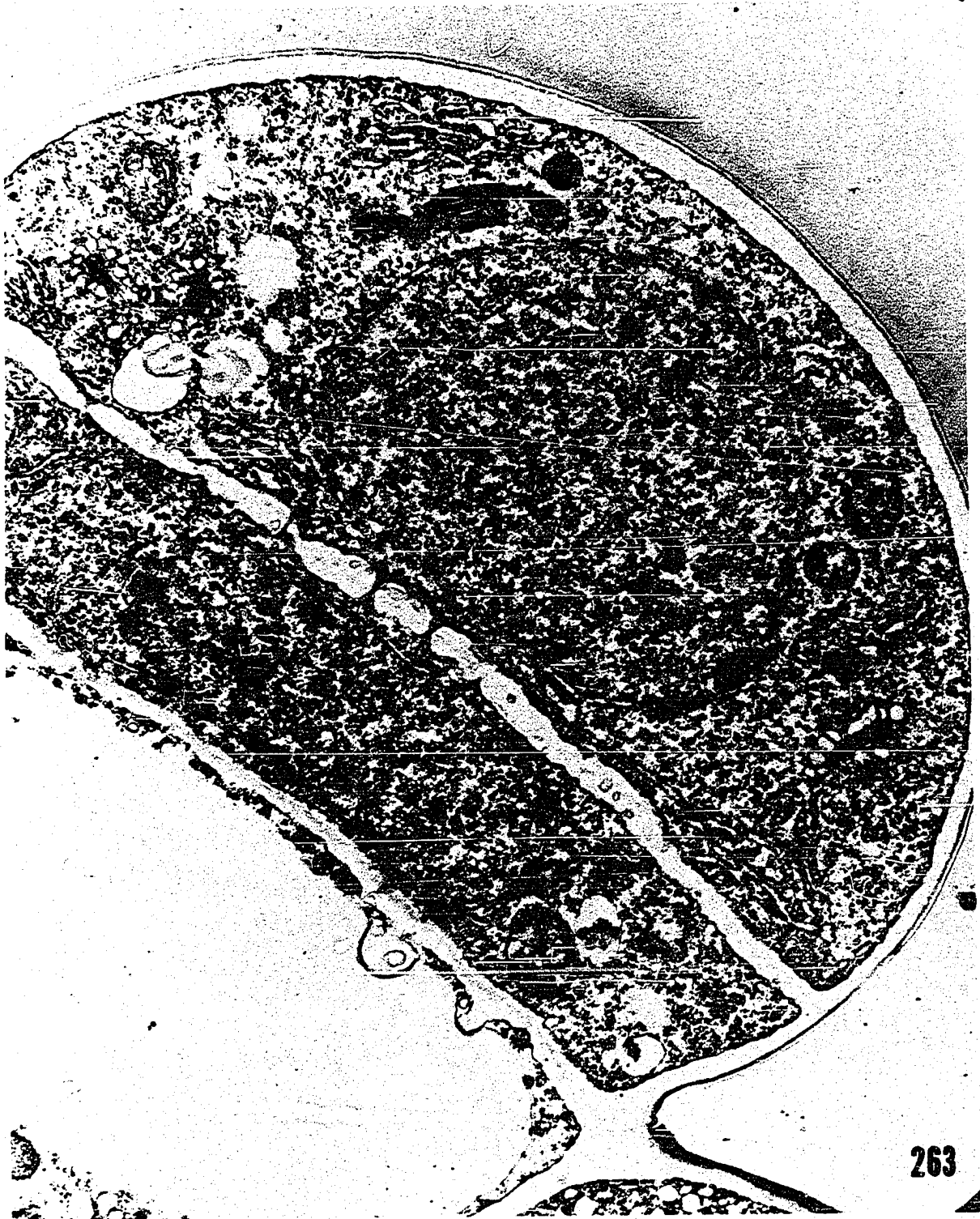
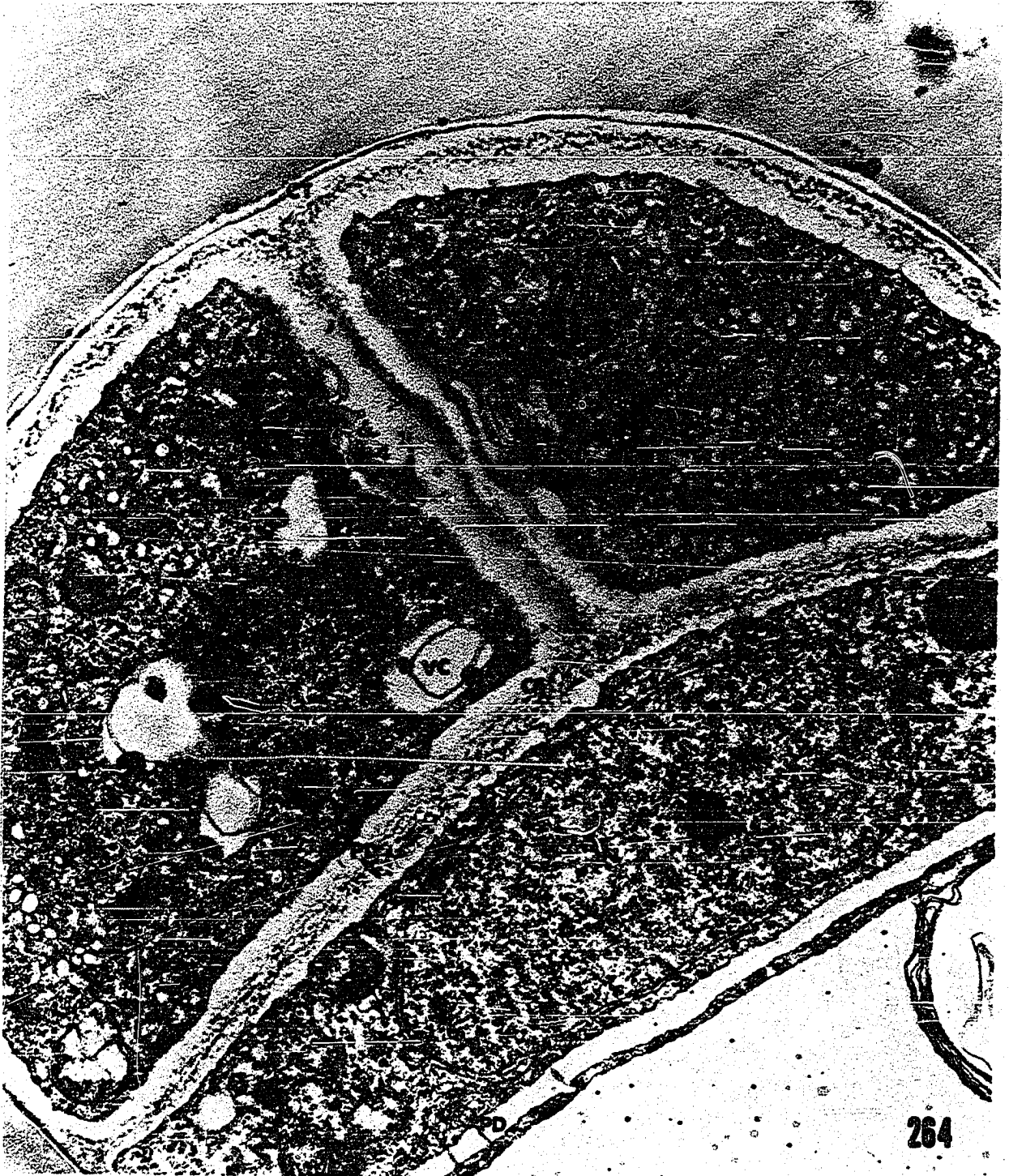


Fig. 264. Longitudinal section through spherical trichome after division of terminal cell but prior to maturity. X 14,000 (SPRING)



- Fig. 265. Longitudinal section through mature spherical trichome with thick callose wall in terminal cell and an endodermoid anticlinal central cell wall and periclinal transfer walls in central cell. X 9,200 (SPRING)
- Fig. 266. Phase contrast view of outer spherical trichomes. X 425 (SLOUGH)
- Fig. 267. Longitudinal section through spherical trichome. X 510 (SPRING)
- Fig. 268. Longitudinal section through spherical trichome. X 510 (SPRING)
- Fig. 269. Spherical trichomes viewed under UV and stained with aniline blue to give positive fluorescence for callose. X 210 (SLOUGH)

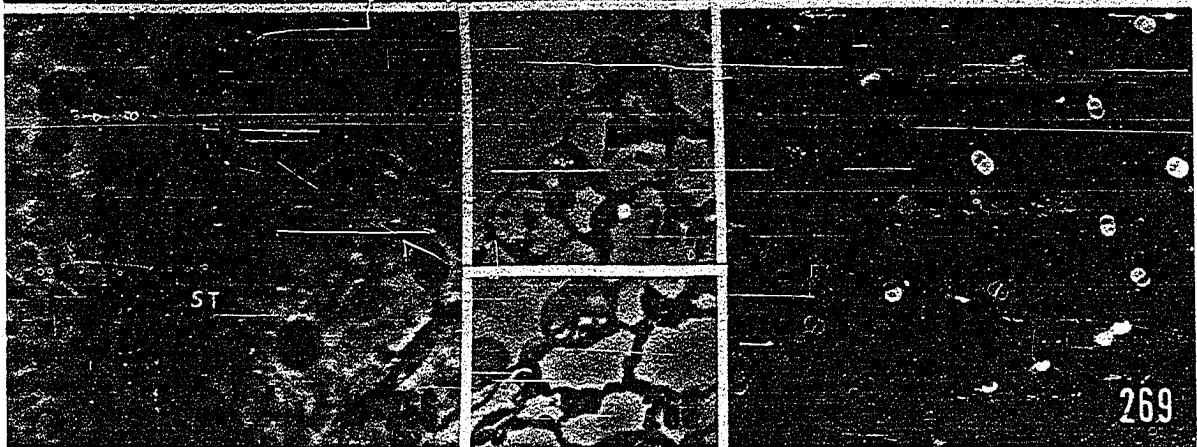
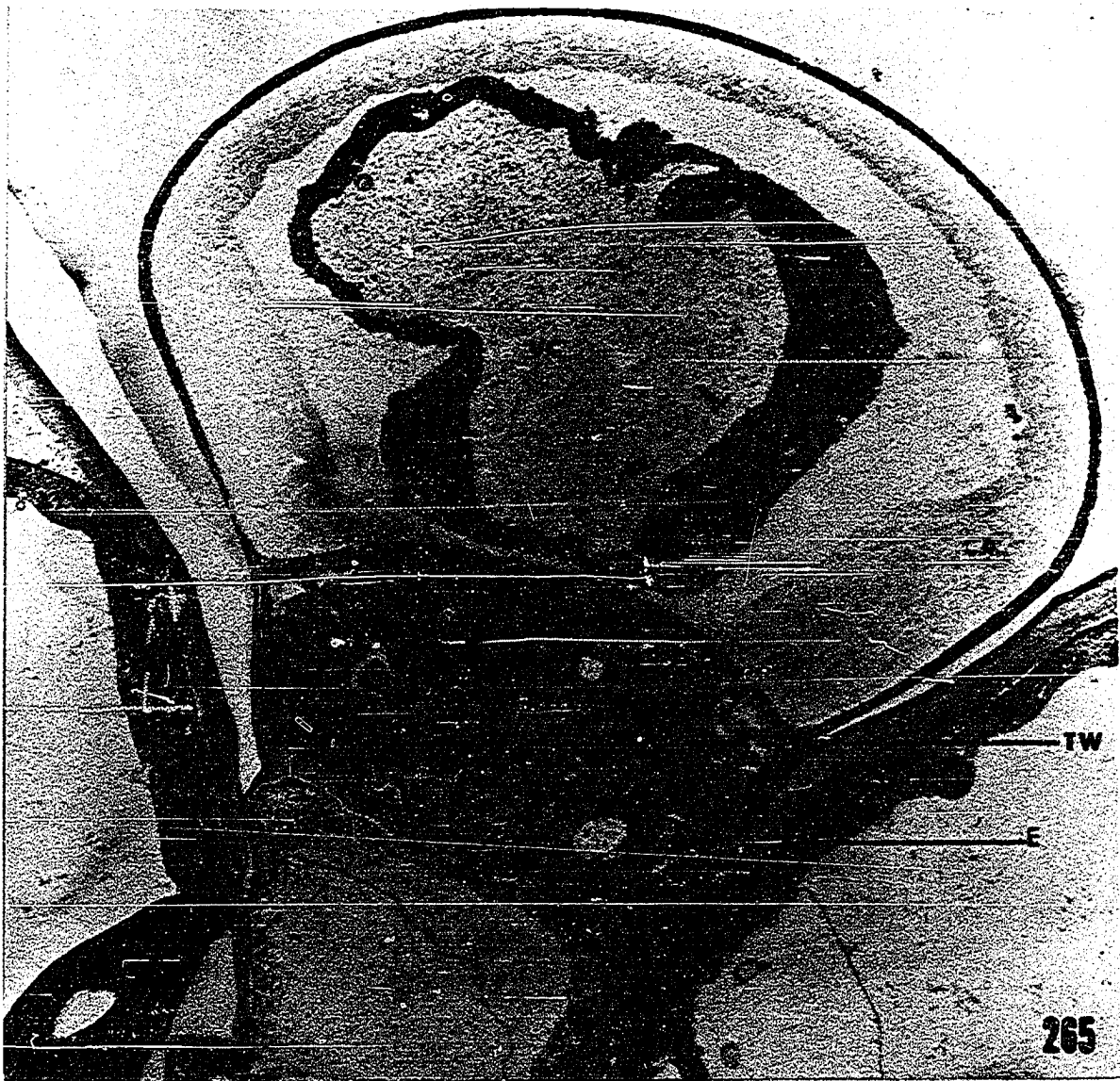


Fig. 270. Longitudinal section through mature spherical trichome showing endodermoid wall and transfer walls of central cell. X 33,000 (SPRING)

Fig. 271. Longitudinal section through terminal cell wall showing callose and cutin cystoliths. X 33,000 (SPRING)

Fig. 272. Longitudinal section through central region of spherical trichome showing plasmodesmata between central cell and terminal cells and between central cell and basal cell. X 14,680 (SPRING)

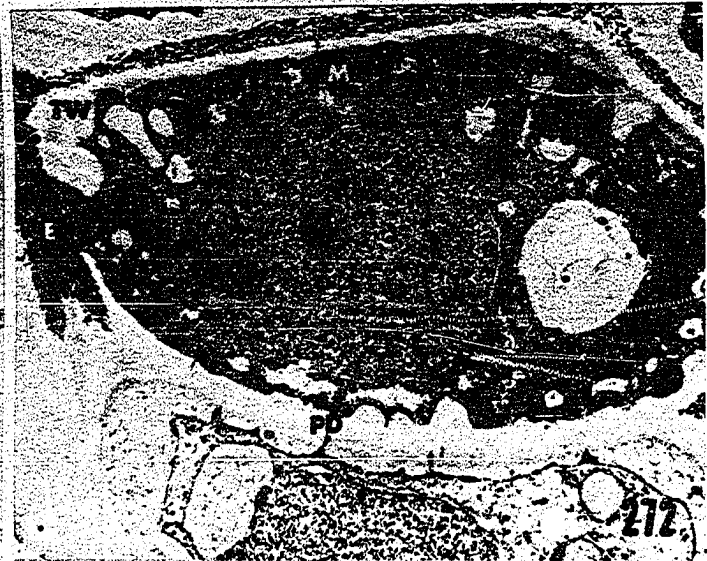


Fig. 273. Longitudinal section through spherical trichome showing peripheral placement of mitochondria in central cell and presence of intranuclear crystals in central cell nucleus. X 28,000 (SPRING)

Fig. 274. Plasmodesmata between central cell and basal cell of spherical trichome. X 30,300 (SPRING)

Fig. 275. Plasmodesmata between central cell and terminal cells of spherical trichome. X 16,800 (SPRING)

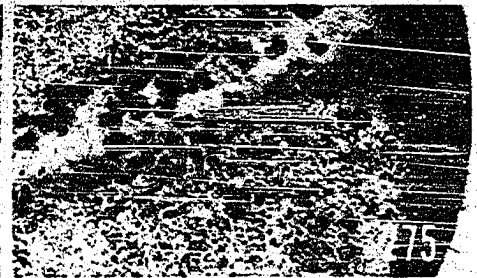


Fig. 276. Intranuclear crystals in spherical trichome central cell. X 37,000 (SPRING)

Fig. 277. Intranuclear crystals in central cell of pavement epithelium trichome. X 37,000 (SPRING)

Fig. 278. Intranuclear crystals in bladder wall cell. X 37,000 (SPRING)

Fig. 279. Intranuclear crystals in bladder wall cell. X 37,000 (SPRING)



Fig. 280. Intranuclear crystals in developing trigger trigger trichome. X 37,000 (SPRING)

Fig. 281. Intranuclear crystals in bladder wall cell. X 37,000 (SPRING)

Fig. 282. Intranuclear crystals in lenticular trichome. X 37,000 (SPRING)

Fig. 283. Intranuclear crystals in bladder wall cell. X 37,000 (SPRING)

