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Light And Electron Microscope Studies Of Erysiphe Polygonl Dc And Its Interaction With Trifollum Pratense

Poo-chow Leong

LIGHT AND ELECTRON MICROSCOPE STUDIES OF *ERYSIPHE POLYGONI DC* AND ITS INTERACTION WITH *TRIFOLIUM PRATENSE L.*

BY

LEONG POO-CHOW M.Sc.

Department of Plant Sciences

Faculty of Science

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ABSTRACT

The development and fine structure of the powdery mildew Erysiphe polygoni DC were studied by means of the light and the electron microscope.

E. polygoni conidia are uninucleate and at maturity contain much glycogen. The glycogen granules are thought to serve as a major energy reserve.

More than half of the mature conidium is filled with vacuoles which contain a variety of contents such as the myelinoid bodies, netted heterogenous material and lipids. It is believed that the myelinoid bodies are synthesizing structures. Most of the water in E. polygoni conidia is believed to be in the vacuoles and the high water content theory of the powdery mildew conidia postulated by Yarwood (1950) appears to be correct. In addition, water may be obtained by oxidation of other food reserves such as lipids and glycogen in the conidia.

Prominent spherosome-like bodies, which vary in size and shape are observed in the hyphae. Lomasomes which are thought to function in cell wall synthesis are present mainly in the germinating conidia and mature haustoria. Woronin bodies, septa, and lateral bodies associated with the nuclei are present in the hyphae.

The germ tube wall appears to originate from the inner wall of the conidium. The mitochondria usually reproduce by direct fission. The growth of hyphal tips is associated with the formation of vesicular structures.

The primary appressoria are lobed and have a thickened wall at the penetration site and a prominent nucleus above the infection peg. They contain many cell organelles such as the mitochondria, endoplasmic reticulum and ribosomes.

Penetration of the cuticle is believed to be by a mechanical means, as no swelling or change in staining properties of the cuticle were observed. The thickened appressorial wall with spine-like protrusion may facilitate such action but the penetration of the cellulose wall and the collar is accomplished by chemical and mechanical means. The change in the staining property and the degradation of the non-fibrillar structure of the wall indicates chemical action.

A sequential development of host penetration was studied. The formation and fine structure of haustoria are described. The host plasmalemma is invaginated by the penetration peg and becomes the sheath membrane, enclosing the haustorium. A criterion for young, mature and old haustoria is suggested. The young haustorium is characterized by a distinct wavy sheath membrane, and a narrow sheath-matrix space which appears

empty or filled with fluid-like material. The plasmalemma of the haustorial central body is highly indented and the central body which is uninucleate, has no or a few haustorial branches. Numerous ribosomes and mitochondria, abundant endoplasmic reticulum and multivesicular bodies are present in the central body.

In the mature and old haustoria the sheath membrane is thicker, denser, and highly invaginated. The sheath-matrix space is large and usually filled with heterogenous material and lipid-like inclusions. Numerous interwoven haustorial branches, lomasomes and vacuoles with various contents are present. Lipid bodies and vacuoles are also more numerous in the old haustoria.

The space containing the sheath matrix, an interface between the host and the fungus, is considered to be a buffer zone or a food reserve region where active absorption or exchange of metabolic material may occur.

Connections between hyphal strands of E. polygoni are observed. The fungus produces penetration pegs which pass through the adjacent hyphal wall of its own mycelium and develop to a limited extent in it. Electron micrographs indicate that entrance is gained by dissolution of cell wall and not by mechanical means.

The quantity of reducing compounds in susceptible and resistant clover cells after infection with E. polygoni differ significantly. Benedict's solution, an indicator of reducing compounds, does not color healthy cells but

turns infected susceptible cells slightly yellowish the first week after infection. The resistant cells immediately after infection turn a deep yellow which remains until death. Thus the mechanism of resistance or susceptibility is related either directly or indirectly to the accumulation of reducing compounds or the redox potential in the host cells.

Azure B produces a degree of coloration quite similar to that of Benedict's solution. Non-infected cells are unstained, infected susceptible cells are slightly stained for about the first week after infection while the resistant cells are deeply stained. The staining is either limited to the infected cell or a faint staining of the neighboring cells. These results indicate that metabolism is drastically altered at the time of infection and that specific cells rather than diseased and healthy areas should be studied.

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TABLE OF CONTENTS

LIST OF DIAGRAMS

LIST OF FIGURES

Note: All figures are electron micrographs except those marked with * which are light micrographs. The scale line represents 1μ in length unless otherwise indicated.

Figures Page

Figures Page

Figures | Page

Figures Page

ABBREVIATIONS

A	Appressorium
C	Conidium
Cb	Central body
Ch	Chloroplast
Co	Collar
Cu	Cuticle
Dm	Densely stained material
E	Epidermal cell
ER	Endoplasmic reticulum
Ew	Epidermal wall
F	Fungus
G	Glycogen
Ge	Gelatinous layer
Go	Golgi apparatus
Gr	Grana
Gt	Germ tube
H	Haustorium
Hb	Haustorial branch
He	Heterogenous material
Hi	Haustorial initial
Hn	Haustorial neck
Hy	Hypha
I	Infected cell
In	Invagination
L	Lipid body
Lb	Lateral body
Lo	Lomasome
M	Mitochondrion
My	Myelinoid body
N	Nucleus
Ne	Nuclear envelope
Nu	Nucleus
Np	Nuclear pore
P	Penetration peg
Pa	Papilla
Pg	Plastoglobuli
Pl	Plug-like substance
Pm	Plasmalemma
R	Ribosome
RER	Rough endosplasmic reticulum
S	Spherosome-like body
Sh	Sheath membrane
Si	Sheath invagination
Sm	Sheath matrix
Sn	Spine-like projection
Se	Septum
Sp	Septal pore
T	Tonoplast
V	Vacuole
Ve	Vesicle
Vi	Vesicular inclusion
W	Wall
Wn	Woronin body.

INTRODUCTION

Erysiphe polygoni DC, an obligate parasitic fungus is a member of the family Erysiphaceae and belongs to the class Ascomycetes. The Erysiphaceae cause plant diseases commonly known as powdery mildews. The name powdery mildew is used because an enormous number of conidia, which are produced on the surface of the host, appear to the unaided eye as a white powdery coating. Thus powdery mildews are best known to crop producers in their conidial stages, but they are usually classified on characters of the sexual fruiting body, the perithecium.

Some of the distinct characters of the powdery mildews are their superficial hyaline mycelium, their luxuriant development in dry weather, the high water content of their large turgid airborne conidia, their xerophytic existence, their diurnal periodicity with respect to several characters, the phototropism of some species, their haustoria in the epidermal cells of their hosts and the compatible association with their hosts.

The powdery mildews cause considerable damage to cultivated crops (Erkinsson, 1930; Stevens, 1942; Yarwood, 1957). The economic importance of the powdery mildews is next to that of the rust fungi.

Powdery mildews have a world wide distribution and a wide range of hosts. They flourish mainly on the

foliage of angiosperms and are obligate parasites on the leaves or young shoots and inflorescences of flowering plants. There are many multi-host species which parasitize species in a number of different families. In Japan 691 host species in 83 families and 32 orders have been found (Hirata, 1969). The host species are abundant in the Gramineae of the Monocotyledoneae, the Salicaceae, Betulaceae, Ranunculaceae, Cruciferae, Rosaceae, which belong to the Archichlamydeae of Dicotyledoneae, the Leguminosae, Labiatae, Caprifoliaceae and Compositae which belong to the Sympetalae of Dicotyledoneae. The above host families which were reported by Hirata (1969), are common to most regions throughout the world. The popular host species are in the families of Gramineae e.g. wheat, barley; Rosaceae e.g. strawberry, apple; Compositae e.g. sunflower; Leguminosae e.g. clover and pea. Many vegetables and ornamental plants are also common hosts of powdery mildews (Moore, 1959).

The highest number of host species for powdery mildew found in Western Asia is 731 and the Canadian and Alaska region is next with 545 species for the following 11 genera. Erysiphe, Leveillula, Sphaerotheca, Cystotheca, Podosphaera, Microsphaera, Uncinula, Typhulachaeta, Pleochaeta, Phyllactinia

and Oidium. The genus Erysiphe has the largest host range and 470 host species are recorded in Romania. Sphaerotheca is next largest and has 180 hosts in Japan. Microsphaera is third, and has 137 hosts recorded in northern central-America. Uncinula, Phyllactinia and Podosphaera have smaller host ranges and 90, 37 and 31 hosts respectively have been recorded. The host range of powdery mildew fungi in Japan is generally considered to be more closely related to that of North America than to Europe (Hirata, 1969). Some powdery mildews are known to attack only their own special host. Sphaerotheca phytoptophila, the most specialized of powdery mildews, attacks only the galls of western hackberry which are caused by a mite (Alexopoulos, 1962).

Powdery mildews usually cause chronic infections which appear every year and induce little loss in most years but occasionally bring about heavy losses. The injury caused by the powdery mildews is partly due to the covering of the affected part by the mycelium, which shades the plant and prevents air movement and partly due to the removal of food material by the haustoria which live in the epidermal cells. The diseased parts of the plant often wilt prematurely and die.

A few of the powdery mildews cause

devastating diseases. Uncinula necator, the cause of powdery mildew of the grape vine under favorable conditions can completely destroy the entire crop in a region. Sphaerotheca morsuvae, powdery mildew of gooseberries, Sphaerotheca pannosa, powdery mildew of roses, Sphaerotheca macularis, powdery mildew of strawberry, Podosphaera leucotricha, powdery mildew of apples, Erysiphe cichoracearum, powdery mildew of cucurbits and many other plants, and Erysiphe graminis, powdery mildew of barley, wheat and other plants can do likewise. On the other hand, Microsphaera alni, the cause of powdery mildew of lilac, does little or no harm to its host (Alexopoulos, 1962).

The records of losses resulting from powdery mildew have been reported by Yarwood (1957). In France the production of wine decreased from about 45 million hectaliters in 1850, in the early stages of the epidemic of the powdery mildew disease of grape to about 10 million hectaliters in 1854 at about the height of the epidemic. He also reported up to a 42 % reduction in the yield of barley, gooseberry crop rendered worthless, 61 to 71 % reduction in yield of hops, 33 to 90 % reduction in yield of grapes, up to 80 % reduction in yield of peaches, 40 % reduction in yield of clover and 75 % reduction in yield of cucumbers in various

countries. Yarwood (1957) also reported a negative correlation between the incidence of the powdery mildew and the green weight per plant in the red clover.

Powdery mildew conidia also act as allergens and are injurious to both animals and humans.

In order to provide a better understanding of the powdery mildew fungus the life history of the powdery mildew and a diagram of its reproductive cycles are given (Diag. I).

Life History:

Spore Germination

The life cycle of powdery mildews is initiated by conidia or ascospores, both of which germinate similarly. Germination usually starts within 2 hours of seeding (Brodie et al., 1942) and is favored by light, suitable temperature and absence of water. Maximum conidial germination occurs if the conidia are collected at a specific time of day and then placed on a specific host (Yarwood, 1936a)(Diag. II).

Under favorable conditions a germ tube is produced from one 'corner' of the conidium, not from the ends or sides (Corner, 1935). The first germ tube is usually short and forms a much convoluted appressorium in contact with the host surface. Growth of germ tubes toward the leaf and their attachment to the leaf may be stimulated by the positive phototropism of the germ tubes to green light (Yarwood, 1932). On

water, agar or glass surfaces, germination is abnormal in that the germ tubes may grow away from the substrate (Corner, 1935) and no appressoria are commonly formed. Appressorium formation on glass slides may be induced by manipulation of light so that germ tubes grow toward the glass surface (Yarwood, 1957). It has also been found that the germ tubes of some species respond positively to unilateral white light (Mitchell, 1967).

Penetration and infection.

A fine penetration tube or peg emerging from the center of the appressorium, grows into the lumen of the cell at the same time as a collar of host-wall material grows around the penetration tube (Corner, 1935; Smith, 1900; Smith, 1938). Within the lumen of the cell a haustorium is formed and expands to its characteristic shape (Diag. III). While the primary haustorium is becoming established, additional germ tubes are formed from other corners of the conidium, and hyphae grow out from the primary appressorium along the epidermis of the leaf. On all hyphae with the exception of the first germ tube, appressoria are formed laterally. Commonly there is one appressorium on every second hyphal cell. Branching is acute and fairly regular.

The mother spore of the powdery mildew colonies remains a living integral part of the colony and does

not collapse after the fungus has established nutritive relations with the host. As the colony grows, new conidiophores are formed farther and farther from the center of the colony. Colonies from single spores rarely become more than 2 centimeters in diameter under the most favorable conditions for growth.

The mycelium of Erysiphe polygoni, E. graminis and E. cichoracearum species is entirely superficial. It consists of a net work of abundant colorless hyphae on the epidermis of the infected parts of the host and is securely anchored by the numerous haustoria which develop in the epidermal cells and obtain nourishment from their protoplasts. Species of Leveillula and Phyllactinia are exceptions because their hyphae penetrate the leaf and grow between the mesophyll cells. Although most of the hyphae of Phyllactinia corylea are superficial, they do not develop haustoria (Alexopoulos, 1962).

Asexual reproduction - Sporulation.

About 4-5 days after inoculation, conidiophores, the specialized hyphae which produce conidia begin to form as swellings from the vegetative cells in the centre of the colony. An illustration of conidiophore development is shown in Diagram II. Conidia are cut off at tips of the conidiophores (Diags. I and II). As this process continues a chain of condia forms as

shown in the species Erysiphe cichoracearum and E. graminis (Diag. II). In Leveillula taurica, the mycelium is endophytic, the conidiophores grow out of the stomata and produce their conidia outside the leaves. In this species as well as in Phyllactinia species, and in E. polygoni the mature conidia fall off individually as soon as they are formed, and no conidial chains are formed (Diag. II). The conidia of Erysiphaceae are hyaline and one-celled (Diag. III). They vary somewhat in shape from species to species, but in general may be described as oval or rectangular with rounded corners. They are the asexual propagating units of the powdery mildews. The conidia are disseminated by wind or other means and by chance come in contact with a suitable host surface. With the deposition of the conidia on an infection court, the conidial or asexual cycle of powdery mildew is completed and starts to repeat itself within 4 or 5 days.

Sexual Cycle - Occurrence of perithecia.

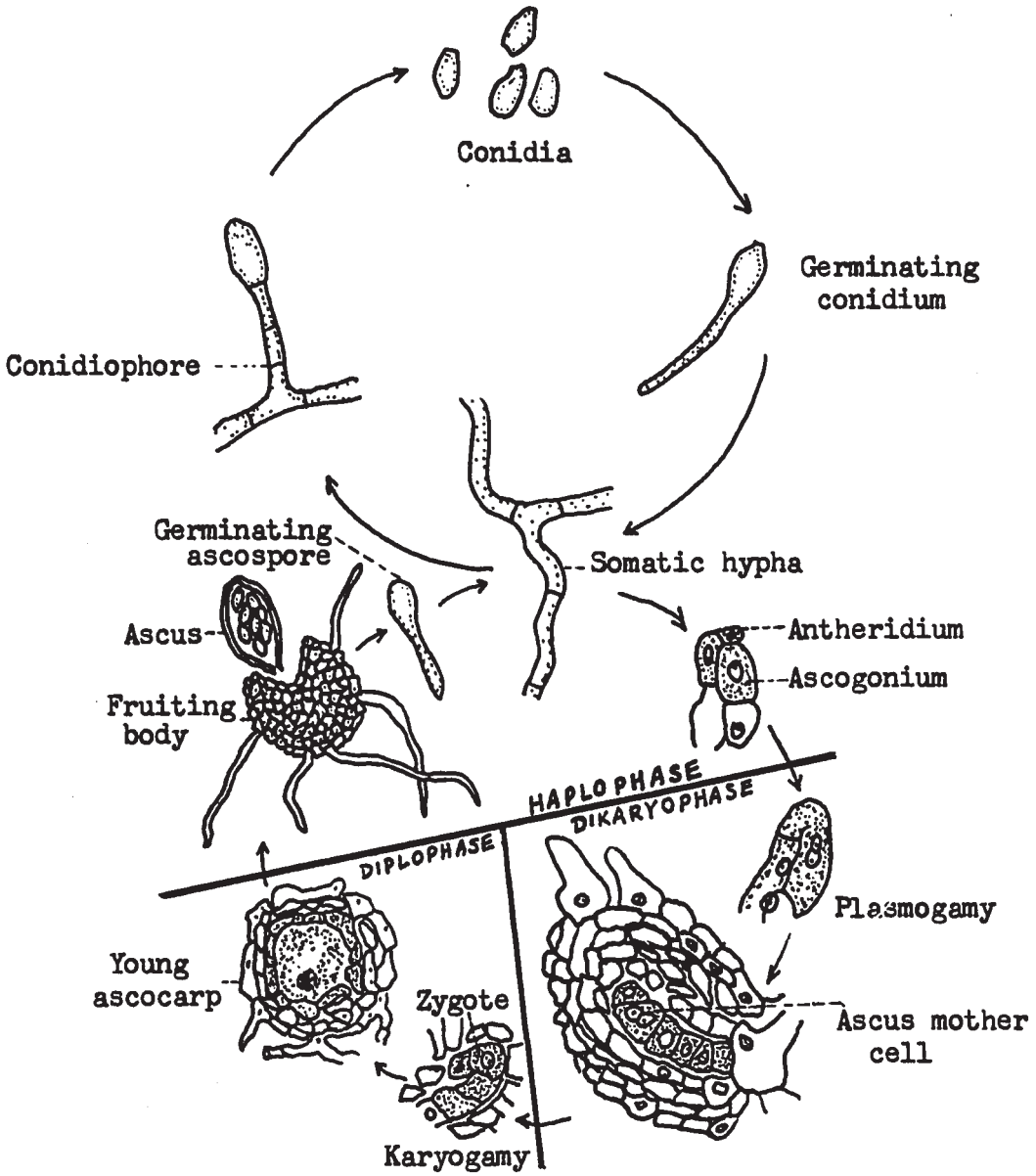
Perithecia are the perfect stage of powdery mildews, and occur either regularly, occasionally or never. The ability to produce perithecia varies from species to species. They are usually first formed after the conidial stage has existed for several weeks, but may be detected at any time in the summer and fall

although they are normally formed in late summer (Yarwood, 1957). The nutritive condition of the host or sexuality are considered the major factors determining perithecial formation. Formation of perithecia is usually preceded by a decrease in conidia production.

The perithecia appear at first white, then orange, reddish, brown, and finally black when mature. Perithecial formation and ascus development requires a long period of time. In many species the asci mature late in the fall and sometimes not until the following spring. Ascospores may be mature when the perithecia become macroscopically mature. Most powdery mildews are relatively inactive during the winter season. Overwintering takes place in the perithecial stage. Sometimes colonies overwinter as mycelia in the dormant buds of their hosts. The mature perithecia of most Erysiphaceae have characteristic appendages which vary considerably in length and shape. The asci of the Erysiphaceae are globose to ovoid. They are often fascicled and spread out like a fan when the perithecium is crushed and they are forced out. The ascospores when mature are released when the asci burst. The ascospores are hyaline, except in Astourella and they are usually unicellular and oval. Two and 4-celled ascospores are also known in some genera. The number of ascospores in each ascus varies between species and within the same species. Uncinula aceris regularly bears 8 rarely 6

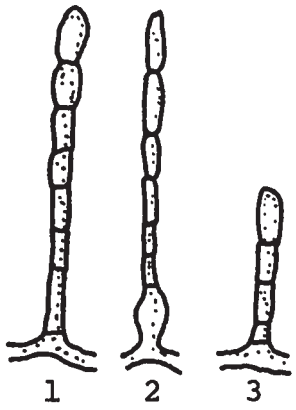
ascospores in each ascus, but Erysiphe cichoracearum has 2, rarely 3, ascospores in each ascus. E. polygoni usually produces 3 to 6 ascospores some have from 2-8 ascospores in each ascus (Alexopoulos, 1962; Patersen, 1938). The mature ascospores when dispersed may germinate and infect the plant host. Few life cycles have been experimentally completed through the perithecial stage, this may be due to the long period of maturation necessary before some ascospores germinate. Because of the longevity of the perithecia which can live up to 13 years (Moseman, 1957) in contrast to the conidial life span of only a few days, it is believed that they may function to carry the fungus over long periods.

Diagram I

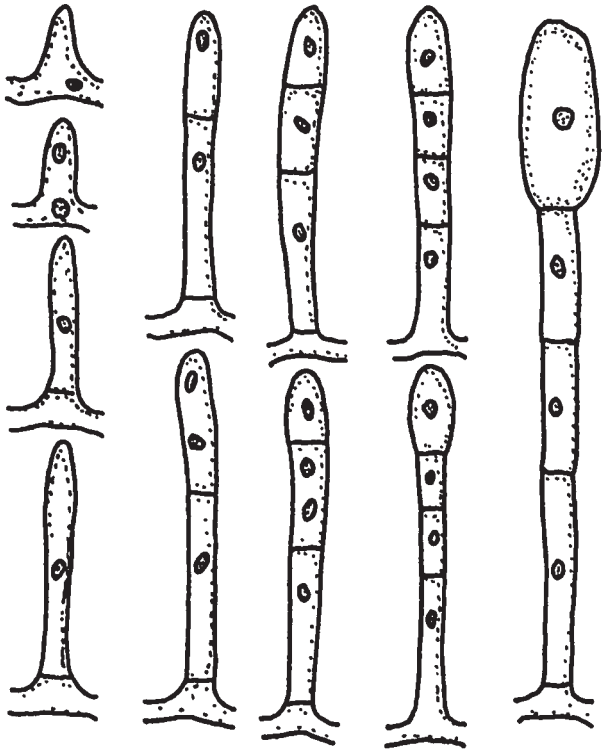


Life cycle of powdery mildew fungus.
Redrawn from Alexopoulos' Textbook
of Introductory Mycology.

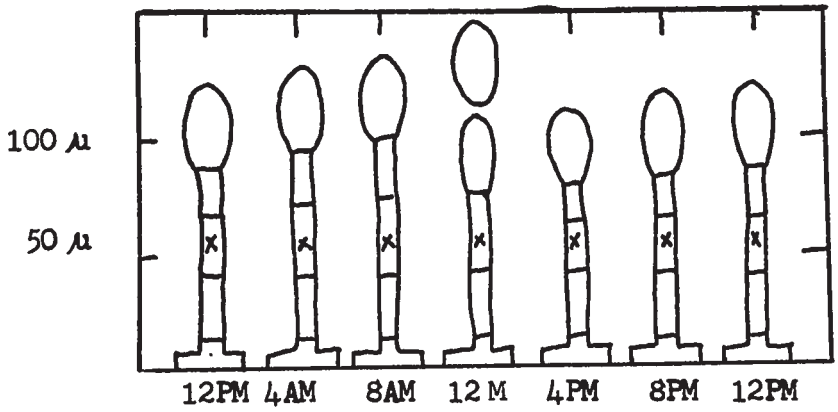
Diagram II



A. Conidiophore of
1. E cichoracearum;
2. E. graminis;
3. E. polygoni.

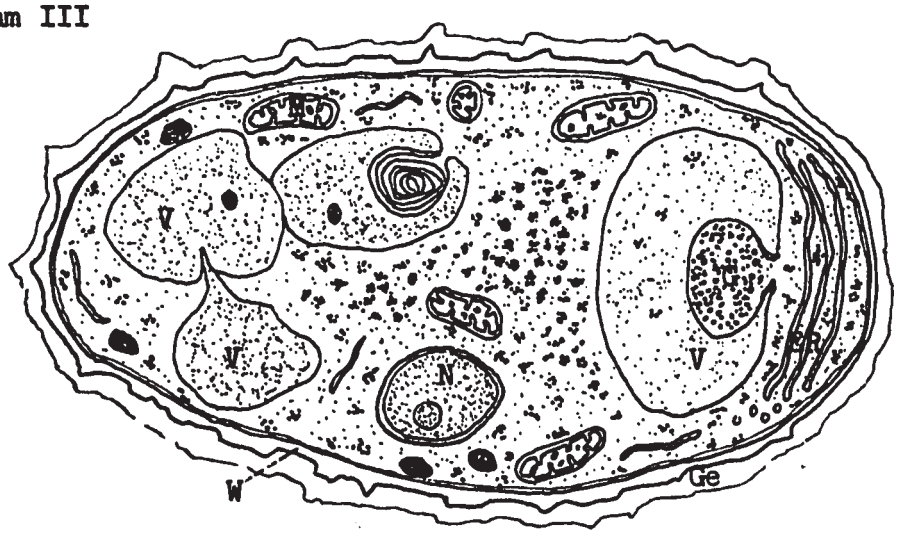


B. The development of the conidiophore and conidium of Erysiphe polygoni.

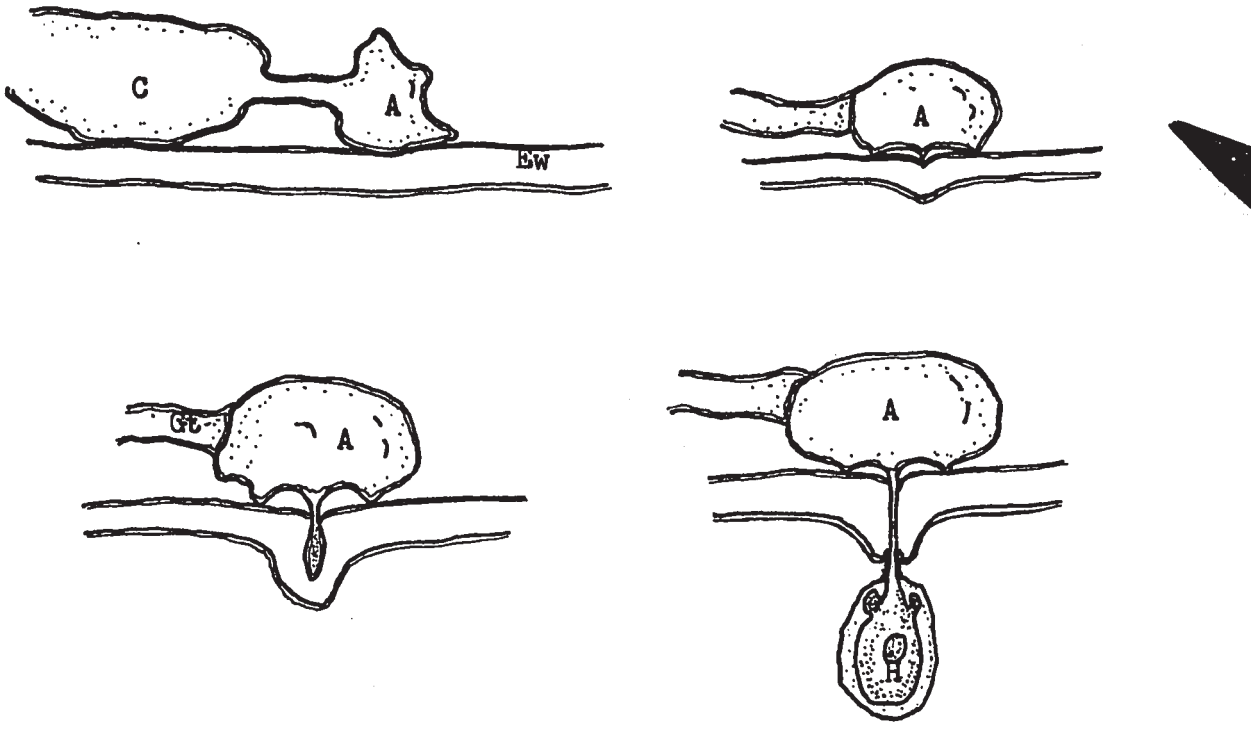


C. The diurnal cycle of spore production and maturation in Erysiphe polygoni.
From Yarwood (1957).

Diagram III



General structure of mature conidium
of Erysiphe polygoni.



Germination, penetration and haustorium
formation of Erysiphe polygoni.

LITERATURE REVIEW

The pathological and economic importance of powdery mildews can be assessed by observing the close attention and keen interest in the research that is paid by many pathologists and mycologists, and by noting the large number of publications on the powdery mildews both in the past and in recent years. Salmon (1900), Smith (1900), Yarwood (1950, 1956, 1957, 1967), Brodie (1942, 1945), Stavely and Hanson (1965, 1966, 1969), McKeen (1966, 1967, 1969, 1970), Jhooty (1965), and Mitchell (1967, 1970), Hirata (1962, 1967, 1969), Moseman (1966) and Schnathorst (1965) are some who have made significant contributions.

Powdery mildews are considered to be a highly specialized group of fungi and have been repeatedly an object of investigation from the standpoint of their general structure and growth habits.

Mitchell (1967) in his studies of Sphaerotheca macularis pointed out that some aspects of the biology of the mildews have been extensively investigated, such as the characteristics and physiology of the various species of powdery mildews. The organization, detailed structure, fungus-host relations have been either neglected or not fully investigated. The conflicting statements from some of the reports of the

biology of powdery mildew have led to a considerable amount of confusion in the literature. One controversial aspect is the water relations of conidia.

Conidia are propagating units produced by most fungi and serve to reproduce and multiply new individuals of the same species and are the principal reproductive units of the fungus. The importance and significance of fungus spores have been stressed by Hawker (1966). They play an important role in spreading of diseases of many plants and are the object of much study. Their structure, physiology, production, germination, variation, dispersal and survival, and their relationship to the epidemiology of diseases of plants are important in both fundamental and applied mycology.

The conidia of some powdery mildews such as E. polygoni and E. graminis are unique from other hydrophilous fungus spores in having the ability to tolerate high moisture stress (Jhooty, 1963). They are capable of germinating at 0% relative humidity (Brodie, 1942; Yarwood, 1936; Cherewick, 1944) on the glass surfaces whereas the spores of Botrytis cinerea, Penicillium digitatum, Puccinia graminis and Venturia inequalis need almost 100% for their germination. Another unique feature is their high water content of about 72 - 75% of the total dry weight of the spore. Other fungus spores have only 6 - 25% when dormant. Based

on moisture requirements the conidia of powdery mildews can be divided into 2 groups. Some such as E. polygoni, E. graminis, Uncinula salicis and Microspora alni, are capable of germinating at moisture stresses approaching 0% relative humidity. Others such as Sphaerotheca macularis, S. pannosa, S. humuli, Erysiphe cichoracearum and P. leucotricha, need more than 90% relative humidity for satisfactory germination on glass surfaces. (Jhooty, 1963; Schnathorst, 1960).

To explain the phenomenon of the tolerance of some powdery mildew conidia to higher moisture stresses, 2 theories have been proposed. A high osmotic pressure theory was proposed by Brodie in 1945, and the high water content theory was put forward by Yarwood in 1950, and supported by Jhooty and McKeen (1965a).

Brodie and coworker (1942) believed that the water content of E. polygoni conidia was not high because they could not stain the large vacuole-like structures in the mildew conidia with a 0.2% solution of neutral red which is believed to be a typical water absorbing stain. They were able to stain the vacuole of the spores of Botrytis cinerea and Fusarium culmorum with neutral red. Because these vacuole-like structures constitute the bulk of the conidia of E. polygoni and because of their non-watery nature, they concluded that the conidia of E. polygoni had a low water content.

In 1945, Brodie also found that the osmotic pressure of E. polygoni and E. graminis was 63 and 68 atmospheres respectively. Therefore Brodie (1945) postulated that the high osmotic pressure may enable the conidium to absorb moisture from the air, by imbibition, and to tolerate high moisture stresses.

The water content theory was first put forward by Yarwood in 1950. He hypothesized that the powdery mildew conidia carry water with them. They were shown to have a much higher water content than the hydrophilous fungus spores. Thus he claimed that the mildew conidia should have a lower density than the spores of the latter mentioned group of fungi.

He found that the conidia of E. polygoni and E. graminis contain 72 and 75 % water respectively. This water content is much higher than that in the spores of Penicillium digitatum, Aspergillus niger, Botrytis cinerea and Monilinia fructicola which contain 6, 13, 17 and 25% water respectively (Yarwood, 1950). Thus Yarwood concluded that the high water content of the conidia of E. polygoni and E. graminis enabled them to withstand high moisture stresses.

Jhooty (1963) in his study of the biology of Sphaerotheca macularis supported the water content theory and disagreed with the osmotic theory. Due to the information obtained from S. macularis and E. polygoni

studies Jhooty and McKeen (1965a)said that the osmotic theory was probably incorrect. They doubted the reliability of the neutral red staining reaction. By using the dry weight method, Jhooty and McKeen (1965a) found the water content of the conidia of S. macularis (average 53%) was higher than that of the Botrytis cinerea which was found to be 17% by Yarwood (1950) who used the same method. They also found that the E. polygoni conidia contained about 69% water which is similar to Yarwood's finding. Therefore Jhooty and McKeen (1965a)believed that the staining method Brodie used was not reliable in determining the actual water content of the spores.

Jhooty and McKeen (1965a)cited Thatcher's (1942) finding that the E. polygoni hyphae had an osmotic pressure value of only 19 atmospheres in contrast to 63 atmospheres found by Brodie (1945) in the conidia of E. polygoni. They believed that the difference in the osmotic pressure value between the conidia and mycelium of E. polygoni could not be so great, because there is an intimate exchange of protoplasmic contents between the mycelium and conidium through the septal pore. They believed that Thatcher's results of 18 atmospheres were more reliable, but Jhooty and McKeen (1965) did not find the actual osmotic pressure value of the conidia of E. polygoni.

Support for the theory that the E. polygoni conidia, which contain about 70% water, have a lower

osmotic pressure than the conidia of S. macularis and E. cichoracearum containing 53% and 52% water respectively, was the report that their osmotic pressure was 18 and 17.8 atmospheres respectively (Jhooty and McKeen, 1965; Schnathorst, 1959).

However the experimental evidence indicates that the water content of the mildew conidia plays an important role in their biological activity, because E. polygoni and S. macularis have widely different moisture requirements. It appears that the conidia of powdery mildews may have to reach a certain water content level before they can germinate.

Jhooty (1963) found that the osmotic pressure of S. macularis conidia (18 atmospheres) is lower than those of the spores of hydrophilous fungi such as Botrytis cinerea, Uromyces fabae and Phoma lingan, assuming that the osmotic value of the spores of the above mentioned 3 fungi is similar to their hyphal osmotic pressure, which were found to be 29.8, 44.25, 41.3 atmospheres respectively (Thatcher 1938, 1942). Moreover fungi such as S. macularis and E. cichoracearum which have a similar water content and moisture requirements for germination also have similar osmotic values (18 and 17.8 respectively) (Schnathorst, 1959). Jhooty (1963) also found that the density of S. macularis is lower than that of the hydrophilous spores (basidiospores) of

Coprinus plicatilis and Psalliota campestris and uredospores of Uromyces phaseoli (Yarwood, 1952). The density of the spores is assumed to be a reflection of their water content. All the evidences shown above seem to support the water content theory.

Jhooty (1963) in his concluding remark said that the conidia of some powdery mildews have a much higher water content than the hydrophilous fungus spores and that there is a gradient of water content between the conidia of powdery mildews, which is reflected in their moisture requirement.

No change of volume was observed during germination of the conidia of S. macularis by Jhooty (1963) and Mitchell (1967) and thus it is believed that the water absorbed may be immediately changed into some form to trigger some physiological reaction necessary to initiate germination. A chemical compound on the surface of the host tissue which stimulates the germination of powdery mildew conidia at high moisture stresses was noted by Yarwood (1944). Jhooty and McKeen (1965b) also obtained a better germination of the conidia of S. macularis on the surface of the host than on glass surface at high moisture stresses. It may be the micro-climate or the combined effect of both stimulation and micro-climate plays an important role in the initiation of spore germination.

Schnathorst (1965) noted the differences in the response of powdery mildew conidia to different moisture stresses and that free moisture was inhibitory to the germination of conidia and Peries (1962) found that it is some times even lethal to conidia of S. macularis.

Gregory (1966) has concluded that fungal spores are characterized by having a small water content and a lack of vacuoles. Brodie (1945) believed that powdery mildews do not contain true vacuoles and contain very little free water. However Corner (1935) found several species of mildews which carry water for germination in the highly vacuolate cytoplasm of the conidia. No agreement has been reached on these 2 views. Mitchell (1967) in his study of S. macularis pointed out that if mildew conidia do contain water to the extent of 70% of their weight they must contain vacuoles or an excessivley hydrated cytoplasm.

Somers and Horsfall (1966) proposed a third explanation for the ability of mildew conidia to germinate at high moisture stress and said that it is the water retaining power and not the absolute water content which is the important factor for the germination of powdery mildews at low humidity.

Mitchell (1967) has pointed out that because a large number do germinate at relative humidities far lower than that required for most other fungal spores a need

for an investigation of their water content, and a further knowledge of the nature of the protoplast is required. Since most previous works have been carried out by the conventional cytological observations with the light microscope it is obvious that cytochemical and electron microscopy studies be conducted.

This prompted Mitchell (1967) to make an electron microscopic study on S. macularis and some other mildew conidia. His study showed that powdery mildew conidia are constructed in ways not only suitable for containing a high percentage of water but also suitable for retaining this water. A close correlation between the percentage of water contained in the conidia and the percentage of vacuolar space to spore volume suggested that most of the water in the conidia is contained in the vacuoles. His findings supported the water content theory suggested by Yarwood (1950).

Mitchell (1967) succeeded in staining the vacuoles with neutral red. He believed that the retention or conservation of water is achieved, not only by the mucilage encapsulation of wall which acts as a barrier or insulation against water loss, but also by the nature of the internal structure of the cell itself. He found that the vacuole consists of several separate compartments each having a protective membrane which would provide an efficient water retaining

mechanism. Thus the water retaining power of the powdery mildew conidia postulated by Somers and Horsfall (1966) seems valid. Mitchell also found that the vacuolar membrane had limited permeability and Goldacre (1952) found that dyes such as methylene blue, Janus green and neutral red penetrate and stain Neurospora cell vacuoles, but Mitchell (1967) found that these dyes stained the cytoplasm only, not the intact vacuole of mildew conidia. Thus it is believed that the vacuolar membrane of powdery mildew conidia is different from that of other cells and that it does not permit the passage of free ions. Mitchell (1967) observed that the vacuolar membrane is more electron dense than the membranes of the E.R. and that the typical unit membrane structures seen in the E.R. was not resolved in the vacuolar membrane.

McKeen (1970) in the study of Erysiphe graminis offered a fourth explanation for the tolerance of the high moisture stress of the powdery mildew conidia and he stated that probably the water needs of the germinating conidium are met in part by the oxidation of fats. He observed abundant osmiophilic lipid bodies in the conidia, conidiophores and mycelium. These bodies are intracytoplasmic, intravacuolar and up to 1 μ in diameter. These large quantities of lipid are oxidized in the process of respiration and the

oxidation of large amounts of hydrogen in the molecule results in the production of a corresponding large amount of water. He found that oxygen which enters through the exposed papillae of conidia, is necessary for germination. He claimed oxygen triggered germination and due to its union with hydrogen derived from lipid added to the large amount of water which is already present in conidia.

Electron microscopy also revealed that granular material is often present in the vacuoles. It was more plentiful in E. polygoni than in S. macularis and E. cichoracearum. Mitchell believed that the vacuoles of powdery mildews contained, in addition to water, important reserves of food and possibly intermediary compounds e.g. enzymes which could be utilized in the synthesis of new cytoplasmic structures during germination. He considered the vacuolar sap as a solution of nutrient material or possibly a colloidal suspension from which the cell could draw needed nourishment and building material for continual synthesis. The association of the myelinoid bodies with the vacuoles was of particular interest. Mitchell thought that these bodies had a synthetic function as did the present author (1967) in an earlier study of the fungus Monilinia fructicola. Mitchell reported also a cytoplasmic change in conidia during germination. He observed an accumulation of glycogen-like material in the germinating conidia but

not in the unincubated conidia. Mitchell claimed that this might be due to the utilization of the lipid reserves in the conidia during the early stage of germination and that their simple sugars were converted to glycogen which could be later converted to glucose for the continued metabolic activity after germination began.

Another unique characteristic of the powdery mildew fungus is its nuclear structure. Harper (1905) described in detail the sexual reproduction and the organization of the nucleus in some mildews. He observed a peculiar and characteristic attachment of the chromatin of the nucleus to a conspicuous deeply stained body which he called the central body. Harper observed this conspicuous granule in Erysiphe and Phyllactinia. He claimed that it constituted a point of attachment for the elements of the nucleus and determined a definite polar organization on the part of the chromatin and of the nucleus as a whole. He showed that the chromosomes were attached to this body by achromatic fibres and that following division of this "central body" the 2 centres separated to opposite sides of the nucleus to form the poles of the spindle. Colson (1938) also claimed that the first sign of spindle formation in Phyllactinia was the division of the small lateral granule, Harper's central body. Because this body always occupies a peripheral position somewhat opposite to the nucleolus,

Mitchell (1967) suggested the term peripheral body be used. The presence of the peripheral body was also observed by Bakerspigel (1959) in nuclei of Neurospora crassa. He noticed that this body was usually doubled at the time of separation of the bar of chromatin, each half moving to a sister nucleus. It appears that this body is of general occurrence in Ascomycetes. Judged by its behaviour, it seems to play a role similar to that of a mitotic spindle, in distributing the chromatin material of the nucleus to the daughter nuclei. No nuclear spindles were seen in the dividing somatic nuclei of S. macularis and E. polygoni (Mitchell , 1967). Mitchell (1967) in his summary said that the division of powdery mildew nuclei involves stages similar to those occurring in ordinary mitosis but an extra-nuclear spindle and discrecely resolvable chromosomes on the metaphase plate are lacking. Segregation of the chromosomes was achieved by the division and subsequent migration of a peripheral body to which the chromosomes were attached by heterochromatic strands. Metaphase groups were aligned parallel to the long axis of the cell and their separation occurred at right angles to it. This form of division shows some similarities to those described for other plant-pathogenic-fungal nuclei (Aist and Wilson 1965, 1967; Brushaber and Wilson, 1967; Robinow and Caten, 1966).

In fungal parasitism, the mycelium of parasitic fungi grows on the surface of the host and penetrates the cells and forms haustoria which comes into close contact with the host protoplasm. Haustoria are specialized absorbing organs and they may be knob-like, elongated or branched. Parasitic fungi usually do not produce haustoria on artificial culture but Dickinson (1949) has shown that certain obligate parasites will send infection pegs through artificial membranes. The production of haustoria is probably a response to the contact stimulus and/or the stimulus of nutrients.

Fungal haustoria are generally considered and accepted as special hyphal branches within a living cell of the host and they absorb food and water from the host cell. The occurrence and significance of haustoria in many groups of fungi were reviewed by Rice (1927). Smith (1900) pointed out that parasitic fungi are nourished in some way by their mycelium "through imbibing juices impregnated with the peculiar principles of the matrix on which they grow." Butler and Jones (1949) and Brown (1936) have suggested that haustoria may be specifically adapted for the uptake of proteins from the interior of the hosts. Yarwood (1956) believed that the haustoria of powdery mildews functioned for food absorption.

Hirata (1967, 1969) in his study of powdery mildews noted some characteristic features of conidial formation. He divided the powdery mildew fungi into 2 types. One such as Erysiphe graminis, produces several mature conidia daily on each conidiophore without diurnal periodicity, and the other, represented by E. polygoni which Yarwood (1936a, 1957) noted produces a single mature conidium from each conidiophore each day. Hirata (1967, 1969) found that the number of conidial chains and number of haustoria of E. graminis was equal on each colony and McKeen (1968) found the same for E. cichoracearum. This suggests that a haustorium nourishes, besides a certain length of hyphae, a conidiophore and its conidial chain. The conidiophore of E. graminis produces 8 to 10 conidia per day, while that of the E. polygoni, produces only one conidium each day. Hirata believed that the difference in the number of conidia produced daily by a haustorium may be due to the difference in the absorbing capacity of the haustoria of different species and/or to the difference in the nutrient supplying capacity of the haustoria. This supposition seems logical and interesting but, some examples appear to conflict with the above suppositions. For example Hirata found that the barley powdery mildew fungus always produces conidia in chains on either susceptible or resistant barley varieties. On undernourished barley plants the fungus never produces conidia singly. Moreover in Sphaerotheca species the

conidial production is not related to the host plant. Such facts led Hirata to suggest that the 2 types of conidial production might have arisen in the early evolutionary stages of the fungus. A related finding was given by McKeen et al. (1967) in their electron microscope study of the haustoria of E. cichoracearum. They revealed that the haustorium is composed not only of a central ellipsoidal body but also of many tortuous exceedingly fine (1μ) hyphae which arise at both ends of the central body and assume a convoluted form around the central body. McKeen et al. (1967) suggested 2 possible reasons for their presence. First, the hyphae are vestigial and have lost their ability to extend straight away, from the body of the haustorium, in which position they are surrounded individually or in groups by the host plasma membrane, as in Erysiphe graminis (Ehrlich and Ehrlich, 1963b; Hirata, 1937). Second, the hyphae are not vestigial but have evolved in a woven fashion around the central body. The surface area of the separating membrane is much smaller in latter type but the surface area of the haustorial body and appendages is several times greater than that of the membrane which surrounds the haustorium in E. cichoracearum. The haustorial body and its branches have a greater surface area for transfer of nutrients than does the membrane surrounding the fungus protoplasm. The above indicates that the haustoria which are different

in structure may have a different capacity for absorption and transfering nutrients.

The haustoria of E. graminis and E. cichoracearum differ in structure, but they both produce conidia in chains. E. polygoni haustoria appear similar to E. cichoracearum and may be assumed to have a similar capacity for absorption, but E. polygoni produces conidia singly. Mitchell (1967) believed that the finger-like processes arising from the ellipsoidal body do not arise exclusively from the ends in S. macularis as shown by Hirata (1937) and as shown by McKeen et al. (1966) for E. cichoracearum. However, he pointed out that in E. polygoni they seem to grow only from the ends and McKeen et al. (1966) found that they only developed from the ends of the E. cichoracearum haustorium. Mitchell (1967) disagreed with the claim made by Dekhuijzen (1966b) that the central body of the haustorium of S. fuliginea is connected with the sheath membrane by finger-like protrusions. He believed that the protrusions are only branches of the central body and are not connected with the sheath membrane.

Smith (1900) observed that only one nucleus was present in a mature haustorium but in one exceptional case he observed 2 nuclei in a haustorium. Berlin and Bowen (1964) reported that haustoria of Albugo candida did not contain nuclei. They attributed this to

the fact that the nucleus could not pass through the narrow penetration peg. Mitchell (1967) disagreed with this view and believed that the nucleus has a capacity to stretch. He observed a conspicuous nucleus in the haustoria of powdery mildews which occupied about two-thirds of the length of the central body. However Mitchell (1967) did not observe such a migrating or stretching nucleus passing into the haustoria.

Ehrlich and Ehrlich (1966) reported that the haustoria of Phytophthora infestans lacked nuclei. They believed that the occurrence of anucleate haustoria was related to the low phylogenetic status of the fungus and that such haustoria were less active physiologically than the nucleate haustoria of obligate parasites. Recently Sivak and Shaw (1969) found that nuclei occurred in the haustoria of P. infestans on potato leaves 24 and 72 hr. after inoculation. Sivak and Shaw (1969) supported the idea that nuclei stretch while entering haustoria, and pointed out that the diameter of the haustorial neck would be large enough, to permit nuclear movement in and out of the haustoria and that the rare occurrence of nuclei in old haustoria might be due to the withdrawal of much of the protoplasm, including nuclei, as the protoplasm moved toward the growing apices. Thus Sivak and Shaw (1969) suggested that it was

essential to specify the stage of development of a parasitic fungus when attempting to study the occurrence of nuclei in their haustoria because age influenced the content of haustoria.

Most Erysipheae confine their absorbing organs to the epidermal cells of the host. The mycelium of Uncinula salicis which is amphigenous on the leaves of the willow (Smith, 1900) may reach the interior tissues of the leaves. The haustoria sometimes may be observed in the palisade cells of the leaf. Phyllactinia which has a different habit from Erysiphe sends hyphae through the stomata into the intercellular spaces of the infected leaves and their haustoria are formed entirely in the interior cells of the leaf. It is clear from the early literature that this minute structure and especially its development is almost entirely unknown. The study of fungal structure has not significantly aided the understanding of fungal functions and relationships.

Up to now although there have been a considerable number of publications resulting from the electron microscope study of fungal haustoria, confusion still exists on the terminology of the haustorial apparatus and the origin or nature of the haustorial sheath. In the past, different authors used different terms to describe the enclosing sheath which is a feature of all the haustoria of obligate parasitic fungi. It represents the physical boundary between host and parasite and it is

a region in which interactions between the 2 organisms must occur.

Bracker (1968) in his study of E. graminis haustoria said that conflicting systems of terminology complicates the task of evaluating the structures around haustoria, and that divergent terminology will encourage divergent concepts of structure, origin and function. He further pointed out that the sheath is merely the non-host-wall zone between the haustorial wall and the host protoplast and is the encapsulation described by Berlin and Bowen (1964) on Albugo candida and Ehrlich and Ehrlich (1963a) on Puccinia graminis, the sack used by Hirata and Kojima (1962) on powdery mildews and zone of apposition described by Peyton and Bowen (1963) on Peronospora manshurica. He said that these structures are unified by their general form and location and the variation reported is not sufficient to consider them anything but homologous. To preserve order in terminology, he proposed that one system should be followed and applied to all haustorium-producing fungi and that the terminology of Smith (1900) should be used because of its priority. The terms sheath, sheath membrane, collar, and papilla describe the general form of the structure and are broadly applicable despite minor variations. In addition Bracker introduced 2 new terms, sheath matrix

and sheath invagination. Smith (1900) used the term sheath to describe the sack-like covering around the haustoria of some powdery mildews and the term sheath membrane for the membrane surrounding the sheath. Bracker (1968) observed in the powdery mildew that the whole haustorium is enclosed in the haustorial sheath. The sheath membrane forms the outer boundary of the sheath and appears as a single membrane enclosing the haustorial elements. The sheath matrix is the granular content between the sheath membrane and the central body of the haustorium. A collar surrounds the haustorial neck and forms at the time of penetration as a papilla against the outer epidermal wall as observed by Smith (1900). As the penetration peg of the fungus grows into the papilla, the papilla grows. Eventually, the peg may grow through the papilla and enter the host cell and form a haustorium. The remainder of the papilla, around the haustorial neck is the collar.

Chou (1970) in his study of Peronospora parasitica disagreed with Bracker's proposition to replace the terms encapsulation and zone of apposition, originally applied to rust (Ehrlich and Ehrlich, 1963) and downy mildew fungi (Peyton and Bowen, 1963) by the term sheath. According to Chou, it is difficult to decide whether or not the collar of powdery mildew is equivalent to the sheath of downy mildew and whether or not the sheath of the powdery mildew is equivalent to

the encapsulation and the zone of apposition in rust and downy mildew fungi. Chou (1970) cited the case of Peronospora parasitica in which he observed that the zone of apposition is not a structure separate from the haustorial wall but is an integral part of it. He did not think that it had any resemblance to the sheath of powdery mildews or the encapsulation of the rusts. Apparently confusion in terminology is unavoidable when different taxonomic groups of fungi and different host combinations are compared or used while little is known about the origin, nature, and function of the various structures. Moreover variation in haustorial structure within one group of fungi on different hosts was also noted by Rice (1927) and Fraymouth (1956). To eliminate the variation due to different hosts, Chou (1970) suggested the use of one host species which is infected by different group of obligate fungal parasites in order to obtain a critical comparison of haustorial ultra-structure of various groups of fungal parasites.

Most studies on the host-parasite relationship of obligate parasitic fungi have been concerned with the structure of the haustorial apparatus and the boundary region of haustoria and host cytoplasm, namely the host-parasite interface. However the origin, nature and formation of the haustorial apparatus have been much debated.

As early as 1900, Smith observed that the plasma membrane of the host cell is not ruptured by the ingrowth of cellulose during fungal infection. McKeen et al. (1966) in their study of E. cichoracearum haustoria, supported the view that the haustoria invaginate but do not penetrate the plasma membrane of the host cells. They agreed with the electron microscope observations of Ehrlich and Ehrlich (1963b) and the light microscopic observations of Hirata and Kojima (1962), and confirmed the view of Thatcher (1943) and Fraymouth (1956), who were able to separate haustoria of Puccinia and Peronospora from invaginated host protoplasts by plasmolysis. They also agreed with the recent electron microscope observations of Shaw and Manocha (1965), Peyton and Bowen (1963), Berlin and Bowen (1964) and Caporali (1960). McKeen et al. (1966), Bracker (1968) and Chou (1970) stated that the connection between the membrane around the haustorium and the host cytoplasmic membrane around the infected cell was never very obvious. Recent electron microscope observations of Bracker (1968), Stavely (1969) and Chou (1970) supported the view that the haustoria invaginate and do not penetrate the plasmalemma.

The enclosing sheath is a general feature of the haustoria of obligate parasitic fungi. The sheath is always present, but some light microscope reports

(Smith, 1900) (Woodward, 1927) have claimed that it was occasionally absent. Bracker (1968) suggested that these need verification with the electron microscope. McKeen et al. (1966) reported that the encapsulation in powdery mildew haustoria was different from that reported by Ehrlich and Ehrlich (1963a), Peyton and Bowen (1963), Berlin and Bowen (1964), and Shaw and Manocha (1965), in other groups of fungi, because it never occurred adjacent to the haustorial hyphae or body and always collected irregularly next to the surrounding membrane. Theories on the origin of the sheath or encapsulation are varied. Smith (1900) thought that the sheath consisted of disintegrated cellulose from the ingrowth of the host wall. Caporali (1960), who worked with Sphaerotheca panosa believed that the sheath was composed of pectic material derived from the haustorium. Ehrlich and Ehrlich (1963b) proposed that the sheath membrane of E. graminis was not merely the host ectoplast but was a membrane "at least partly of fungal origin". The support for this view is based on the fact that Hirata and Kojima (1962) were able to pull the haustorium from the host with its sheath intact and Dekhuijzen (1966a) was able to isolate the intact haustorium and sac from cucumber leaves. Others such as Shaw and Manocha (1965) working with Puccinia graminis, Peyton and Bowen, (1963) working with Peronospora manshurica and Berlin and

Bowen (1964) working with Albugo candida, Kojima and Hirata (1961) and Hirata (1937) working with Erysiphe graminis and Sphaerotheca fuliginea tended to agree that the sheath is of host origin. With regard to the nature and appearance of the sheath membrane, some workers have claimed that the sheath membrane is reinforced. Smith (1900) and Hirata and Kojima (1962) reported a thickening of the sheath membrane in the powdery mildews. Ehrlich and Ehrlich (1963a) observed that the encapsulation membrane around the haustoria was sometimes twice as thick as the host ectoplast, suggesting 2 separate membranes. Bracker (1968) working with E. graminis, stated that the sheath membrane was reinforced along the sheath side of the membrane. He thought that the reinforcement was the cause of the toughness and resistance of the sheath membrane and that the resistance of the sheath membrane would permit the sheath environment to be maintained for the haustorium even when the host cell has been markedly changed. Hirata and Kojima (1962) considered calcium to be responsible for the firmness of the sheath membrane and found that leaves could be made more susceptible by calcium treatment. Their experiments also indicated that an unbroken sheath membrane was necessary for the host cell to remain alive.

Davison (1968a) in her cytochemical and ultrastructural work of P. parasitica found that in the periodic acid-Schiff stained preparations a small

percentage of lightly stained haustorial wall, and sometimes one lobe of a haustorium was surrounded by a densely stained layer, while the others were not. She attributed the difference in staining to a possible age factor and she said if the encapsulation consisted of carbohydrate material, the haustoria with only a lightly-stained wall may have lacked this region, and been younger than those with a more intensely stained boundary. She occasionally observed the carbohydrate callose nature of the partly covered sheath around the haustoria. She considered the formation of a callose sheath to be a possible attempt by the host plant to wall off the invasive haustorium by an impermeable barrier. She also found by using staining techniques, that in P. parasitica the distribution of substances and organelles, such as nuclei, mitochondria, lipid material, protein, and RNA were more or less evenly distributed throughout the hyphae and haustoria whereas glycogen was not detected.

Peyton and Bowen (1963) and Berlin and Bowen (1964) observed connections between the host cytoplasm and the encapsulation. They found numerous vesicles with dense contents (secretory bodies) in the host protoplasm, connected to the enclosing membrane and suggested that this layer may be formed by host secretory activity. Chou (1970) and Davison (1968) failed to detect secretory vesicles around the haustoria of

P. parasitica but secretory tubules and vesicles were occasionally seen around those of Albugo candida by Davison. Ehrlich and Ehrlich (1963a) also showed that in rust there was continuity of the material from the haustorial cytoplasm through small channel-like areas in the haustorial wall and into the encapsulation. Chou (1970) found that the enclosing layer in P. parasitica was well developed at the very early stage of haustorial development. Consequently Chou expressed the view that no relation could be established between the stages of development of the haustorium and the occurrence of the zone of apposition. He proposed that the so-called zone of apposition was simply an integral part of the haustorial wall, because he observed that the haustorial wall was a three-layered structure like the hyphal wall. Chou (1970) thus suggested the term zone of apposition be replaced by the term dense zone, outer dense, and inner dense zone.

Bracker (1968) observed that the thickness of the sheath matrix of E. graminis varied up to about 5μ and seemed to increase with age. The sheath was restricted inside the collar channel by the limited space and the clustered membranes. The sheath matrix was not continuous with the host surface. The matrix of the sheath appeared amorphous and flexible. According to Bracker (1968), the sheath matrix had no recognizable cytoplasmic components and lacked continuities between haustoria and host

cytoplasm. He believed that the sheath matrix was neither fungal nor host protoplasm. McKeen et al. (1966) believed that the encapsulation was composed of by-products which result from the host-parasite interactions and served no useful function. They expressed the view that the increase in amount of the encapsulation might be related to age.

McKeen et al. (1966) believed that the space between the haustorial body and enclosing sheath membrane was probably filled with a nutrient solution. Hirata and Kojima (1962) believed that this space gradually increased when the water pressure, which fluctuated in this space, exceeded that of the haustorium and host protoplast. They thought that at times there was a higher osmotic concentration in the gap than in host and fungal cells and that water moved in and gradually separated the sack from the haustorial surface. The close contact between the peg and cuticle would prevent a leak. McKeen et al. (1966) believed that the sack was held back by mechanical pressure from the haustorial hyphae, because the sack does not have a symmetrical contour but is draped around the hyphae. They suggested that there would seem to be active transport from the bathing liquid. They further said that the fungus must secrete some substance which caused the host to secrete or release food materials into the sack because the inter-cellular spaces in the host tissue were empty. Hirata and

Kojima (1962) mentioned that the semipermeability of the sack controlled the diffusion of the constituents of the dead haustorium and prevented the death of the host cell and consequently was an indispensible structure for successful parasitism by the powdery mildew. McKeen et al. (1966) in their concluding remark said that the nutrition of the mildew may be due to a specific activity of the host and non-specific absorption by the parasite. Calonge (1969) in his study of the ultrastructure of haustoria of 4 fungi found connections between the host endoplasmic reticulum and host plasmalemma delimiting the encapsulation in P. hordei. Occasionally the tubules connected the haustorial cytoplasm with the encapsulation, and passed through the haustorial wall. In Exobasidum japonicum, a facultative parasite, the sheath surrounded some portions of the haustorial branches, exactly where the fungal cell wall seemed to present "open" areas which showed a direct connection between the parasite and the surrounding sheath. Calonge observed in the finger-shaped rudimentary haustoria of P. palmivora a facultative saprophyte, several vesicles with granular contents between the haustorial wall and the plasma-lemma, and a direct connection between a vacuole in its haustorium and the host cell through the haustorial wall.

Ehrlich and Ehrlich (1966) compiled the results of the current literature on the ultrastructure of

haustoria in different fungi, and made a comparative study of the fine structure of the facultative parasite (Phytophthora parasitica) and a facultative saprophyte (P. infestans). They found that the parasitic fungi exhibit no unique cytoplasmic features when compared with non-parasitic fungi, and the ultrastructure of the haustoria-producing facultative saprophyte was similar to that of the obligate parasites.

Recently Mount and Ellingboe (1969) demonstrated the transfer of P^{32} and S^{35} from host to parasite during primary infection by E. graminis. Plants were fed the isotope for 2 or 4 hr. at various times after inoculation. Five separate stages of transfer were noted. Twelve to 16 hr. after inoculation there was a measurable, slightly increasing amount of transfer from host to parasite. The rate of transfer increased very sharply after the 16th hr. This was approximatley the time (18 hr. after inoculation) that the haustorial bodies with nuclei attained full size, appendages began to form on the haustoria, and the haustorial sheath was first evident with light microscopy, but before the initiation of secondary hyphae. This suggested that transfer from epidermal cell to surface hyphae might be other than passive movement to a swelling appressorium.

The ability of the pathogenic fungi to invade hosts may be attributed to the appressorium which is a special organ differentiated from germ tube or hypha.

The mode of appressorium formation differs in different pathogenic fungi. Akai and Ishida (1967) reported that the conidia of Helminthosporium oryzae form appressoria after the elongation of germ tubes, whereas about 90% of the conidia of Colletotrichum lagenarum produced appressoria immediately after germination and about 10% of the conidia formed long germ tubes which sometimes did not form appressoria when gfown on a glass slide. Yarwood (1957) reported that in the parasitism of powdery mildew, the germ tubes commonly form appressoria prior to host penetration. It appeared that these structures were necessary antecedents for host penetration, and the initiation of host invasion. However, little attention was paid by many researchers to their organization and ultrastructure and their role in host penetration.

Pathogenic fungi invade host plants by the direct penetration through the cuticle and walls of the epidermal cells or by intrusion through natural openings such as stomata, lenticels or wounds. Whether the former method or penetration is accomplished by mechanical or chemical means or both has been discussed for a long time. The process of the infection has not yet been completely analysed. In the case of powdery mildew the causal fungus can penetrate directly through the epidermal cell wall.

McKeen et al. (1969) have pointed out that in the

past many plant pathologists speculated and believed that the mode of entry through the host wall was mechanical. However, with regard to this mode of penetration of host cells by plant pathogenic fungi, as stated by Mitchell (1967), opinions varied from total enzymic dissolution of both cuticle and cellulose wall (Caporali, L960; Woodward, 1927) to its being totally mechanical (Peries, 1962). Between these 2 extremes were those who believed that penetration of the cuticle was mechanical whereas penetration of the cellulose layer was both chemical and mechanical (Blackman, 1916; Corner, 1935; McKeen et al. 1966; Mitchell, 1967).

Woodward (1927) working with Podosphaera leucotricha powdery mildew reported that chemical dyes showed an enlargement of the passage through the cuticle. His objection to mechanical penetration was that some means of attachment of the fungal hyphae to the host epidermis would be necessary to produce the relatively great force required for a purely mechanical penetration. He could not find evidence of such attachment in the mildew. However Mitchell (1967) in his electron microscopy studies found no evidence for chemical lysis of the cuticle in 3 species of powdery mildews, namely, S. macularis, E. polygoni, and E. cichoracearum. He observed that the passage made through the cuticle of strawberry, clover and

sunflower by the penetration peg was always the same diameter as the peg. No difference in electron density of the cuticle was observed at the point of penetration and elsewhere. Thus he believed that penetration of the cuticle was purely mechanical. Besides he observed a mucilaginous coat that surrounded the hyphae which he thought would aid the firm attachment of the fungus to the host cell during penetration.

Others who favored the mechanical theory such as Brown *et al.*(1927) showed that the extracted juice of parasitic fungi did not dissolve the cuticle of their hosts. Wood (1960) reported that no one had demonstrated that plant pathogens were able to degrade the cuticle chemically. Nour (1958) showed the effectiveness of the attachment by mucilage in preventing conidia from being washed off their hosts by rain or direct watering. Blackman and Welsford (1916) also claimed that the mucilaginous coating held the spores of *Botrytis cinerea* to the leaf even before penetration. Besides, Brown and Harvey (1927) while experimenting with artificial membranes, demonstrated that fungal hyphae could penetrate relatively hard substances by mechanical pressure. Apparently the pressures for penetration were achieved by anchoring at the basal end

and by the close attachment of the hypha to the leaf and by extension in length. Flentje (1957) showed that mechanical penetration of host plants by Pellicularia filamentosa was preceded by a firm attachment of the fungus to the host by a mucilaginous sheath and that failure to achieve this attachment on the host resulted in failure to infect the host. Therefore the mucilaginous sheath of the fungus provided the necessary attachment of the fungus to its host, so that adequate pressure could be developed for puncturing the cuticle of the host. Smith (1900) in his study of Erysiphe communis on geranium leaves observed in the stained section, the cell wall around the point of penetration was more or less altered and dissolved, indicating a chemical penetration. There was an area surrounding the point of penetration which was entirely colorless, clear, and shiny. The remaining portions of the epidermal wall stained with safranin. The outer surface of the colorless area was usually depressed also, the depression being deepest at the point of penetration, as though a part of the cellulose had been dissolved away. Smith thought that an enzyme was probably produced by Erysipheae for the dissolution of the cellulose. The first sign of the beginning of penetration was a deep staining of the inner surface of the outer wall of the

cell immediately under the point where the hypha came in contact with the epidermis. The next step observed in penetration was the thickening of the epidermal wall toward the interior over an area coinciding roughly with the clear space mentioned above. The collar of the haustorium was dense and remained as a permanent structure in the cell. Smith observed that when the minute penetrating tube grew, its distal end entered the wall and a thickening of the wall kept pace for a time with the growth of the tube.

The tube continued its growth through the increasing or swelling cellulose, a part of which remained permanently encircling the neck of the mature haustorium as the collar. Apparently there was a stimulus, chemical or mechanical which excited the cell protoplasm to unusual activity in the production of cellulose over the region of penetration. The thickening of the wall ceased after a considerable U-shaped ingrowth had been formed. The slender penetrating tube was seen piercing the basal portion and extending into the disintegrating distal extremity. The gradual disintegration of this ingrowth of cellulose formed the first material from which the true structure of the haustorial sheath was made. The end of the penetrating tube enlarged into the body of the mature haustorium. Smith (1900)

reported that the ability to disintegrate cellulose was probably generally possessed by fungi, parasitic and saprophytic. The mature organ showed no unmodified host-cellulose surrounding it except the collar around the neck of the haustorium. Smith observed that the plasma membrane was not ruptured by the ingrowth of cellulose.

Corner (1935) in the investigation of E. graminis also observed a change in the infection zone. He attributed this to a cytase enzyme which diffused from the penetration apparatus and altered the cellulose wall.

Other workers such as Smith and Blair (1950), Lupton (1956), Dekker and Van Der Hoek-Scheuer (1964), and Akai and Ishida (1967) tended to support the chemical theory. They reported that the halos observed on the epidermal cell wall of powdery mildew leaves were the results of degradation of cell wall constituents. McKeen et al. (1966) also had suggested that changes in the epidermal host wall might be due to the enzyme(s) produced by the infection peg. Mitchell (1967) believed that in the penetration of the cellulose wall, chemical dissolution was likely involved. He observed a difference in the chemical nature of the wall surrounding the peg. An intense electron density of the wall immediately surrounding the penetration peg was observed in contrast

to the rest of the wall which was of low electron density. The production of additional wall material by the host to form a collar around the penetration peg was considered also suggestive of chemical reactions. The fact that the collar was produced in advance of the penetrating peg indicated the diffusion of a stimulating substance which induced the host to react in this way. Mitchell also believed that the penetration of the cellulose layer was partially mechanical, due to the fact that the peg often took a tortuous path while penetrating the host wall.

Recently McKeen and Bhattacharya (1969) in their histochemical and electron microscope study reported chemical alteration as well as the morphological changes of the epidermal wall by a variety of powdery mildews. Their results corroborated the findings of Smith (1900) and Corner (1935). They observed specific staining or altered zones around infection pegs below the appressorium and above the haustorium in the leaf strips of barley, clover, strawberry and sunflower infected by different mildews.

Lupton (1956) who worked with E. graminis on barley believed that the presence of halos about infection pegs depended upon environmental conditions and the susceptibility of the host. He found that blue halos occurred, after strippings were stained with cotton blue, in resistant barley varieties and in

susceptible varieties which were kept under conditions unfavorable to infection. However McKeen and Bhattacharya (1969), after staining, found that all infected resistant and susceptible hosts, regardless of environmental conditions, had halos with the same range of sizes (up to 30μ in diameter) around the infection pegs. Lupton believed that halo production was the manifestation of a "bruising" of the epidermis below the point of penetration and that it might be caused by the diffusion of an enzyme produced by either host or parasite, or might be a purely mechanical affect of the physical action of the fungus as it attempted to penetrate the epidermal cell wall. McKeen and Bhattacharya doubted the mechanical action as suggested, because if it were so, the mechanical bruising should have occurred in all resistant and susceptible hosts regardless of environmental conditions. Wood (1967) suggested a need for further study of the question of whether cell wall degrading enzymes function in diseases caused by obligate parasites and facultative saprophytes such as the smuts. Although Van Sumere et al. (1957) had reported the presence of polygalacturonase, cellulase and a hemicellulase in uredospores of Puccinia graminis var. Tritici Wood thought that there was a possibility the enzymes might have originated in contaminating bacteria. Goodman et al. (1967) thought the penetration through

the cell wall of the host plant by a pathogen might require the participation of cutinases, pectinases, pectin-methylesterase, cellulases, peptidases, and proteases. Recently Akai and Ishida (1967) in their histochemical study on the infection of barley leaf by Erysiphe graminis observed chemical modifications in the cellulose walls around the penetration tubes. This modified cellulose layer corresponded to the halo of the wall. They therefore suggested that the halo could be attributed to the degeneration of the cellulose layer by the fungal enzyme, probably cellulase. Their electron micrographs showed that the cell wall around the penetration point was electron dense which they thought indicated an enzymic degeneration of the host cell wall. Akai and Ishida (1967) also reported a crack across the center of the halo and they thought that the penetration of the cell wall might be in part mechanical rather than enzymic, alone. However, McKeen and Bhattacharya (1969) did not observe any crack in the halo zone when viewed with either the light or electron microscope. Because they observed that the altered area was proportionately more extensive in the lateral wall than in the epidermal wall, they thought this would indicate that a chemical diffusion rather than a mechanical stress was the cause of the halo.

Bracker (1968), in his investigation of powdery mildew of barley mentioned that his electron micrographs indicated that the epidermal wall was not altered. However as pointed out by McKeen and Bhattacharya (1969), it seemed there was some contradiction in his own observations and a few of his micrographs indicated that some alteration had occurred in the host wall.

By using cytochemical tests McKeen and Bhattacharya (1969) found that cellulose was lacking in the halo or disc-shaped zone with the peg as the central point. The amount of polysaccharide was reduced around the penetration tube. After differential extraction of polysaccharides the PAS-negative halo was always surrounded by a PAS-positive band even after pectin, hemicellulose, and noncellulosic polysaccharides had been removed. Their electron micrographs showed that the morphology of the epidermal wall was altered and electron density was increased in the disc-shaped zone. They sometimes observed a swelling of the host epidermal wall around the penetration peg and a change in the ultra-structure of the cell wall. The area was more wavy and the microfibrils were more prominent. McKeen and Bhattacharya (1969) thought that the evidences obtained indicated that the cellulose wall in the zone around the infection

peg was degraded by enzyme(s), such as cellulase, produced by powdery mildews. The cellulase might be secreted from the infection peg. Because the microfibrils remained, they believed perhaps only the amorphous or crystalline cellulose was affected.

It was known that most microbial pathogens were capable of secreting polysaccharide degrading enzymes into their environment (Wood,1967). This seemed to be a wide spread occurrence and was considered to play an important role for the pathogens in the infective processes. English and Albersheim (1969) in their study of host-pathogen interaction of Collectotrichum lindemuthianum on beans obtained a correlation between α-galactosidase production and virulence. Their results suggested that α-galactosidase, which was secreted by the fungus and was capable of removing galactose from the cell walls of the bean, had a crucial role in the establishment of a successful infection. They believed that other enzymes which were not yet identified might be equally important in this connection. Calonge et al. (1969) in their study of facultative parasitism stated that it has long been recognized that the observed effects of fungal infection of plants are a reflection of changes brought about in the fine structure of the host tissues. These structural

changes are in turn due to physico-chemical processes in which enzyme-catalysed reactions are often important. They noted the presence of extracellular fungal enzymes in the infected tissue in the following host-parasite relationships: Sclerotinia fructigena on apple and bean caused localized degradation of the wall region, S. sclerotiorum on cucumber caused extensive wall degradation, and infection by Phytophthora palmivora was characterized by disintegration of plasmalemma and tonoplast. These effects appeared to be correlated respectively with pectolytic enzyme activity in vivo with S. fructigena, cellulolytic activity with S. sclerotiorum, and the in vitro lysis of membrane by culture filtrates of P. palmivora.

Chou (1970) in his electron microscope study of host penetration by Peronospora parasitica found no electron microscopic evidence either of cuticle erosion in the immediate vicinity of the appressorium or of wall degration around the penetrating hypha. Thus he believed that penetration of the epidermis was solely mechanical, but he did not rule out the possibility of chemical agents aiding penetration. According to him, the fungus may need only a limited amount of wall degradation at a very early stage of penetration. And indeed he found that some initial wall degradation was involved during haustorial formation. He suggested that the host wall was degraded

only enough to allow the incipient haustorium to pass through the hole which was subsequently sealed off by the expanding haustorium, leaving no obvious sign of wall degradation.It seemed to him that further attention or research was required in order to obtain evidences of the ultrastructural changes of the host wall during infection especially in the early penetration stages.

Recently Edwards and Allen (1970) who conducted a fine structure study of the penetration apparatus of powdery mildew, Erysiphe graminis, reported that 2 major mechanisms had been suggested to explain the penetration process of pathogenic fungi into healthy host cells. Edwards and Allen (1970) attempted to make a systematic study of the initial penetration process in powdery mildew infection of barley with the aid of the electron microscope. They showed that the initial penetration in the mildew host-parasite combination was a two-stage process. The first stage was an enzymatic digestion of the cuticle and cellulose portion of the epidermal wall by enzymes apparently secreted by the developing mildew infection peg. The second was a mechanical pushing of the infection peg through a layer of material (papilla) formed on the underside of the host cell wall in response to the formation

of the appressorium or prior to the formation of the infection peg. Although the function of the papilla was not known, it was thought that it might function in the formation of a mechanical barrier through which the infection peg must penetrate to gain access to the cell lumen (Temmink and Campbell, 1969). The tissue, Edwards and Allen (1970) used for examination of the primary penetration process was obtained from primary leaves inoculated 48-72 hr previously. However, the primary infection of powdery mildews have been observed to occur as early as 9-12 hr after inoculation (Leong et al., 1970b; Stavely et al., 1969; Hirata, 1967; Masri and Ellingboe, (1966 a, b); Mount and Ellingboe 1968; Ellingboe, 1968). Hirata (1967) in his study of the barley mildew fungus Erysiphe graminis, reported that the primary haustorium began to develop 10 hr after inoculation, irrespective of the time of inoculation, whereas the secondary and subsequent haustoria began to develop at about midnight (mostly from 10 p.m. to 2 a.m.) of the following days. Ellingboe (1968) reported that the penetration of host epidermal cells was completed by the 14 hr after inoculation in primary infection by E. graminis. The body of the haustorium attains full dimensions, and appendages attain full size by about the 35th hr. The secondary appressoria began to form at about

30 hr after inoculation. Ellingboe believed the necessary criteria for parasitism are satisfied somewhere between 8 and 27 hr after inoculation. It seems that this is the critical period for a systematic and sequential study of the primary penetration process.

Sempio (1950) reported that among the many resistance mechanisms exerted by plants against their parasites, there were some of a strictly metabolic type. Fundamentally, every resistant mechanism was linked, more or less indirectly, to a specific type of metabolism.

Kaul and Shaw (1960) reported that the rapid increase in the respiration of the wheat leaf that followed rust infection was believed to be partly a response of the host to the presence of the parasite. This might be due to increased synthetic activity at the infection sites (Shaw, 1957). Respiration would be increased if infection with rust resulted in the formation of a catalyst which participated in the chain of hydrogen transport and controlled and accelerated the speed of the over-all transfer of hydrogen to molecular oxygen.

Kaul and Shaw (1960) in their study of oxidation reduction changes in wheat-leaf sap caused by rust infection found a reversible rise in potential

during the course of rust infection on Little Club, a susceptible species; but with Khapli, a resistant species the rise was similar but was not reversed. After infection the oxidation reduction balance of susceptible tissue was reorganized by the introduction of new systems which were found in the reduced state, only. After infection the oxidation-reducation potential of resistant tissue was only poorly poised as indicated by a rise in the average potentials and as demonstrated by oxidation-reduction titrations. From the results, they speculated that a parasite-induced catalyst might play a role in the acceleration of respiration at infections of obligate parasites or 'short circuit' the chain of hydrogen transport, thus initiating all the observed redox changes.

Benada (1968b) found that loose smuts infected particular tissues only and the disease was dependent to a great extent on the environment. His results showed that the loose smut did not tolerate the redox potential that occurred in kernels or in cereal leaves in the field. The main problem in this field of research results from the difficulty of measuring the redox potential. Several methods have been used. One group of methods involves redox dyes which have the advantage of the

disturbing the tissue very little. The disadvantages are that they alter the redox properties in living cells, partly by their own electron capacity and catalytic action and partly by their toxic effect on metabolism. The other method requires the use of potentiometric techniques. This has a disadvantage of bringing the electrode into contact with cell content. Any traces of air may cause serious oxidative changes in the redox potentials of the cells.

Benada (1968a) in his study of redox potential and the change of varieties in relation to the changes in environment, mentioned that 2 sorts of resistance were distinguished in obligate parasites on cereals. One was the resistance to physiologic races, and the other was the change of resistance in accordance with the ontogeny of plants and/or the environmental conditions. In experiments with seedlings, Benada found that distinct limits of redox potential in host tissues were needed for successful infection in barley and wheat powdery mildew. The symptoms appeared on the host only after the incubation period had passed, but the success of infection was determined by the state of metabolism at the beginning of infection. Another factor complicating the determination of the limits, as pointed out by Benada, was the ability of the para-

site to regulate the redox condition in the host in infected tissues. Therefore Benada stressed that in breeding for resistance it was necessary to distinguish between these 2 sorts of resistance as their genetic base seemed to be different. A particular redox-potential range may exist for a compatible mildew-clover relationship.

Edwards (1970) in his histochemical study of the penetration process found a substance which stains with basic protein stains (Bromophenol blue) and occurs in papillae of susceptible and resistant barley infected with powdery mildew. It was first detectable after the infection peg penetrated the host cell wall. In susceptible host cells this substance was confined to the papilla, whereas in resistant host cells this substance spread throughout the penetrated cell and to adjacent mesophyll cells beneath the infected cell. It was thought that the spread of this substance correlated with the expression of resistanee in the host cells and its effect might be on the development of the haustorium and not the penetration process, because Edwards doubted the infection peg was able to push through the papilla in the resistant reaction. The chemical nature of the Bromophenol blue-positive material was not known. Edwards thought it might be

the basic protein which stained, and that other non-protein compounds containing basic groups could also bind with these stains. One such compound isolated from barley by Stoessl (1967) was known to possess antifungal properties.

Allen (1954) stated that infection with both obligate and facultative plant parasites was followed by marked increases in the rate of respiration of the host tissues and a decrease in photosynthesis within a few days after infection. With rusts and powdery mildews, increased respiration and decreased photosynthesis were accompanied by the accumulation of various substances (Allen, 1942).

With regard to the mechanism of host resistance, several workers have shown that excessively thick cuticle layers may prevent penetration by fungal parasites (Jhooty, 1963), (Melander, 1927), (Peries, 1962). Jhooty attributed the greater resistance of Fragaria chiloensis to infection by S. macularis to thickness of its cuticle, as compared with that of the more susceptible F. ovalis. Mitchell (1969) believed that the thickness of the cellulose wall itself was of even greater importance. A thick cell wall would be a serious barrier to infection, because it might delay penetration long enough for the germ tube to die before the peg

entered the cell. Those fungi having the conidia which could not make use of external sources of food or water at germination would be dependent upon early establishment within the host.

In the past, very little information of the nature of host resistance was available at the ultrastructural level. This was due to the smallness of the infection pegs and their relatively great distance apart. Consequently there was extreme difficulty in obtaining thin sections of attempted infections on the resistance hosts. As pointed out by Day (1966) in his study of the genetics of the host-parasite system only 2 studies, Shaw and Manocha (1965), Ehrlich and Ehrlich (1962), have so far included inoculation of a resistant host of obligate parasites for comparison. Shaw and Manocha (1965) found a more rapid development of the encapsulation around the stem-rust haustoria in leaves of the resistant variety, Khapli, than in the susceptible variety, Little Club. The breakdown of the plasma membrane and other subcellular structures of the invaded host cells took place in 6 or 7 days in Khapli, while taking 15-20 days in Little Club. Many of the haustoria on Khapli quickly became necrotic even though surrounded by an encapsulation, while the few haustoria found on Little Club became

necrotic. When uninfected cells of both varieties were compared, lomasomes were found in Khapli but not in Little Club (Manocha and Shaw, 1964). Whether or not the lomasomes in the resistant variety Khapli played any part in resistance was not known. Ehrlich and Ehrlich,(1962) reported that haustoria of stem rust in susceptible wheat varieties had more mitochondria and endoplasmic reticulum than haustoria in resistant varieties. Hilu (1965) in his light microscope observations of Puccinia sorghi on corn, also described premature death and encapsulation of haustoria and mycelial lysis in resistant reactions. An increase in the size of the host nuclei in both resistant and susceptible reactions was noted also.

Although some light microscopy studies have been made on the development of Erysiphe species (Smith, 1938 ; Stavely and Hanson, 1966a, b, c), little was known about the fine structures of host or parasite in resistant reactions. Despite the increased use of the electron microscope, most of the fine structure studies of the Erysiphe species and infected host cells were done on the susceptible hosts (Bracker, 1965; Ehrlich and Ehrlich, 1966; McKeen et al., 1966).

However, recently McKeen and Bhattacharya

(1970) made a study on the infection of a susceptible and resistant barley to Erysiphe graminis f. sp. hordei with the aid of the light and electron microscope. Information was obtained on ultrastructure and on the degree and type of resistance displayed by the resistant barley. Their electron micrographs revealed that in the infections attempted in the resistant line, the penetration peg passed through the barley epidermal wall but grew little further into an aggregation of the host cytoplasm and (or) reaction products which occurred also in the cell of the susceptible coleoptile. They believed that the interaction between the host and fungus must have been very rapid because the infection peg had developed to the same extent as one in a susceptible host about 11 hr after inoculation. Two types of resistance were found, one which was effective during or immediately after host wall penetration, and another which was effective 2-4 days later.

According to McKeen and Bhattacharya (1970), either mechanical resistance or an incompatible reaction at the penetration site could prevent development of the infection peg. They believed that mechanical resistance was not the cause of the death of the peg because they observed that the infection

peg of the 73% of the conidia which germinated but never formed a haustorium passed through the wall in all the thin sections. This view was supported by the result that enzymes were secreted by the pathogen at the earliest stages of infection (Akai et al.,1968a), (McKeen and Bhattacharya, 1969). McKeen and Bhattacharya (1970) stated that even if some infection pegs did not pass completely through the host wall it did not necessarily indicate that resistance was mechanical. Ellingboe (1968) stated that the development of the pathogen might be arrested upon contact of the parasite with the plasma membranes of the host epidermal cell and there would be a very rapid reaction of adaptive or constitutive genes to prevent the formation of small, partially developed haustoria. McKeen and Bhattacharya (1970) suggested that interaction began as soon as the enzymes which altered the wall at the point of penetration, reach the plasmalemma and that the pathogen might not be arrested until 2-4 hr after the initial interaction.

Some infection pegs did form haustoria which resulted in the formation of secondary growth. However these young colonies died after 3 or 4 days. It was believed that not only primary but also secondary haustoria had a functional host-parasite relationship before growth of the fungus was inhibited. The finding of Bushnell et al. (1967) that secondary

haustoria were never found after the primary haustorium was removed tended to support the belief that there was a degree of compatibility established with the host before death. McKeen and Bhattacharya (1970) thought that the fact that the whole colony ceased growth at the same time indicated that the whole colony was affected at the same time and that there might be only one mechanism for resistance. If the appressorial and germ tube cytoplasm escaped into the host cell and formed a young haustorium which occurred in a very short time, 2-4 days were required before activity of the large amount of cytoplasm in the parasite could be inhibited.

Recently Stavely et al. (1969) found that the resistant reaction was due to lack of response of the host cell and that no nutritional relationship was developed by the fungus which infected the resistant host. Their electron micrographs revealed no differences in penetration of resistant and susceptible clones of Trifolium pratense by E. polygoni. Resistant and susceptible reactions could be differentiated soon after the fungus had penetrated the host cell wall and passed through the collar. In resistant host cells, the plasmalemma and nuclear membranes were rapidly destroyed. Chloroplast membranes were destroyed and the grana dissociated from their normal

stacked arrangement. Mitochondria and most of the haustorial sheath remained intact in resistant cells at the latest stage observed after penetration. Haustoria in resistant cells were smaller, less branched, and had less well-developed endoplasmic reticulum than in susceptible host cells. The sheath membrane in resistant cells never developed the vesicular invaginations that developed in sheath membranes in susceptible cells. However their comparison was made between the young or immature haustoria, 20 hr after inoculation and the mature or old haustoria which were present at 120 — 144 hr after inoculation. Apparently a sequential or developmental study was lacking. It will be interesting to ascertain whether there is any difference between the susceptible and resistant haustoria at the same age or at a specified early stage after penetration.

In recent years, electron microscope techniques have been available, but because of limited use of these, the study of the above mentioned problems is still incomplete. Despite the recent advances of the electron microscope which have led to a large number of publications on the fine structure of fungi, as reported by Hawker (1965) and Bracker (1968), there are relatively few works which have been done on the host-fungus interactions, especially those on the

obligate parasites, such as the powdery mildews. Only a limited amount of work has been done on the ultrastructure of the conidia, the early initiation of penetration and the susceptible and resistant reactions.

Bracker (1967) in his review of ultrastructure of fungi pointed out that most of the material used for the study of host-parasite interactions were one or more weeks old. A lack of developmental analysis was evident. Bracker (1968) also stated that many structural features such as the appearance of the sheath, neck and the cytoplasmic structure of the haustoria might be related to age of haustoria and that a correlation of the structure of the haustorial apparatus with haustorial age was desired in order to obtain an accurate analysis of the haustorial apparatus and the host-parasite interactions. However in an early developmental study, difficulty arises due to the low density of infections and haustoria in the very young colonies. Also a time lapse study between inoculation and sampling does not necessarily indicate the age of any given haustoria in a colony. It seems that a refined technique is required for the approach of this problem. An effort towards this was made in the present study. It was hoped a better under-

standing of the mechanism of penetration and the haustorial structures would be obtained, and that any finding or additional information that might be obtained from the present study would help to explain some peculiar characteristics of this group of fungi and would help to clarify, if not solve, some of the difficult problems mentioned above.

Erysiphe polygoni, one of the well known powdery mildews, was chosen as the fungus pathogen and the resistant and susceptible red clover plants were chosen as the host for the present study. It was hoped that light and electron microscopy along with histochemical studies would prove to be valuable in elucidating controversial problems in ultrastructure, resistance, infection and host-parasite relations.

MATERIALS

For the present investigation Erysiphe polygoni DC powdery mildew was the fungus pathogen used and the red clover Trifolium pratense[1] Linnaeus var. Ontario Double Cut was the susceptible host.

T. pratense var. Lakeland[2], isogenic line 138 obtained from a cross between Lakeland variety type clone 26 (Rr) and clone 27 (RR) was the resistant variety.

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1. Seeds obtained from W.A. Jenkins MFG. Co. Ltd. London, Ontario.

2. Seeds were obtained from Mr. Jon Watterson, Research Assistant, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53706. The Lakeland is a variety bred by Dr. E.W. Hanson at Wisconsin and is known to be resistant to all the races of Erysiphe polygoni found in the state of Wisconsin.

EXPERIMENTAL METHODS

I. Growth and maintenance

The clover plants were grown from seeds in the greenhouse. The temperature ranged from a minimum of 60°F to a maximum of 75°F during the winter. However during the summer the temperature fluctuated with the outside atmospheric temperature and varied between 60° 90°F in the day time and between 60° - 70°F at night.

After growing for about 1-2 weeks in soil bed boxes, the young clover plants were transferred to 6" x 6" clay pots. The soil, used in the pots and beds consisted of loams : peat moss : black muck : sand in the following proportions 6:3:2:2. The plants were fertilized once a week with a 28:14:14: NPK fertilizer mixture and 20:20:20 mixture in the winter months. The main parasites prevalent on the leaves of the greenhouse clover plants were mites, aphids and mealy bugs, and they were more prevalent in the summer months. They were kept under control by spraying the plants regularly with Tedeon Chlorabenzilate 4E and/or Malathion + Lindane. The Malathion mixture was useful for the control of mealy bugs. All these chemicals were also effective in killing the mildews as well. It took about 2-3 weeks for the reappearance of the fungus which usually occurred on the freshly formed leaves.

II. Collection of conidia

Conidia were collected from the leaf surfaces which usually had been infected 2-4 weeks. To assure that only fresh and viable conidia were being collected, the old or existing conidia were shaken off the infected leaf the night before collection. The following methods were employed for the collection of conidia, each one served its specific purpose.

(a) Dry glass method:

The infected leaf was pressed directly on to the slide or cover-slip surface. This method was useful for most purposes. For fresh spore observation or fixation, the spores were shaken off the leaves onto a clean glass slide.

(b) Coating glass method:

The surface of the glass slide or clover slip was coated with a thin layer of viscous or adhesive material.

1. An egg albumin solution.

When dried it was a good holding material for the conidia, especially when fixation was required before staining. It held not only the conidia but also the mycelium firmly while fixation, washing and staining procedures were carried out.

2. Sucrose coating.

A 2-4% sucrose solution was used. The solution was wetted on the surface of the glass slide. When dried, the adhesive coating was used for collection and germination of conidia.

3. Agar coating.

This coating was made up of 2-4% agar. It was used for the collection of conidial chains and for spore germination.

(c) Cello-tape methods (Butler and Mann, 1959)

This was employed for removing the superficial growth of the whole colony from the leaf surface, especially the conidiophore and branched mycelium. This method was used before or after fixation but unfortunately the tape wrinkled when it was wet.

III. Germination of conidia

The germination of conidia was carried out either on a slide, the surface of distilled water, sugar solution (2-6% sucrose solution), or on the suface of an agar (2%) medium in a Petri dish.

When the dry glass slide and cover slip were employed they were raised above the water in the Petri dishes which were covered and placed in an incubator at 20°C. A period

of 4-6 hr was adequate for the production of germ tubes 2-4 times the length of the conidia.

IV. Method of inoculation

Inoculations were made on the lower side of the leaf. At first conidia were collected with a soft camel's hair brush and then dusted evenly on to the surface of the leaf. For best infection and a high rate of germination the inoculation was carried out in the afternoon usually at 4 pm. After inoculation, the plant was covered with a plastic bag for a few hr or overnight to create a saturated atmosphere which allowed a higher rate of infection.

V. Light microscopy method

For light microscopic observations, the conidia and mycelium were collected by the method described above. For the observation of the intact fungus with the host, infection and development processes, the following methods were applied.

(1) Lactophenol cotton blue clearing and staining method. (Shipton and Brown, 1962). The infected leaves were cut into small pieces about 1/2 - 3/4 in. in length and immediately immersed in 10-15 ml of alcoholic lactophenol cotton blue 1 pt. lactophenol cotton blue to 2 pts of 95% alcohol. Lactophenol cotton blue was prepared as follows:
phenol 10 g, glycerine 10 ml, lactic acid 10 ml,

aniline blue 0.02g, distilled water 10 ml. The solution containing the leaf sections was brought to boiling and the leaf sections submerged for 1 min. After the leaves sank, the solution was brought to boiling again for 1/2 min. Leaves were allowed to remain in the stain for about 2-48 hr at room temperature and then they were removed, rinsed in water, and placed in chloral hydrate for 15-50 min and then mounted on a microscope slide in 50% glycerine. This method was useful for light and phase microscope observations especially for the cytological host-pathogen studies.

(2) Epidermis peeling method:

With the aid of a fine forceps (No. 4), the lower epidermis was carefully stripped from the branch vein out to the edge of the leaf so that one layer, or rarely 2 layers of cells were obtained. The strips were then immediately floated on water or fixed in a formalin-acetic acid-alcohol (50% ethyl alcohol) solution (5:5:90) or stained with different dyes as required. This peeling technique proved to be very useful in diagnostic work in the study of host-pathogen relationships, colony development, haustorial development and in locating the

infection sites. This technique was used in the histochemical studies after treatment of Azure B and other stains and in the Benedict's Test. It also was applied in the electron microscopic study.

(3) Microchemical procedures:

Strips of epidermis were removed from the lower surface of susceptible and resistant clover leaves at selected times after inoculation. Some strips were fixed in formalin-acetic acid-alcohol (5:5:90) were stained in Azure B 0.25 mg per ml of solution, in a citrate buffer at pH 4 for 1-12 hr (Jensen, 1962). Other strips, some fresh, some fixed in FAA were boiled for 2-5 min in Benedict's quantitative solution (Hawk, 1953). The strips were then observed under the light microscope. The powdery mildew fungus which infected sunflower and barley epidermal cells were treated in a similar way.

VI. Electron microscopy

(A) Fixation procedures.

(1) Conidia.

Because conidia will not wet and sink in water, a prewarmed 2% agar solution from a micro-pipette was dropped on them after they had been placed on a slide. The solidified agar

was then cut into small cubes and fixed immediately in the fixatives.

Sometimes conidia were collected in the glass homogenizer filled with distilled water, and they were plunged down slowly a few times with a glass rod. This did not break or damage the conidia, but helped a great deal in temporarily submerging the conidia. They were then centrifuged at low speed (2-5000 g) into pellets.

(2) Leaf:

Healthy and infected portions of whole leaves, were cut into (1 x 1 mm) squares, or punched with a hypodermic needle (1 mm in diameter), to provide leaf disks. They were put immediately in fixatives.

(B) Fixation.

(1) Potassium permanganate.

The material was fixed in 2-4% $KMnO_4$ solution, unbuffered at room temperature for 1/2-2 hr.

(2) Osmium tetroxide.

The material was fixed in 1-2% osmium tetroxide phosphate buffered sucrose solution at pH 7.2 for 2-4 hr at $4^{o}C$. A 0.5%

sucrose concentration was used.

(3) Glutaraldehyde-osmium tetroxide.

Material was fixed in a 3-5% glutaraldyhyde 0.1 molar phosphate buffered solution pH 7.2 for 3-6 hr at $4^{o}C$. It was then washed in buffer solution with 3 changes for 3-6 hr or overnight. Before post-fixation, the epidermal strips were flattened on a glass slide prior to selection of early infection sites. A 2% prewarmed agar solution was dropped on each of the strips in order to spread and hold the strip flat and to hold the fungus in place on the host cell and to prevent the appressorium from separating from the host cell during the dehydration procedures. The embedded agar strips were then examined under the light microscope and cut with a scalpel into small pieces (1mm cubes). The uninfected ones were eliminated. For post-fixation, materials were fixed in 1% $0s0_4$ in 0.5% sucrose solution, unbuffered, for 2-6 hr or overnight, then washed in distilled water. Healthy tissues were treated similarly except all pieces were saved.

(C) Dehydration

Materials were passed through the ethanol alcohol series in ascending gradients from 25% to 50%, 75%, 95% and absolute alcohol, with 15 min for each and 2 changes for the absolute alcohol.

(D) Infiltration

After clearing with the propylene oxide, with 3 changes, and 30 minutes for each change, specimens were then mixed with 1/3 epon plastic (6A:4B) Epon mixture A: Epon Resin 8.2 (62 ml) + DSA (Dodecenyl Succinic Anhydride)(100 ml). Epon mixture B: Epon Resin 8.2 (100 ml) + MNA (Methyl Nadic Anhydride)(89 ml), or Epon Araldite mixture (Stavely, 1966). To each plastic mixture, 1.5-2% of DMP 30 accelerator was added before mixing. The Epon-Araldite mixture, was kept in 60°C oven for about 3 min in order to warm it before the accelerator DMP 30 was added. It was mixed thoroughly for 5-10 min. with an electric blender fitted with a wooden stirrer. The well-mixed plastic was allowed to settle for a while and then added to the material in propylene oxide. This was then shaken in order to thoroughly mix it before leaving it overnight. The next day, 1/2 of the mixture was poured off

and replaced with another fresh batch of plastic and again mixed and kept for about 6 hr to overnight.

(E) Embedding

Before embedding, the material was transferred first to fresh pure plastic for 2-6 hr. It was then embedded in No. 00 gelatin capsules or a flat plastic holder in order that it might be properly orientated. It was kept in the oven at 60°C for polymerization for a period from 14-24 hr. The hardened blocks were then examined under the microscope and kept for future use. They were later trimmed with razor blades so that they could be sectioned. When necessary, the desired material was cut out of the block while it was on the stage of the light microscope and re-embedded on to another capsule block in order to obtain the proper orientation. Usually epidermal strips were embedded and orientated in such a way that the leaf was cut transversely, so that the chances of getting the appressorium, penetration peg and haustoria in one section were higher.

(F) Sectioning

After trimming, the blocks were cut with glass knives on a Porter-Blum ultra-microtome. The

knives were made either by a knife maker or by hand. The water troughs of the glass knives were formed by fixing a narrow Scotch-tape around the back of the glass knives. To avoid water leaking from the trough, the edge was sealed with dental wax applied with a camel's hair brush. Thin sections of 500 - 1000 Å thick (gold-silver-grey color) were cut and floated on glass distilled water or on a 5% acetone solution in a trough. The acetone was used for spreading the sections. The sections were then picked up by means of a pair of fine forceps (No. 5) on 200 mesh copper grids. The grids were dipped into the water and flipped up. The sections were kept in a Petri dish. Thicker sections were cut also and stained with toluidine blue and checked under the light microscope.

(G) Staining

The thin sections were stained with uranyl acetate (6% in 70% methanol) for 15-20 min at 45°C in a covered spot glass. They were then washed with 100% methanol, 50% methanol and glass distilled water respectively. Several drops of each solution was used. The sections were then dried on clean blotting paper and counter stained with a lead citrate solution

(Venable and Coggeshall, 1965) (0.035 gm in 10 ml of distilled water + 0.1 ml 10N NaOH) for 45-60 seconds. Some sections were stained with uranyl acetate others were stained with lead citrate and some were not stained.

For lead citrate staining, the clear lead citrate solution was dropped on to paraffin wax, which half filled a Petri dish. Sodium hydroxide pellets were placed in the bottom of a small Petri dish which sat in the larger Petri dish. The sodium hydroxide absorbed the moisture and carbon dioxide from the air inside the Petri dish, and thus minimized contamination due to carbonate precipitation. Each grid was floated face down on the surface of the drop of lead citrate solution and the Petri dish was then covered. After counter staining for 45 seconds the grid was picked up with a sharp forceps and washed quickly with 10-15 drops of glass distilled water. The grids were then dried and kept on a blotting paper in a Petri dish.

(H) Electron microscope examination

The stained sections were observed in the

Philips 200 Electron Microscope at 60 KV. The double condenser lenses were used. Photographs were taken either with 30 mm high contrast film or 3 1/4 x 4 in. plates at different magnifications as required.

(I) Photography and Magnification

Exposed films or plates were developed with Kodak D19 developer. Pictures were printed with D72 developer. Different grades of Kodabromide single weight printing papers (F2-F5) were used depending on the contrasts of the negatives. Normally F3 and F4 papers were used. The approximate magnification was calculated by using the calibration in the electron microscope and the magnification produced by the enlarger.

(J) Isolation and test of particulate glycogen

Particulate glycogen was isolated by a method similar to that of Foerster et al. (1965). Fresh spores free of mycelium, were collected in pellets according to the method described above with a homogenizer. All the solutions used were maintained at $0^{o}C$ and the preparative steps were carried out at $0^{o}C$ as well as the centrifugations which were carried out in a Sorvall superspeed

automatic refrigerated centrifuge (model RC-2B).

The harvested spores were broken in 100 ml of cold glass-distilled water in a glass homogenizer fitted on to a 'Caframo' stirrer type R2Ri-64 with an instrument rheostat setting at 8 for 10 min. The homogenate was rapidly adjusted to pH 5.2 by the addition of cold 0.1M potassium phthalate-sodium hydroxide buffer at pH 4.8, allowed to stand in the cold for 5 min. and then centrifuged at 5000 g for 5 min. The pellet was discarded and the supernatant was again centrifuged at 50,000 g for 30 min. The white opalescent supernatant was saved.

The chemical composition of the final white opalescent fraction was investigated by the use of the following:

(1) The iodine reaction test according to Hawk (1954).

(2) The alcoholic Periodic Acid - Schiff (PAS) Reaction for polysaccharides (Humason, 1962) was carried out on ethanol-fixed fungal cells.

(3) Bauer-Fuelgen Reaction. (Humason, 1962) to demonstrate the presence of glycogen.

(K) Electron micrographic method for identification of glycogen particles.

This method was used for the isolated fungal carbohydrate. The electron microscopic

preparations were prepared as follows: Pellets of spore were fixed in 2% osmium tetroxide, phosphate buffered 1.5% sucrose solution for 2 hr at 4°C, centrifuged and washed twice with buffer solution. They were then collected with 2% agar at 4°C, cut into 1 mm cubes and then dehydrated in ethanol and propylene oxide and embedded in Araldite according to Glauert et al. (Sjostrand, 1967). Post staining with lead citrate solution was optional, and is indicated in the description of figures.

The fungal-glycogen preparation was observed directly under electron microscope without staining, after the lead citrate staining or after negative staining with 2% Phosphotungstic acid (PTA) in an aqueous solution (Kay-Desmond, 1965) for 2-5 min. The carbohydrate preparation was mixed in the cold in equal portions with PTA solution. A small drop of the mixture was placed on a formvar coated grid and allowed to stand for 5 min, after which excess liquid was removed and the preparation on the grid was allowed to air dry. This preparation was carried out at 4°C.

(L) Enzyme treatment with α- Amylase.

An attempt was made to digest the particulate glycogen from the suspension on the formvar grid. The grid with the dried glycogen suspension was dipped in the α-amylase solution in a spot plate covered with a glass plate and placed in the incubator at 37°C for 2-5 hr. The α-amylase used was 250ug/ml in 0.003N $CaCl_2$ in a half saturated NaCl solution. Control tissues were incubated in the solution without the enzyme. The grids were rinsed several times in the distilled water, air dried, then stained with lead citrate solution for 2 min and observed in the electron microscope.

The fixed fungal spores or mycelium which were embedded in Araldite were sectioned and then the sections were stained in the lead citrate solution and examined in the electron microscope. Fixed material stained with uranyl acetate (0.5%) prior to embedding was used also.

(M) Lipid Identification.

The method used by Eurenius and Jarskar (1970) was used to demonstrate the lipids in Epon-embedded ultrathin sections. Lipids were

extracted in a solution of sodium methoxide dissolved in a methanol-benzene mixture, an effective solvent for epoxy resins and lipids. This was likely due to a combined effect on the osmium-lipid complex of the Na-methoxide reagent and the methanol-benzene mixture as the lipid solvent. In addition the acetone extraction method (McKeen, 1970) was also used.

OBSERVATION AND RESULTS

I Comparison of different fixation methods used in fixing the conidium.

Three basic fixation methods were used, namely:

(1) Double fixation method. The material was first pre-fixed with glutaraldehyde and post-fixed in osmium tetroxide. For the purpose of convenience this method is called the glutaraldehyde and osmium fixation method. The quality of this fixation is best represented in Figure 1. The cytoplasm was best preserved by this method.

(2) Osmium fixation method. The material was treated in an osmium tetroxide solution. Although some details were lost and the membraned structures frequently appeared irregular in outline, the clarity of the picture was good (Fig. 2).

(3) Permanganate fixation. The material was either fixed in potassium permanganate solution alone or post-fixed with permanganate after fixing with another fixative, such as glutaraldehyde. Figure 3 shows the results of permanganate fixation in which some of the cytoplasmic materials are lost. The ribosomal particles are destroyed and distorted membranes occur.

II The fungal ultrastructure.

(A) Mature conidia.

The general morphology and fine structure of mature non-germinated conidium were as follows: (Diag. III)

The conidium was generally spherical, oval or oblong in shape and varied in size from 7-14 x 15-25μ. When a mature conidium was released from the cross wall of the conidiophore, a papilla was left at the abscission region (Fig. 4). This papilla usually appeared at the tip of the conidium with a plug in the center. The conidial wall measured from 0.25-1μ in diameter and was irregular in outline. The wall appeared slightly irregular and spine-like projections were present on its surface (Fig. 5). Spines were characteristic of the mature conidia, but the surface of the wall of immature conidia was smooth (Figs. 69-73). There was a mucilaginous layer covering the wall (Figs 3,4 and 5) and this gelatinous matrix was about half the thickness of the wall. The conidial wall was divided into an outer and inner layer (Fig. 5), but at the tip of the conidium only a thin layer of inner wall was observed (Figs. 4,17, and 20). The outer wall appeared amorphous or granular in appearance and was more densely stained by osmium than the inner wall (Figs. 3 and 5).

Plasmalemma:

The plasmalemma, a unit membrane measuring about 75 -120Å in width was inside the wall

(Figs. 6,7,38 and 39). It appeared generally smooth, sometimes indented or wavy. The membrane appeared closely associated with the E.R. and mitochondria. Invaginations of the membrane was observed in certain areas of the cell (Figs. 6 and 7).

Lomasomes:

The Lomasomes (Fig. 9) were membrane bounded structures with vesicular contents outside the plasma membrane and inside the wall in the mature conidium.

Nucleus:

Inside a conidium only one nucleus was observed. The nucleus was approximately 3 x 5 μ in size, often appeared spherical and sometimes irregular in shape and was enclosed in a double membraned envelope which had pores (Figs. 2,3,18, 40 and 42). A nucleolus with denser granular material was observed in the nucleus (Figs.2 and 40).

Vacuoles:

These were the most prominent feature in a conidium. The number varied from a few to as many as 15 in one conidium (Figs. 1,3 and 17-20). They were frequently round in shape and sometimes irregular in outline and occupied about 40-80% of the cell area in a section (Figs. 17-20). These vacuoles contained a variety of contents which consisted of granular or fine netted granular substances (Figs. 1 and 18),

lipid bodies, (Figs. 20 and 23) or various membranous structures (Figs. 8,9,10,20-22 and 24). Packages of glycogen particles were also observed inside the vacuole and were membrane bounded (Fig. 27). Membranous structures with many concentric lamellae or myelinoid bodies were observed not only in the vacuoles but also in the cytoplasm of the conidia (Figs. 8 and 11).

Glycogen:

Glycogen particles were prominent and frequently observed in mature conidia in both the light and electron microscope preparations (Figs. 29 and 25-28). These particles were mainly in the form of a rosette (α particle) in which each rosette was formed from about 6-10 units of individual particles (Figs. 25 and 26). A dense aggregation of the rosettes into groups of glycogen particles were frequently observed (Figs. 3 and 25-28). The particles occupied ½ - 1/3 of the area of the conidium in many sections (Figs. 25,29 and 30). In some parts of sections, they were somewhat evenly distributed throughout the cytoplasm (Figs. 1,2 and 11). Each particle was 200 - 300Å in diameter (Fig. 26). The glycogen particles were descernible from the ribosomal particles in the non-germinated conidium (Figs. 1-3). Morphologically they were larger in size, and had a tendency to aggregate into rosettes or groups of rosettes. The glycogen particles were

more densely stained especially with lead citrate (Fig. 2) than the ribosomes. With potassium permanganate fixation, only glycogen particles were seen (Figs. 3 and 28), and the ribosomes and polyribosomes were not visible.

Identification of glycogen:

After fractionation the glycogen suspension was observed under the electron microscope. The particles appeared densely stained with lead (Fig. 34) or negatively stained with phosphotungstic acid (Fig. 35). They were identical to those observed in sections of the fungal material, in which the particles were either densely stained with lead citrate (Fig. 25 and 26) or negatively stained with uranyl acetate (Fig. 30). They were in the form of roughly isodiametric particles and about 150-400Å in size and slightly irregular in outline. They were 0.1-0.2μ in diameter. When the preparations of both the glycogen suspension (Fig. 36) and the spore sections (Figs. 31 and 33) were treated with α-amylase enzyme and then stained with lead, blank spots were observed instead of densely stained particles as had been observed in the suspension in Figure 34 and in the section in Figure 32.

Observations in the light microscope showed that the fractionated granules gave a brown, golden

yellow or golden brown color when treated with an iodine solution (Lugol's) as did a commercial preparation of glycogen from animal tissue. When the whole mature conidia were treated with an iodine solution, similar brown, golden yellow or golden brown materials appeared in the cytoplasm.

With the Bauer Fuelgen reaction and the periodic acid-Schiff treatments, patches of reddish spots were seen inside the conidia (Fig. 29). These spots represented the glycogen polysaccharides. The cells or conidiophore below the mature conidium was less intensely stained, while the mycelium was only faintly stained pink.

Ribosomes:

These particles were about 150-200Å in diameter. They were observed evenly distributed throughout the cytoplasm (Figs. 1 and 2) and were discernible from the larger glycogen particles as described above.

Mitochondria:

They were usually situated close to the periphery of the cell (Fig. 1) and in close association with the plasma membrane (Figs. 1, 12-16 and 37-39). The sizes varied from 0.5-1 x 2-6μ with sparse inward growing tube-like cristae (Figs. 1,2,38 and 39). Curved or dumb-bell shaped mitochondria were frequently seen (Figs. 40 and 41). Glycogen particles were observed

to occur within the mitochondria (Figs. 38 and 39).

Endoplasmic reticulum (E R)

Short and occasionally long smooth E.R. elements were observed scattered throughout the cytoplasm (Figs. 1-3,6,7, and 27-41). They were adjacent to the mitochondria and plasma membrane (Figs. 6,7, and 37-39). At the tip of the conidium, groups of long E R profiles were situated parallel to the plasma membrane (Fig. 4).

Lipid bodies:

These were osmiophilic bodies which darkened with osmium tetroxide and appeared densely stained in sections. Lipids were scattered mainly along the peripheral area of the spore and appeared to be in close association with the plasmalemma (Figs. 1,3,25 and 30). They were frequently enclosed in vacuoles (Fig. 1). Ten to 20 lipid bodies could be counted in a transverse section of a conidium (Fig.30). They were either spherical or irregular in shape and were 0.4-0.8μ in diameter. In the glutaraldehyde-osmium fixed material, they appeared densely stained (Fig. 1) but in the permanganate-fixed material, the lipid body had a dark and slightly irregular outline and a blank center (Fig. 3). Blank spots were observed when these osmium-darkened bodies were extracted with acetone or sodium methoxide dissolved in a methanol-

benzene mixture.

(B) Germinating conidium:

The basic cell components of the non-germinating conidia were observed in the germinating mature conidia but several differences were observed. Twelve hours after germination, the conidia had fewer vacuoles which were larger in size and occupied a greater area (Figs. 19,20,42 and 43). The cytoplasm often appeared to be pushed aside or against the cell wall by the vacuoles and as a result only thin layers of cytoplasm were observed in a cell (Figs. 19,20 and 43).

The glycogen particles in the germinating conidia were evenly distributed and less aggregated and fewer in number (Figs. 16,43 and 44). Light microscope observations showed that the germinated conidia after the Bauer Fuelgen treatment were a lighter red but were more uniformly stained. There was one nucleus in each germinated conidium (Figs 19,42 and 45). The nucleus was sometimes elongated and compressed between the vacuole and the conidial wall (Fig. 53).

Germ tube:

The germ tube wall appeared to arise from the inner wall of the spore (Fig. 54), usually adjacent to the papilla (Fig. 4). The germ tube became 2-4 times the length of the spore 10 hr after germination

at 20°C on slide or culture medium. They grew up to about 7-10 times the length of the original spore in 2-3 days (Figs. 48,51, and 54), then the growth rate slowed but the fungus lived for as long as 2 weeks. Short branches, hooked structures or appressoria were observed at the tip of the germ tube (Figs. 89-91). No sporulation could be obtained when the conidia were germinated on artificial media, even when the conidia were treated with the special culture medium which induced the sporulation of the obligate parasite Puccinia graminis (Williams and Scott, 1967). The germ tube usually had a relatively transparent cytoplasm (Fig. 50). Large vacuoles (Figs. 51 and 56) and some glycogen particles (Fig. 52), a few short E.R. (Fig. 49) and mitochondria mainly parallel to the long axis of the tube (Figs. 49,54 and 56) were observed. Ribosomes were evenly distributed (Figs 48 and 51). The lipid bodies were observed at the periphery or a short distance away from the tip of the germ tube (Figs. 48,49 and 51-52). Vesicles from 300-600Å were observed chiefly at the tip area (1-2μ) of the germ tube (Figs. 48 and 49). Little E.R. was observed at the tip of germ tube. Mitochondria were not observed from 1-2μ from the tip.

The germ tube remained either cylindrical pointed (Figs.48,49 and 51) or expanded at the tip to form a knob or 2-5 lobes of disk-like structures,

appressoria (Figs. 89-91). These lobed appressoria were observed only when the conidia were germinated on the host surface. A large nucleus was always seen in the expanded tip.

Septum:

The septum was formed by the centripetal growth of the hyphal wall (Figs. 55-59). The septum was slightly thinner than the hyphal wall and it tapered towards the pore (Figs. 55 and 56). The septum between the developing conidia (Figs. 51, 69 and 74) appeared slightly thickened before tapering towards the pore. A simple pore which is characteristic of the Ascomycetes was present in the center of the septum and had a diameter of 0.1-0.2μ. It was sometimes plugged with an electron-dense Woronin body which appeared to be different in nature from the normal lipid bodies shown in Figures 58 and 59. They appeared electron dense throughout instead of electron transparent in the center with a densely-stained outline, as do lipid bodies.

Hyphae:

The hyphae obtained from the infected leaf were denser than the germ tubes (Figs. 58-62) and the vacuoles usually contained lipid bodies (Figs. 60-63). Sometimes small groups of glycogen rosettes were present in the cytoplasm adjacent to the wall. Abundant E.R., mitochondria (Fig. 59) and some lipid

bodies were observed in the cytoplasm along the wall (Figs. 59-61). Most mitochondria were orientated parallel to the long axis of the hyphae and were elongated in longitudinal sections (Fig. 62) and oval in cross section (Fig. 60). Dense ribosomes were observed in the hyphae (Fig. 59-62). Nuclei with a lateral or peripheral bodies were seen (Fig. 61). The lateral bodies which were slightly curved were situated close to the nuclear envelope and measured 0.3μ in length. The hyphal wall was thinner than the conidial wall and measured from 0.2-0.4μ in thickness. The walls were smoother in outline and had no spine-like projections as do the conidia.

Spherosome-like bodies:

Spherosome-like bodies were frequently observed in the hyphae (Figs. 63-66) and ranged in size from 0.2 to 1μ and somewhat resembled lipid bodies which were osmiophilic. They were usually in very large groups which consisted of about 20-60 bodies. The bodies were tightly packed (Fig. 63) or slightly separated (Fig. 64) and each group was bounded by a membrane. This membrane appeared similar to the vacuolar membrane and appeared interconnected to other vacuoles (Fig. 64). Each body was again bounded by its own membrane and these bodies had variable staining-properties. They appeared either

slightly stained (Fig. 63) or heavily stained (Fig. 64) and degrees of staining occurred within one group (Fig. 63). Usually the peripheral granules were more heavily stained than the rest of the group. The spherosome-like bodies varied also in shapes depending on the fixation method used. They appeared irregular, polygonal (Fig. 63), or oval (Fig. 64) when fixed in glutaraldehyde and osmium and stellate when fixed with potassium permanganate alone (Figs. 65-67). The electron density of the interior of the spherosome-like bodies varied from uniformly greyish or dark after osmium tetroxide fixation to almost transparent after permanganate fixation (Figs. 63-67).

(C) Formation of conidia:

The conidia started to form or develop when septa were formed in the aerial conidiophore (Fig. 68-69), all but the basal one or 2 cells of the conidiophore were young developing conidia. Usually 3-5 cells were observed in one conidiophore, each with a nucleus, numerous vacuoles with abundant granular and some lipid contents, E.R. and mitochondria (Fig. 68-71). The young developing conidia were cylindrical in shape (Fig. 68-69) and the terminal cell later expanded in width to form an oval-shaped and mature conidium (Figs. 71-73). While the terminal conidium matured its wall increased in

in thickness, the septa curved, and the conidium was surrounded by a gelatinous layer (Figs. 71-72). A large number of glycogen particles formed in the cytoplasm (Figs. 72-73). The granular content of the vacuoles decreased as the conidia matured (Figs. 68-72). Only one conidium (i.e. the terminal one) matured each day. The mature conidium was later abstricted or detached from the lower conidium and became the mature conidium, ready to germinate and infect the host cell.

In the species of Erysiphe graminis, each conidiophore bore a chain of conidia usually from 10-15 in number (Fig. 74). It appeared that the conidia were matured in a sequence from its tip to its base and several conidia were observed to mature at the same time. Three terminal fully developed oval-shaped conidia were observed (Fig. 74), each with a nucleus and an accumulation of abundant glycogen particles which resembled those of the E. polygoni (Figs. 72-73). No glycogen particles were observed in the immature conidia below the 4th and 5th conidium (Fig. 74). Instead a number of lipid bodies were present as shown in the 5th conidium.

III Formation and structure of appressorium:

Primary appressoria, the expanded and modified tip of germ tubes (Figs. 89-92) were observed to form at about 6-9 hr after germination on the host leaf surface.

The form of the appressorium varied greatly, ranging from a slightly enlarged knob-like or hook-like tip of the germ tube (Fig. 91) to a multiple lobed disk-like structure (Fig. 89). The secondary appressoria appeared as protrusions on the hyphae (Figs. 93-95). They differed from the young hyphal branches (Fig. 50) because they were larger, rounded and their walls were uneven in thickness near the penetration peg. The appressoria were 6-8μ in length.

A peg was formed underneath the protrusion or the appressorium (Figs. 92 and 96) and was observed on appressoria produced by conidia which germinated on the culture medium or on cover slips. Occasionally a granular secretion was seen surrounding these pegs. The secretion stained deeply with Azure B or methylene blue. This secretion was observed around the appressoria (Figs. 89, 91 and 112). A cap-like dense extracellular secretion was seen around the secondary appressoria (Fig. 93).

A large nucleus was usually found inside each appressorium (Figs. 90-92 and 94). The mitochondria, numerous in number, appeared to be randomly arranged (Figs. 89-90, 94 and 96). Some lipid bodies, ER, vacuoles with granular and lipid contents were observed also. The cytoplasm was filled with dense ribosomes (Figs. 89-92, 95

and 96). The mitochondria changed shape and increased in number at the time of infection. Before infection they were elliptical (Figs. 89-90) and during or after infection they became a highly irregular dumb-bell shape with a narrow constriction at the center, and appeared to be dividing (Figs. 94, 96, 110 and 124). The ER also appeared more prominent and longer (Figs. 96, 110, 111 and 124), and some had flared ends (Fig. 111).

Near or at the point of penetration, the fine structure of the appressoria was characteristic. The plasma membrane was withdrawn slightly from the wall near the penetration tube leaving an electron-transparent space, (Figs. 96, 97, 110 and 111). Granules of densely stained material were always present at the tip of the penetration peg (Figs. 92 and 97), in the peg (Figs. 110 and 111) or near the side of the penetration peg (Fig. 98). Similar densely stained granules were observed in the collar (Figs. 92, 96, 99, 100, 104, 105-107 and 111), some of which appeared membrane bounded (Figs. 96, 103 and 105-107). Small densely stained granules were observed in the host wall (Figs. 97, 103, 104 and 111) and were scattered in the wall. Densely stained 'spines' which were formed by the extended wall of the appressorium

were seen close to the penetration area (Figs. 98, 99 and 104), some of which were anchored or attached tightly to the epidermal wall (Figs. 99 and 104). Extracellular secretion was observed near the 'spines' (Fig. 104).

IV Healthy host clover cell.

Both the susceptible and the resistant clover cells were similar in structure. In a healthy clover epidermal cell, a thin layer of cytoplasm surrounded the peripheral area of the cell, and a large vacuole was present in the center (Figs. 84-86). The nucleus was normally situated at the lower side of the epidermal cell (Fig. 84). The cytoplasm consisted of a few, usually 4 chloroplasts (Figs. 86 and 87). Mitochondria, rough ER, dense ribosomes and a Golgi apparatus were observed in the thin layer of cytoplasm (Figs. 85, and 86), which were situated between the plasma membrane and the tonoplast. The mesophyll cell possessed denser cytoplasm (Fig. 88) than that of the epidermal cell.

The outer wall of the epidermal cell measured 0.5-0.7μ in thickness and was about twice that of the inner wall. The outermost cuticular layer was about 0.5μ thick (Fig. 85).

V Host-pathogen interactions.

(A) Host penetrations.

The earliest infection was detected by means of the light microscope about 9 hr after inoculation. Most of the electron microscope observations on the early infections were made on the tissue 14 hr after it had been inoculated.

First the appressorium came in contact and adpressed to the host cuticle (Fig. 103). The attachment covered an area of about 3-5μ in diameter (Fig. 103). This was equivalent to the diameter of the collar material formed as a result of penetration (Figs. 92, 96, 100, 103-104 and 110). An indentation of the cuticle was seen at the mid point of attachment (Fig. 97, 103 and 104). A peg was formed at this indented area (Fig. 103). Immediately below the cuticle, densely-stained material, was seen in the wall (Figs. 92, 103 and 104). This material was similar to that observed in the penetration area or peg (Figs. 92, 97, 98 and 110). Similar densely-stained material was seen in the collar (Fig. 104) or formed the thick dark sheath of the haustorium initial (Figs. 105-107). The orientation of the parallel microfibillar structure of the cellulose wall was changed at or near the penetrating point (Figs. 103 and 111). No indication of drastic

disruption of the host wall was observed during penetration (Figs. 103-104 and 110-111), and the penetration peg fitted the hole in the wall. It appeared that during penetration, the outer wall of the appressorium was dissolved while the newly formed inner wall was extending and penetrating into the host wall (Figs. 97 and 111). This newly formed wall appeared to be derived from the wall-like material of the electron-transparent zone at the mouth of the penetration peg (Figs. 96-98 and 110-111). The host wall staining properties changed over a relatively wide area about 3-6μ in diameter at site of penetration. Cracks were observed in the wall in the older infected cells beside the penetration site (Fig. 102). The densely-stained wall had a gradation in staining and the denser area occurred next to the penetration peg as well as at the region about 2μ distant from it (Figs. 92, 100-102, 104 and 111). This area (Figs. 100-102) was equivalent to the halo or blue circular area observed when stained with Azure B and viewed under the light microscope. No change of structure or staining property of the cuticle was observed during penetration (Figs. 97, 98, 103, 104, 111, 84 and 85).

A host cell infected with 2 haustoria was

frequently observed (Fig. 159). The haustoria were close to each other but their sheath membrane never touched each other and was separated by a thin layer of host cytoplasm (Fig. 159).

(B) Resistant and susceptible reaction during infection.

In the resistant cells, during early penetration, the peg appeared to stop advancing at the collar (Fig. 112) and the appressorium appeared dead, the host cytoplasm degenerated into clumps of material. Even when the peg which appeared bat-shaped in structure had passed through the host cell wall, coagulation of the host cytoplasm was observed and resembled older infections with collapsed collar, peg and coagulated cytoplasm (Fig. 113). Typical light micrographs (Fig. 108 and 109) and a typical electron micrograph (Fig. 110) are shown. The host plasma membrane was disrupted by the penetration peg, about 5μ in length, after passage through the collar. Part of the wall of the penetrated peg appeared broken near the tip (Fig. 110). In the susceptible infected cell an oval haustorial initial, about 1x2μ in size, surrounded by a thick layer of densely-stained material was observed (Figs. 105-107). The infected host plasma membrane

was invaginated by this development (Figs. 114-116). The host cell organelles, in the susceptible infected cell appeared normal except for a slight increase of cytoplasm at the infection site (Figs. 105, 116, 117 and 92). Mitochondria, Golgi apparatus, vesicles and lipids were observed.

(C) Host cell reaction during infection.

A collar was always formed beneath the wall of the penetrating site in response to the invasion of the fungus (Figs. 92, 96, 103, 104, 105-107, 110 and 111), and as a result the host plasma membrane was invaginated (Figs. 92, 103-104 and 105). The collar usually covered the entire area below the halo. It was often a V or U shape and frequently 2 layers of collar material were found (Figs. 96 and 103) as if a new collar material was being added to an old one. The collar was 6-9μ across at the host wall, and about 2.5μ in thickness. The collar often had a fibrous appearance and consisted of fibrillar and fine granular material, permeated with irregular densely-stained material, some of which appeared membrane bounded (Figs. 103-105). The invaginated plasma membrane followed the contour of the collar (Figs. 92, 103, 104, 105 and 134).

The cell organelles in the susceptible infected cell appeared normal (Figs. 92 and 105) and contained lipid bodies, dense ribosomes and mitochondria (Fig. 92). The oblong young haustorium was surrounded by a thin layer of cytoplasm in the susceptible cell (Fig. 116). No such covering was observed on the haustorium in the resistant host cell. After the penetration peg passed through the collar in the resistant clover cell the cytoplasm began to change (Fig. 110). In the infected cell, the thin layer of host cytoplasm was withdrawn slightly from the wall (Figs. 92, 103, 133 and 145).

The thin layer of host cytoplasm surrounding the young haustoria in the susceptible host contained ribosomes and vesicles (Fig. 118, 120 and 126). The host nucleus was always situated close beside the haustorium in an infected susceptible cell (Fig. 114 and 147) and it appeared slightly enlarged in an older infected cell (Fig. 147) compared to that in the normal host cell (Fig. 84). Organelles of the host cell other than the nucleus such as the chloroplasts and mitochondria appeared normal (Figs. 147 and 148). Vesicular invaginations of the host plasma membrane were observed in the infected susceptible cell which contained well

developed haustoria (Figs. 147, 148 and 149). In an infected susceptible cell, 2 weeks after inoculation all the cell organelles were disintegrated in the dead cell (Fig. 168) and the cytoplasm was coagulated around the dead haustorium.

(D) Formation and ultrastructure of haustorium.

A peg was formed after the appressorium attached itself to the host surface and it soon penetrated the host wall (Figs. 97, 100, 102, 102, 110-111 and 140). The peg which passed through the host wall was about 0.25μ in diameter (Fig. 135). The peg hole was sometimes filled with a densely stained material and surrounded by a layer of less densely-stained material. The peg in the host wall varied slightly in size from 0.25 to 0.4μ in diameter with the narrow regions at the point of entering and leaving the host wall. The mid-region was wider and about 0.5μ in diameter (Figs. 100, 102 and 111). The penetration peg which became the haustorial neck later, in the infected cell, had an irregular outline. Sometimes the peg was empty (Fig. 136), other times it was filled with unknown material (Fig. 138). The fungal plasma membrane in the penetration peg or neck was either smooth (Fig. 136), slightly convoluted (Fig. 138) or highly indented (Fig. 137) with numerous

tube-like invaginations into the peg hole. The penetrated peg was surrounded by a densely-stained material, a collar with 2 distinguishable layers of fibrous-like material, and the host plasma membrane (Figs. 136-139).

The haustorial initials or haustoria obtained before 14 hr after inoculation were considered young haustoria, 3-5 days as mature and there after as old haustoria.

Neck and septum of haustorium:

The septum which appeared between the narrow neck and the central body was observed in the very young haustorium, 14 hr after inoculation, (Fig. 141) as well as in the older haustoria (Figs. 142 and 143). Woronin bodies were usually present near the septum or in the septal pore in the older haustorium (Fig. 143).

General and fine structure of haustoria:

The haustorial initial was formed by the expansion of the tip of the penetration peg and formed while still encompassed in the collar (Figs. 105-107). It was oval in shape covered by a thick layer of densely-stained material which sometimes appeared indistinguishable from the wall of the haustorial initial (Fig. 106). The haustorial initial was 1 x 2 μ in size and contained dense ribosomes and

only a few cell organelles such as vacuoles and lipids. The central body of the young haustorium consisted of a prominent nucleus (Figs. 118, 121, 123 and 127). A nucleus is shown sandwiched between 2 mitochondria (Fig. 123). Vacuoles with multivesicles, 150-350Å in diam., and myelinoid bodies (Figs. 118-119, 126 and 128), numerous mitochondria, E. R., dense ribosomes and a few lipid bodies were observed (Figs. 118 and 126). The plasma membrane in the central body appeared highly indented (Figs. 118, 125-126 and 128). Vesicles similar to that observed in the vacuoles of the central body were present in and outside the wall (Figs. 125 and 118), or on both sides of the sheath membrane (Fig. 120). The entire central body of the haustorium was surrounded by a wavy sheath membrane (Fig. 118) which at high magnification appeared highly convoluted (Fig. 120). Its thickness was about 120-150Å and at certain areas, the membrane appeared double (Fig. 130). The host plasma membrane in the same cell was about 90-120Å in thickness (Fig. 117). The sheath membrane was surrounded with a thin layer of host cytoplasm which in turn was surrounded by a tonoplast (Fig. 120). The sheath matrix, a zone between the sheath membrane and the central body, appeared

empty or electron transparent except for the presence of some vesicular structures (Figs. 118 and 121-123) and sparse granular material near the sheath membrane (Fig. 122). The haustorial branches were observed to arise from the end of the central body in the sheath matrix of the young haustorium, 14 hr after inoculation (Fig. 114-116). Both the anterior (proximal) and the posterior or distal haustorial branches were observed in the haustorium 18 hr after inoculation (Fig. 131). They originated from either end of the central body. Numerous tortuous branches were observed in the fully developed haustoria (Figs. 133, 144, 147 and 149), and appeared to arise from many regions of the central body (Fig. 133). These tortuous finger-like haustorial branches, each surrounded by its own wall, occupied most of the space of the haustorial sheath matrix.

Fully developed haustorium:

In the fully developed haustorium the basic organization was similar to that as described above in the young haustoria. However, it had denser cytoplasm and vacuoles, and both were observed in the central body and the branches. Vesicles were also seen in the cytoplasm or in the vacuoles (Figs. 145, 148, 149 and 158). Myelinoid bodies were present in the cytoplasm (Figs. 145 and 148), as well as numer-

ous mitochondria (Fig. 152). Only one nucleus was observed in each haustorium (Figs. 149, 151 and 153). The plasma membrane in the central body showed fewer convolutions than that of the young haustorium (Figs. 148, 149 and 151). The sheath matrix was mostly filled with granular material (Figs. 148, 149 and 153) which sometimes appeared net-like in structure (Figs. 155-158 and 161-162). Sometimes small groups of densely-stained deposits in the sheath matrix were present at the inner side of the sheath membrane (Figs. 148, 149, 160 and 161). The sheath membrane of the fully developed or older haustoria was more highly invaginated than that of the young one (Figs. 146, 148, 149, 160 and 161). It was thicker, about 150-180Å, than the one in the young haustorium.

Haustorial lomasomes:

Lomasomes were frequently observed in the actively growing or fully developed haustorium. They were present in the central body as well as in the appendages of the haustorial branch (Figs. 154-158 and 162-163). The vesicular contents of the lomasomes were similar to those observed in the conidia (Fig. 12-16). The lomasomal vesicles of the central body (Figs. 145 and 158) were also similar to the vesicles in the cytoplasm (Figs. 145

and 158). The lomosomal vesicles contained granular contents resembling those in the wall (Figs. 154 and 162-163).

Old haustoria:

In the old haustoria (Fig. 164-167) there was an increase of vacuoles and vacuolar contents, and numerous masses of densely-stained material were observed in the vacuoles (Fig. 167). More lipid bodies were observed in the central body and haustorial branches as well as in the haustorial sheath matrix (Fig. 166), than in the young haustorium. There was an increase of depositions on the inner side of the sheath membrane and also in the sheath matrix (Figs. 164, 166 and 167). The nucleus appeared fuzzy and often the nuclear envelope was disrupted. The dead haustorium was surrounded by the coagulated host cytoplasm (Fig. 168). The sheath membrane of the dead haustorium appeared intact.

(E) Infected resistant and susceptible red clover after treatment with Azure B and Benedict's solution.

The infected epidermal cells in the resistant Lakeland variety became more deeply stained than in the susceptible variety. At 9-12 hr a light blue-green color was present around the margin

of the cell (Fig. 78). By the sixth day the color intensity had increased and the whole cell was completely colored (Fig. 79). The cytoplasm had coagulated and the cell had died. Susceptible epidermal cells turned a light blue color as early as 9 hr (Fig. 75) after inoculation when they were stained with Azure B. Usually the blue color disappeared during the second day after infection but reappeared 4-10 days later and gradually increased in intensity and finally became a deep blue or blue-green after approximately 3 weeks. The adjoining cells showed either no color change or a less intense coloration (Figs. 76 and 77).

Resistant cells which had been boiled in Benedict's solution turned yellow in color 48 hr after inoculation. The intensity of coloration increased until death resulted at or prior to the sixth day (Figs. 82 and 83). The susceptible infected cells were a light yellow 3 days after inoculation (Fig. 80) and the coloration gradually increased up to 3 weeks (Fig. 81).

Infected sunflower and barley epidermal cells showed either no coloration or a very slight coloration after staining in Azure B or boiling in Benedict's solution.

VI Attempted infection of Erisphe polygoni hyphae by its own mycelium.

While studying the E. polygoni powdery mildew fungus and its host-parasite relations, connections between hyphal strands were observed. Infection pegs developed in the hyphal strands (Fig. 169, 170 and 173) or germ tubes (Fig. 174) at points of contact with other E. polygoni hyphae. These pegs passed through adjacent pores in the fungal walls and developed to a length of 1μ in the host mycelium (Fig. 175). The protoplasm surrounding the penetration tube was withdrawn up to a distance of 0.5 to 2μ. In some instances the penetration tube enlarged after penetration, to approximately 4 times the diameter (Fig. 174). In others it remained constant in diameter (Fig. 175).

An electron-dense zone was always formed around the penetration tube and the plasma membrane of the invaded hypha was invaginated proportionately. The penetration tube appeared electron dense (Figs. 170, 172 and 173) or sometimes electron transparent (Figs. 171 and 175). The vesicular structures in the cytoplasm and vacuoles of the invading hypha (Figs. 170 and 173) were similar to those present in the penetration tubes (Figs. 170, 172 and 173).

DISCUSSIONS

In this study it has been shown that electron microscopy studies must not stand alone but must be complimented by light microscopy and cytochemical studies if one wishes to obtain a complete picture. Also different procedures help to add more information. Pre-fixation with glutaraldehyde, and post-fixation with osmium tetroxide of fungal organelles proved superior to fixation with glutaraldehyde, osmium tetroxide and potassium permanganate alone. Similar results were also obtained by the present author in a previous study on the fungus Monilinia fructicola (Leong, 1967). However the other fixation methods were employed, despite their weaknesses, to provide special information on the fine structure of different cell organelles because different fixatives can produce different images of certain organelles in electron microscopy. The use of different fixatives helped give a complete picture of the material studied. For example the identification of the glycogen particles and their recognition from the ribosomal particles was made possible. Both of these particles were observed after glutaraldehyde and osmium fixation, but ribosomes were not observed after potassium permanganate fixation.

Light microscopy was employed, to provide general information when necessary and to complement electron

microscopy. Because only a very minute amount of material is studied in electron microscopy incorrect or misleading interpretations may readily be made.

The possibility was considered both from the chemical and ultrastructural point of view, that dense granules present in the mature conidia of E. polygoni were glycogen. The results obtained from the cytochemical tests, such as the conventional iodine test on the fresh spores and the Bauer-Feulgen reaction (Humason, 1962) showed that these granules were glycogen-polysaccharides. Previously in fungi glycogen has been identified cytochemically and morphologically (Foerster et al.1965), (Zalokar 1960, 1965) (Prusso and Wells, 1967). Prusso and Wells (1967) used the Bauer-Feulgen technique to show the presence of glycogen granules in the fungus Sporobolomyces roseus in which they found abundant glycogen in young buds and spores. Ehrlich et al.(1968) reported glycogen in fungi on the bases of likeness of particles to those in the animal tissues presented by Revel et al. (1960). Foerster et al.(1965) identified the fungal glycogen chemically and morphologically and showed that it was identical, with or very similar to that in animal cells.

In the present study little modification occurred in the isolation of the glycogen particles. It was found that α-amylase removed glycogen particles from suspensions and sections. The morphological evidence or

ultrastructure of glycogen shown in the electron micrographs was similar to that described in fungal (Foerster et al., 1965) and animals cells (Revel et al., 1960; Revel, 1964; Ryley, 1969 and Childress, 1970). Thus the evidence presented both by light and electron microscopy showed that the dense granules present in E. polygoni conidia were glycogen particles.

In the past, several polysaccharides have been described (Cochrane, 1958), but with few exceptions, the criteria for identification were inadequate and the nature of the objects remained in doubt. However, the substances may be identified due to the advance of electron microscopy techniques.

Zaracovitis (1966), in his paper on the germination of conidia of powdery mildew fungi, stated that powdery mildew conidia are fundamentally different from other fungal spores because they may not require an external supply of water for their germination. The ability to accumulate large amounts of reserve material, glycogen in this case, is probably a unique feature of the powdery mildew conidia.

Glycogen is generally considered a primary storage polysaccharide in animals (Fawcett, 1966) and fungi (Zalokar, 1965; Bracker, 1967). Glycogen particles commonly aggregate into rosette configurations, alpha particles. Glycogens have been reported in several fungal

species described by Cochrane (1958). They have been found in different parts of the fungal thallus, such as in cytoplasm of hyphae (Zalokar 1960, 1965; Foerster, 1965; Hashimoto 1966 and Ehrlich et al., 1968b) in reproductive structures, e.g. basidia (Zalokar, 1965), asci, sporangia (Bracker, 1967), and spores (Prusso and Wells, 1967; Akai et al. 1968b). However Zalokar (1965) found that glycogen was more abundant in mature or aged portions of a thallus than near the growing hyphal tips. Foerster (1965) found that one of the metabolic activities in the fungus Collybia velutipes was glycogen biosynthesis and that the glycogen granules appeared to increase with age.

The present finding, in the conidia of E. polygoni and E. graminis showed glycogen accumulated during maturation. Only dense glycogen granules were observed in the mature conidia. There were fewer granules in germinating spores, appressoria and young hyphae. Electron micrographs showed that glycogen accumulates in the conidia while they are undergoing maturation and a large amount of glycogen appears only when they are fully mature.

The view that fungal glycogen is a reserve food was supported also by the data of Ergle (1948) on glycogen utilization during the germination of sclerotia. Chrzaszcz and Tiukow (1929) stated that polysaccharide is a form in which fungi can store energy, contrary to an earlier view of polysaccharide formation as a system of a deranged metabolism.

Cochrane (1958) reported that glycogen comprises about 5% of the mycelial or spore weight, but in the sclerotia of Phymatotrichum omnivorum the glycogen is 36% (Ergle, 1947). Recently Foerster et al.(1965) reported that only 1% glycogen was found in 29 day old Callybia velutipes mycelia. However the present study showed that about 50% of the cell volume of E. polygoni contained glycogen. This indicates that these conidia contain substantial amounts of glycogen by weight. If fungal-spore glycogens comprise about 5% in weight as mentioned above, then the constituents of the conidia of E. polygoni powdery mildew are greatly different from other fungus spores.

It is interesting to note that recently glycogen has been characterized from the sclerotia of Phymatotrichum omnivorum by Gunasekaran and Lyda (1970 a,b). They found that Phymatotrichum glycogen consists of aggregates of small spheres and that the average chain length of Phymatotrichum glycogen is 13 glucose units. They also found that there was a direct correlation between growth and glycogen concentrations, with most growth occurring in liquid or agar medium containing 4% glycogen. The large amount of glycogen in E. polygoni may play an important role in the initiation of germination and subsequent growth of the conidia.

Zalokar (1959) claimed the glycogen forms the main reserves of carbohydrate for the endogenous respiration in fungi. Glycogen is believed to break down to yield glucose and during the enzymatic degradation of glucose, energy for spore germination and growth is produced. Also supplies of short chain carbon skeletons which are reutilized in the synthesis of various basic components of protoplasm are produced. This process may enable powdery mildew conidia to grow on dry glass slides and on water for a limited time without any external source of food. In fact E. polygoni conidia continue to grow for as long as 1 to 2 weeks and to form a germ tube as long as 150-200 μ i.e. 5-8 times the length of the conidia, without a food source.

Mitchell (1967) claimed that glycogen aggregates were quite abundant in germinated conidia of powdery mildews and were only occasionally seen in sections of hyphae and not in unincubated conidia. In fact, his electron micrographs (Figs 2,5,8,9,14,16,17 and 62) of unincubated conidia of S. macularis and E. polygoni show dense aggregations of glycogen particles which appear negatively stained or blank. This probably resulted from the negative staining of the uranyl acetate solution. Revel (1964) reported that aqueous uranium salt solutions greatly enhanced the contrast of ribonucleoprotein particles but did not stain glycogen.

Revel (1964) also reported that large polysaccharides

such as glycogen contain relatively few chemical groups which are capable of binding or reducing osmium tetroxide. Owing to this lack of interaction, the preservation of glycogen for electron microscopy studies is often unsatisfactory, and even if the polysaccharide is preserved, it has a very low electron density in thin sections. The difficulties encountered in fixing and staining glycogen have been so great that in one instance in Escherichia coli studies, glycogen was identified by its absence, as circular holes in thin sections of the material (Cedergren and Holme, 1959). However, Revel (1964) stated that glycogen particles occasionally have a considerably greater density, and reported that glycogen polysaccharide seems to be unusually well preserved or may even be stained during potassium permanganate fixation. He also stated that glycogen could be stained by phosphotungstic acid, and stained exceedingly well by lead hydroxide. Revel (1964) commented that critical identification of glycogen should be based on fractionation studies and one should establish that such fractions have the same morphology and staining characteristics as those of intracellular glycogen, until better or more convenient methods are developed. It seems further investigation is necessary to varify Mitchell's finding on the glycogen content in different powdery mildew conidia. However in the present study, the large amount of glycogen was present only in the fully mature conidia in E. polygoni and not in the young or

immature conidia during the developing stage. On the other hand, there was much less glycogen present in the germinated conidia after 12-36 hr incubation and glycogen was only occasionally seen in the germ tubes and mycelium.

In animal tissue, it has been suggested that the membrane of the agranular reticulum may have a role in glycogenolysis (Fawcetts, 1966), both in synthesis and degradation, but no direct role of the smooth reticulum in glycogenosis has been established. Hashimoto and Yoshida (1966) reported an association of the membrane system with glycogen synthesis in Geotrichum candidum, an imperfect fungi. They concluded "that glycogen is synthesized in this fungus within the mesosomes (created by intrusion of the cytoplasm into the distended or expanded endoplasmic reticular cavities) and is finally translocated into the cytoplasm through the agranular endoplasmic reticulum ". The present author found that the glycogen was distributed evenly throughout the cytoplasm and occasionally in mitochondria. Glycogen granules have been observed also within the mitochondria by Hohl and Hamahote (1967) in the zoospores of Phytophthora parasitica. However there is a close association of the glycogen particles with the vacuoles because packages of glycogen were observed frequently in the vacuoles. The membranous myelinoid bodies frequently present in the vacuoles of E. polygoni resemble the membrane system in the mesosome described by Hashimoto and Yoshida (1966).

McKeen (1970) observed large amounts of lipid in conidia of E. graminis and thought that they were oxidized and used in the process of respiration during germination. Perhaps the glycogen in conidia is mainly derived from the conversion of the numerous lipid bodies in the conidiophore and in conidia.

It is reasonable to assume that the accumulation of glycogen in the conidia can be considered as a criterion for the stage of maturation in the powdery mildew. As mentioned earlier, Hirata reported that the conidium of E. polygoni matured singly and the conidia of E. graminis matured in chains. The fact that the results obtained in this study showed only the top expanded conidium contained glycogen in E. polygoni while several top conidia contained glycogen in E. graminis, bear out the above view.

The reason for the different number of conidia formed per day between different powdery mildews is not known. Since spores of the obligate parasite must depend upon their reserves for germination a knowledge of their components may provide a guide to the nature of their growth requirements. The materials present in the spores at maturity must provide the essential ingredients for the growth of a germ tube and sometimes for the differentiation of limited hyphal structures. Allen (1965) reported that the germination of some fungal spores such as ascospores of Neurospora and Ascobolus and probably also the germination of resting sporangia of Phycomycetes and teliospores of

rusts and smuts occurs at the expense of stored reserves but direct experimental evidence for the latter is scarce.

Allen (1965) also reported that in saprophytic fungi, the nutrients required for germination are taken up by the spores and rapidly transformed within the spores. Sporangia of Phytophthora infestans germinating in glucose media showed increases in glycogen content. Blastocladiella spores consumed glucose rapidly and, during the early stages of germination, converted it to a glycogen-like polysaccharide which is used in later stages of development. It appears that in the saprophytic fungi, the carbon source used for germination is taken up and converted to some temporary reserve, and is later used for continued growth. A transitory build-up of materials from exogenous compounds is believed to occur in the spores of saprophytic fungi. These changes in the composition of a spore during germination may provide a clue to the nature of the reserves needed to support germination. There is as yet little information concerning the changes in spore constituents which occur during germination of powdery mildew conidia. The present anatomical evidence provides additional information about the constituents of the powdery mildew conidia. Perhaps the difference between the powdery mildew fungi which are unable to utilize exogenous compounds during germination and growth, and the saprophytic fungi is that powdery mildews are able to build up a pool of reserve

material in their conidia at the time of maturation which is required for germination and for future growth.

Shu et al. (1954) reported that lipid was the important reserve of rust uredospores. The lipids dropped appreciably during germination. Allen (1965) also reported a decrease in lipids and an increase in carbohydrates in spores of Puccinia graminis during incubation or after germinating spores had mainly used their fatty acids or polyalcohols. The powdery mildew conidia may be considered more advanced or active in building up the reserve food than the rusts.

Gregory (1966) has stated that powdery mildews are xenospores. That is they are spores without a resting period after maturation. It is likely then that the metabolic activity is relatively active during and after maturation, and that they are able to utilize or convert the lipid bodies into carbohydrate during maturation or before germination. While the rusts are only able to do so in the later state or after a short period of the incubation of the spores.

Another prominent and consistently observed structure in the conidia of E polygoni were the vacuoles. The conidia were highly vacuolated. More than half of the space was occupied by vacuoles in fresh conidia. Gregory (1966) claimed that fungal spores are characterized by small water content and lack of vacuoles. If this is so,

then the powdery mildew conidia are different from most fungal spores. Mitchell and McKeen (1970) showed that about 53% to 70% of the weight of conidia of S. macularis and E. polygoni is water. Scott (1957) found, by using the method of equilibration at 25°C and 0% R.H., the water content of non-germinated conidia of E. graminis from oats is 65% of their initial fresh weight. This evidence indicates that powdery mildew conidia possess a great deal of vacuolar space and supports the postulation of Yarwood (1950) that the powdery mildew conidium has the capacity to carry water and to germinate in the absence of an external source of water. Moreover, the results of Schnathorst (1959) and Jhooty and McKeen (1965a) also suggest that conidia of powdery mildew fungi have a low osmotic value. These facts indicate that germination of powdery mildew conidia at low humidities cannot be the result of water uptake caused by a high osmotic pressure as postulated by Brodie (1945).

Somers and Horsfall (1966) suggested that it is the water retaining power and the way in which it is bound and not the amount water which is the important factor for germination. Therefore it is interesting to know the manner in which the water is bound within the powdery mildew conidia and the way in which it differs from that in other fungi. A correlation was found between the percentage of water contained in the conidia and the percentage of vacuolar space in the conidium. This

indicates that most of the water in the conidium is contained in the vacuoles. The evidence from the fine structure of the conidia also indicated that the powdery mildews have the ability to conserve or retain the water within the conidia. The mucilaginous layer of wall material around the conidia, except the conidial tip acts as an insulation or a barrier against water loss. The nature of the internal structure of the condium itself also helps to retain the water. The electron micrographs showed that the vacuole was divided into different compartments which were usually interconnected. Each had its own protective membrane, the tonoplast. This is considered to be a more efficient water retaining mechanism than would be possible in a single compartment vacuole (Mitchell, 1967). The electron micrographs in the present study did not reveal any significant difference in the staining property of the vacuolar membrane from other cytoplasmic membranes as has been reported by Mitchell (1967) and others (Frey-Wyssling and Muhlethaler, 1965; Brown and Bertke, 1969). Therefore no significance could be attributed to the structure of the tonoplast. The difference may be due to the fixation procedures used. However, Mitchell (1967), in experimenting with various dyes including neutral red, observed that the vacuolar membrane had a very limited permeability, and he considered it served effectively for water retention. The net-like heterogenous materials observed in most vacuoles may also

play an important role in retaining thewater within the compartment. Other features such as the early germination of the powdery mildew conidia after abscission also insure against the depletion of water supply in the conidia. In E. polygoni the germ tube is produced within 3 hr after liberation from the conidiophore.

Zaracovitis (1966) classfied powdery mildew fungi into 3 groups according to their rate of germination, and rate of appressorial formation on glass slides when incubated in a saturated atmosphere at 21-23°C. The time required for group A including E. polygoni and most others was within 5 hr, for group B, including E. graminis, E. cichoracearum was less than 10 hr, and for group C, including E. macularis was about 10 hr or longer. Zaracovitis claimed the powdery mildew fungi in group A which show a fairly high percentage germination, were able to form appressoria even at 0% R.H. and were able to survive due to the fact they germinated rapidly. This seems to relate to water economy in the conidia.

The vaucoles in the conidia do not have a classical structure. Electron micrographs revealed that dense netted granular heterogenous material is often present in the vacuoles and in the early conidial maturing stage it is denser. Mitchell and McKeen (1970) observed similar granular structures in conidia of S. macularis and E. cichoracearum. They believed that the water in the

vacuole was not pure water, because when the conidia were suspended in water the vacuoles soon broke, and disappeared. They thought that the vacuolar sap must have been a solution capable of absorbing water and might also contain suspended particles of insoluble waste or reserve material. Although the nature of the vacuoles is not known, several investigators have reported the isolation of various compounds from plant cell vacuoles. Buvat (1963) reported that vacuoles accumulated various metabolic products in the form of true solutions of crystalloids or colloids. Matile (1966) found several enzymes localized and bounded with vacuolar membranes in corn seedlings. In yeast cells Matile and Wiemken (1967) also reported the presence of hydrolytic enzymes in isolated vacuoles possibly localized in the vacuolar sap. Therefore they postulated that the yeast vacuole was a lysosome. Svichla et al. (1963) in a study with yeast showed that both waste and storage materials were found in vacuoles. Therefore these vacuolar granular contents may be the reserve material, waste product or enzymes. However these heterogenous materials are denser in the maturing conidia and less dense in the mature or germinating spores of M. fructicola (Leong, 1967). Brown and Bertke (1969) reported that certain cells in most seeds produce specific vacuoles that accumulate protein which becomes solid and granular. Such reserve deposits of protein in vacuoles, which are often enzymes are called aleurone grains. In barley, these

enzymes are used by beer industries in the conversion of starch to sugar. Frey-Wyssling and Muhlethaler (1965) considered that vacuoles served as receptors of excretion. Apart from the metabolites such as phenols, anthocyanins, alkaloids, valuable assimilates such as sugars and protein were secreted into the vacuoles where they were stored as reserves, but could be reactivated and re-introduced into the metabolic system of the cell when required. Therefore it is reasonable to believe that they serve as reserve for future development during germination. The fact that they are more prominent at maturity suggest that they are temporarily stored in this form until they are used for the synthesis of cytoplasmic organelles. Another vacuolar content, such as the intravacuolar lipids may also serve as reserve material.

Powdery mildew conidia, reproductive bodies of obligate parasites, must store in their conidia all the food reserves, enzymes and other materials necessary for germination and subsequent growth, until the mycelium is successfully established upon the host. This requirement is mostly met by the large amount of glycogen reserve present in cytoplasm of the conidia. For continual synthesis, the fungi need some enzymes and building materials. They may be present in the vacuolar sap of the conidia. The vacuolar heterogenous substance is one such material mentioned earlier from which building materials can be withdrawn and new cytoplasm can be synthesized during

growth.

The association of the myelinoid bodies with the vacuoles is considered significant. These myelinoid bodies are especially abundant during the early stages of germination. Electron micrographs revealed the myelinoid body projected from the cytoplasm into the vacuole and consisted of a large number of concentric membranes that enclosed, or partly enclosed a cavity with granular materials. The membrane and the material contained between the membranes was similar to and continuous with the cytoplasm adjacent to the vacuole. Although their exact function is not known, it is logical to assume that they are synthetic in function. The occurrence of myelinoid bodies in association with vacuoles or vesicles in fungi has been reported by several investigators (Buckley et al.1966; Jarvie, 1966; Linnane et al. 1962; Zachariah and Fitz-James, 1967; Greennawalt, 1965). Their function has been interpreted in various ways, sometimes functioning like mitochondria (Linnane et al., 1962; Zachariah et al. 1967); sometimes in enzyme associations (Linnane et al. 1962); sometimes in membrane synthesis and sometimes functioning in a synthetic way (Mitchell and McKeen, 1970; Leong, 1967).

It is known that the large number of folds that make up the concentric membrane system, which are bathed by the vacuolar fluid, provide a greatly increased surface area for enzyme activity. Therefore during rapid

growth the vacuolar contents can be mobilized quickly and withdrawn from the vacuoles. The materials from the vacuole may thus pass through the myelinoid membranes, during synthesis of new material and later become incorporated in the cytoplasm.

Because mildew conidia are xenospores, they must be prepared to begin their new life at the time of dispersal. A predigestion of material is thought to take place in the mature conidia in preparation for germination. This intracellular digestion is believed to take place in the vacuoles of the conidia.

The fine structures of the conidia of E. polygoni are generally similar to those in E. cichoracearum conidia described by McKeen et al.(1967) and Sphaerotheca macularis conidia described by Mitchell and McKeen (1970). The wall of the conidium is composed of 2 layers, the outer layer is thicker and gelatinous, whereas the inner layer which possessed spine-like structures is usually thinner and electron dense. In some cases this inner layer can be sub-divided into an outer spine layer and an inner amorphous layer. The wall at the tip of the conidia appears thinner and has a homogeneous electron density. The plug that appears at the point of abstriction at the end wall of the mature conidium has an incomplete coverage of mucilage. Mitchell and McKeen (1970) thought this area was more permeable than the rest of the wall, because dyes entered only through the end wall of the

conidia. They believed that the outer slime layer of the wall played a significant role in water relationships and fungicidal properties of mildews and prevented intake or loss of water except at an opening at the ends of the conidium where no layer is usually present. The spines on the inner layer of the wall may be the points at which the capsular material is excreted.

The electron micrographs showed that the germ tube wall is continuous with the original inner layer of the conidial wall which may stretch to form the germ tube wall. This type of germination has been observed in Botrytis cinerea and Penicillium frequentaus (Allen, 1965; Hawker, 1966).

The spherical, elongated or dumb-bell shaped mitochondria appeared to reproduce by division. Spherical lipid bodies which served as reserve food, were usually situated next to the conidial wall and some appeared in the vacuole.

The nucleus of E. polygoni possessed a lateral granule, opposite to a prominent large nucleolus. The lateral body that occurred in E. polygoni was similar to that observed with the light and electron microscope by Mitchell (1967) and Mitchell and McKeen (1970) in the Sphaerotheca macularis powdery mildew. Recently Wells (1970) in a study of the nuclear divisions in the ascus of Ascobolus stercorarius also reported a similar structure which he called the centriolar plaque. This centriolar

plaque was detected at all stages of the nuclear cycle in the ascogenous hyphae, croziers, and asci. Usually the centriolar plaque was closely associated with the outer membrane of the nuclear envelope. A tubular structure of the centriolar plaque was evident in some of his electron micrographs. He suggested that the centriolar plaque was a modified centriole. Wells (1970) reported that the centriolar plaque had been detected only in species of Ascomycetes. Light microscope observations (Olive 1953, 1965) have indicated that the plaque is present in taxonomically diverse groups of Ascomycetes. However Beckett and Wilson (1968) reported that there are other configurations than those observed in Ascobolus sterorarius and Pustularia cupularis. It should be of considerable phylogenetic interest to determine the exact structure and function of the centriole-like bodies in the major taxa of the fungi.

Septa between conidia changed shape as the conidia matured and were always distinct from those in germ tubes. The septa are similar to those described in S. macularis by Mitchell and McKeen (1970). The septum narrows at the center where it approaches the pore and in other areas it is about twice as wide as the exterior wall. It is a typical Ascomycete septum.

As mentioned above the septum of E. polygoni belongs to the simple, single-pored type of Ascomycetes, except for those observed between the maturing conidia.

It is different from the more complex dolipore apparatus of the Basidiomycetes observed in Rhizoctonia, (Bracker and Butler, 1963) and the Polyporus (Giesy, 1965).

Reichle and Alexander (1965) reported that the multiple perforations in the large hyphae of Fusarium species have never been observed in a conidium. He believed that the multiple perforations were produced by a digestion process, in which parts of the septal wall were removed to facilitate greater translocation of materials. This wall digestion resembles the process of the anastomoses in hyphae and crozier formation in ascogenous hyphae. No comparable observations were made in the present study.

Associated with this is the septal plug or Woronin body. Many authors reported that septal pores are frequently plugged by electron-dense material, the Woronin bodies (Moore and McAlear, 1962; Reichle and Alexander, 1965; Marchant, 1966; Carroll 1967; Brenner and Carroll, 1968; Kreger-van Rij, 1969).

Bracker (1967) reported that inclusions near ascomycetous septa, observed by many authors, have been equated with the refractile Woronin bodies observed by light microscopy. In electron microscopy, they appear spheroid, electron dense, and membrane bounded. Reichle (1965) believed these round bodies were the same structures as those described by Woronin in 1866 and thus named them Woronin bodies. Their location and behavior are similar and they remain confined to the immediate

vicinity of the septum. Woronin bodies are larger than septal pores and function as pore plugs. They are unique to Ascomycetes. Although the nature of Woronin bodies is unknown, they have been variously interpreted as lipid bodies (Moore and McAlear, 1962) and as bodies of unknown nature associated with pores (Dickson, 1963). They resemble septal plugs in their density and in being bounded by a membrane. However in E. polygoni these bodies were different in nature from lipid bodies, The lipid bodies, when fixed with potassium permanganate appeared blank in the center with a dark outline, whereas the whole of the Woronin bodies were uniformly and densely stained. Similar pictures have been shown by Reichle and Alexander (1965) in their electron micrographs in which potassium permanganate fixation was used. Further investigation is necessary to determine the exact nature of these bodies.

Reichle and Alexander (1965) suggested that the Woronin bodies function as a safety valve, which protect the hyphal cell contents by plugging the septal pores when adjacent cells are injured. They stated that the constant close association of these bodies with septal pores made them appear to be the logical plugs. When an adjacent cell was ruptured the pressure in the other cell forced one of these balls into the pore and plugged it. Several balls per pore increased the effectiveness of the safety device. Similarly the plug of E. polygoni

conidia at the abscission region of a detached mature conidium may also serve to protect the cell contents. However liquid and air is believed to be able to pass into the conidia. Oxygen is necessary for the conidia germination (McKeen, 1970). Excessive water may be drawn in and disrupt the delicate vacuoles and may produce a fatal effect on the fungus as has been shown by immersing the conidia in the water. The conidia are destroyed in a way similar to that caused by the fungicides (Zaracovitis, 1966). Plugging was observed in the haustorial neck of E. polygoni. At times plugging was observed also by Bracker (1968) in the septa of the haustorial necks of Erysiphe graminis. In this instance, it is not believed to function in a protective way. If this is so, it may have detrimental effects due to obstructing translocation. It would be interesting to know whether the fungus possesses the ability to selectively control the plugging and unplugging by Woronin bodies.

Groups of spherical spherosome-like bodies were found in the hyphae of E. polygoni. They were different from that of the individual lipid bodies that appeared in the conidia. The lysosome-like organelles, often called spherosomes have been reported in both fungal and higher plant cells (Frey-Wyssling, 1963; Jacks et al., 1967; Wilson et al., 1970). The development of the spherosome in fungi may be similar to that described by

Frey-Wyssling (1963) in the plant cell. According to Frey-Wyssling the so-called spherosomes in plant cells evolved or differentiated from vesicles produced by the E.R. into oil droplets. The process involved the detaching of vesicles from terminal strands of E.R. which formed juvenile spherosomes then fat bodies and then oil droplets.

Spherosomes were named by Perner (1953), and described as the small refractive dense body in the cytoplasm, with a diameter of 0.5 - 1.0 μ. The sphersome stained like fat droplets with the usual fat dyes i.e. Sudan black, sudan III and Nile blue sulphate. In addition, they showed the Nadi reaction, which is the synthesis of Indophenol blue from α-napthol and dimethyl-p-phenylene diamine through O_2 -transfer. Perner (1953) concluded that the spherosomes were not ergastic particles like fat droplets but enzyme-active organelles and that they contained cytochrome oxidase. The spherosome acted as selective storage sites. Frey-Wyssling (1965) reported that sphersomes were lipid rich (4%) and after their lipids were extracted a protein ghost remained. There were several differences between lipid droplets and spherosomes. Both particles varied in size between 0.2 and 1.3 μ. They lost their spherical form as a result of the preparative treatment, and appeared irregularly shrunken. Both granules were surrounded by a unit membrane, which appeared to

develop from E.R. Spherosomes when fixed with osmic acid or permanganate showed a fine granulation, while the oil droplets appeared optically void. This granulation of the spherosomes indicated a proteinaceous stroma, with a definite affinity for the electron stains. The oil droplets seemed to lack the ability to reduce osmium tetroxide or potassium permanganate and remained unstained, like starch granules. Mishra and Colvin (1970) described the variability of spherosome-like bodies in Phaseolus vulgaris cells fixed with different fixatives. Their electron micrographs of spherosomes in the cytoplasm of the plant cells varied widely with different fixatives. The variation in the appearance and distribution of spherosomes varied from tissue to tissue within the bean plant even when the same fixative was used. A variation was found also even within the same cell.

The spherosomes reported in fungal and plant cells (Jacks, 1967; Stiers and Wilson, 1969; Wilson et al. 1970) sometimes are called lysosome-like bodies probably because of their close resemblance to the lysosomes generally found in animal cells (DeDuve, 1959). Lysosomes are regarded as a group of particles which are rich in hydrolyic enzymes. They have a lipoproteinic membrane, a densely granulated stroma (Novikoff et al. 1956) and a large central vacuole when fully differentiated and appear like small bags. These bags which contain various enzymes have consequently been called lysosomes by DeDuve (1959).

Avers et al. (1965) thought the spherosomes must be related to the lysosomes and were organelles storing enzymes such as acid phosphatase. This would be correct, if the lysosomes are defined merely as bags of enzymes. Frey-Wyssling (1963) pointed out that if we consider their lytic capacity, we must ascertain first whether the spherosomes are used only for the breakdown of cell substances, catabolism, or whether they also participate in synthesizing, anabolism, as in the case of fat synthesis. The phosphatases are effective not only in splitting off but also in the transfer of phosphate groups. Therefore the presence of acid phosphatase alone can scarcely be used for the identification of lysosomes. It is believed that spherosomes and lysosomes are phytogenetically the same organelle which store and isolate enzymes from their substrates in the cytoplasm.

It is interesting to note that variations also exist within and between groups of spherosomes or lysosome-like bodies found in the hyphae of E. polygoni. They may also participate in the synthesis of fats, if so they are probably the main source of the numerous intravacuolar lipid bodies formed in the hyphal cells and also in the conidia. Whether these spherosomes contain enzymes or not is not known. Further investigation is worthwile in order to understand the nature and function of these organelles.

Mishra and Colvin (1970) claimed that the variability in morphology of spherosomes in bean cells and their heterogeneity may be used to support the suggestions that the spherosomes are sites of lipid synthesis (Frey-Wyssling et al. 1963), the botanical equivalent of primary lysosomes (Sorokin, 1968) and sites of reserve protein storage (Srivastava and Paulson, 1968). The finding that spherosomes were widely distributed among a large number of cell types and tended to be most prominent in actively dividing cells and to disappear as the cells mature, supports the suggestion that spherosomes are storage or transport organelles which are consumed during growth of the organism. McKeen (1970) in studying the lipid of Erysiphe graminis hordei, observed numerous intracytoplasmic, intravacuolar lipids, in the conidiophore, conidia and mycelium except in the haustorial mother cell and the growing hyphal tip, which have a distinct metabolism of their own. He suggested that lipids in powdery mildew are storage bodies, and would be immediately mobilized when needed. In E. polygoni, the electron micrographs also showed many intracytoplasmic and intravacuolar lipids and with lesser amounts in conidiophores and conidia. They were probably derived from the spherosome observed in the hyphae. McKeen (1970) postulated that at least part of the water needs for the germination of powdery mildew conidia are met by the oxidation of fats.

Lomasomes were observed in the germinated conidia as well as in the actively growing haustoria. They were mainly vesicular in form, but multivesicular bodies were observed also in the haustoria. Lomasomes were first reported by Girbardt (1958) and he showed that they appeared as aggregations of membranes in a matrix between the plasma membrane and the cell wall. These were later named lomasomes, meaning border bodies by Moore and McAlear (1961) on the basis of their location at cell peripheries. Recently Marchant and Robards (1968) suggested that all membranous or vesicular structures associated with the plasmalemma are classified under the general term, paramural body, regardless of their origin. They subdivided the paramural bodies into 2 classes according to their derivation. Lomasomes are derived from cytoplasmic membranes, while plasmalemmasomes are formed entirely from the plasmalemma.

Lomasomes and structures resembling them are found in both lower and higher groups of plants (Marchant and Robards, 1968). They are observed in various fungi (Foerster et al., 1965; Giesy and Day, 1965; Hawker, 1965; Hendy, 1966; Moore 1965; Shaw and Manocha, 1965; Wells, 1965; Wilsenach and Kessel, 1965; Zachariah, 1967; Manocha and Colvin, 1968) as well as in algae (Barton, 1965; Crawley, 1965), and higher plants (Esau et al., 1966; Hanchey and Wheeler, 1966; Manocha and Shaw, 1964). The configurations of lomasomes vary from tubular (Peyton and

and Bowen, 1963), vesicular (Wilsenach and Kessel, 1965) to dilated (Wells, 1965). The matrix may consist of wall or wall-like material or ambient fluid between the plasma membrane and the wall. It has been suggested that lomasomes may participate in secretion (Moore and McAlear, 1961), wall formation (Crawley, 1965; Wilsenach and Kessel, 1965; Zachariah and Fitz-James, 1967; Marchange et al. 1967) absorption by haustoria (Peyton and Bowen, 1963) glycogen synthesis (Hashimoto and Yoshida, 1966) membrane proliferation (Zachariah and Figz-James, 1967), cytoplasmic degeneration (Wells, 1965), stress response (Bracker, 1967) and may maintain basidial turgor (Wells, 1965). Marchant and Robards (1968) proposed that the paramural bodies may in some cases be involved in wall synthesis, either as a transitory stage during the incorporation of wall precursors or as the site of incorporation of enzymes for extracellular syntheses.

The function of lomasomes in E. polygoni is not known and their occurrence has not been reported in the haustoria of the powdery mildews (Ehrlich and Ehrlich, 1966). Although Bracker (1967) in his review of fungal fine structure has questioned the reality of lomasomes as normal components of living cells and said their formation could even be influenced by stress. However Marchant and Robards (1968), using different methods of fixation and with an analysis of similar structures illustrated in other published results have concluded that

the formations of vesicular and membranous structures associated with the plasmalemma in both lower and higher plants are not artifacts. In the present study they appeared consistently in the germinated conidia of E. polygoni as well as in the haustoria. In view of this and their close association with the actively developing cells and the fungal walls, it is reasonable to assume that the lomasomal structures observed in E. polygoni are real and may function in wall formation as described by Marchant et al. (1967) and Marchant and Robards (1968). Marchant et al. (1967) found 2 vesicular systems associated with wall synthesis in Phycomyces blakesleeanus, Fusarum culmorum and Coprinus lagopus. These fungi have chitin in their walls. They believed that a system of vesicles produced by the endoplasmic reticulum is responsible for 'primary' wall synthesis. These vesicles fused with the plasmalemma. A similar type of vesicle has been observed in the germinated conidia of E. polygoni. Another vesicular system Marchant et al. (1967) observed, was the multi-vesicular bodies from which lomasomes are formed. These were thought to be associated with 'secondary' wall synthesis, and may be concerned with chitin deposition in the wall. However this type of miltivesicular body was observed in the developing haustoria of E. polygoni after its wall was formed. They may function in deposition of microfibrils, as proposed by Marchant et al. (1967) or participate in the synthesis of several

fungal-wall components (Calonge, 1969).

The fact that there are vesicles at the tip of the germ tube and at the tip region of the hyphal branch indicates their role in the production and deposition of the wall material. Similar observations were reported by Girbardt (1969) and Grove et al. (1970) and both support the generality of tip growth by vesicular addition. Grove et al. (1970) in their study of Pythium ultimum tip growth found that the apical zone of the hyphal tips is characterized by an accumulation of cytoplasmic vesicles, often to the exclusion of other organelles and ribosomes. Vesicle membranes were occasionally continuous with the plasma membrane. They described secretory vesicles which were released from dictyosomes, migrated to the hyphal apex, fused with the plasma membrane, and liberated their contents into the wall region. This allowed a plasma membrane increase at the hyphal apex equal to the membrane surface of the incorporated vesicles as well as a contribution of the vesicle contents for surface expansion. However, no dictyosomes were present in E. polygoni. The vesicles observed seem to originate directly from the E.R. The size of these vesicular structures was similar to that observed in the lomasomes.

As mentioned earlier, the ability of pathogenic fungi to invade their host may be attributed to the appressorium which is a special organ differentiated from hyphae. The mode of appressorium formation differs in

different fungi. E. polygoni, usually formed a long germ tube when the conidia were grown in distilled water or on dry glass slides. They did not form appressoria even after 2-3 days. This is called the germ tube type of germination. On the other hand, when the conidia were incubated on the host leaf surface the appressoria could be observed on the short germ tubes about 6-8 hr after inoculation. Early differentiation of appressoria was observed also when the conidia were grown in a 2 to 6 % sugar solution. This variation in germination indicated that E. polygoni responded to external stimulation at a very early stage and that the host surface and the sugar solutions were stimulatory. On the host surface stimulation is probably due to the exudate from the host epidermal cells. E. polygoni germ tubes were easily distinguished from those of other powdery mildews such as E. cichoracearum and Sphaerotheca fuliginea, because they had short, lobed appressoria. Hirata (1969) claimed this to be the typical E. polygoni type of germ tube.

The appressoria always had a prominent nucleus with a distinct nucleolus and nucleolar vacuole but a nucleus was not usually observed in the germ tube. The tight packing of organelles, such as mitochondria, E.R., ribosomes and lipid bodies in the appressoria compared to the sparse arrangement in the germ tube indicated that more metabolism was taking place in the

appressorium during infection than in the germ tube.

It is interesting to note that the glycogen granules, which were abundant in the dormant conidia, were not observed in the appressoria. They were likely utilized or converted into simple sugars during the early stage of germination, and presumably they were used for growth and differention of the appressoria. Akai and Ishida (1967) reported that the glycogen-like particles, in Colletotrichum legenarium conidia seemed to flow into the germ tubes, and consequently they thought these particles were utilized during the germination of conidia for the synthesis of cell walls. Yasumori (1964) believed that during the process of appressorium formation lipid globules moved from the conidia into the appressoria, but the lipid globules did not disappear during the spore germination. Akai and Ishida (1967) agreed with this finding, after making electron microscope observations, and assumed that the lipid globules might be utilized during germination and appressorium formation. Manocha and Shaw (1967) also reported numerous lipid globules which appeared in rust uredospores, they tended to disappear as germ tubes grew. Akai and Ishida (1967) observed a marked difference in nuclear division and in cell wall synthesis between the appressorial type and germ tube type of development. They found in the former case that the nucleus divided at the base of the germ tube, and then a septum was formed.

In the latter case, the division of the nucleus was not observed. The cell wall of the germ tube in the former case became very thick, the outer layer becoming electron-dense, but in the latter instance the cell walls were thin and relatively electron-transparent. The wall of the appressorial initial in E. polygoni did not appear significantly different in appearance or thickness from the wall of the germ tube, but the appressorial wall was greatly thickened at the site of penetration in both primary and secondary appressoria.

The basic structure of the secondary appressoria was similar to the primary appressoria except the spherosomes, cytoplasmic lipid bodies or intravacuolar lipids were often observed in the secondary appressoria. McKeen (1970) reported that the haustorial mother cell lacked osmiophilic bodies until the adjoining haustorium was 3 or 4 days old, then some lipid usually appeared.

It is believed that active fungal wall synthesis and secretion of materials including gelatinous substances and the wall degradating enzyme(s) occurs at the infection site where the appressoria and the host come in contact. This covers an area of 1-2μ in diameter where a thickened appressorial wall or a spine-like projection could be observed. At the beak-like mid-region of the infection site, the appressorial wall appeared 2-4 times thicker than the rest of the wall. In some cases the plasmalemma of the appressoria appeared to be pushed inwards. This

area is responsible for pressure necessary for penetration. The spine-like structures, developing from the area of contact of the appressorium may serve for anchoring to the host, and perhaps for the secretion on the host surface of gelatinous material which enables the appressorium to hold firmly to the host surface during penetration. A close contact of the appressorium with the host surface always has been observed.

The cuticle in the present study appeared intact, flexible and slightly invaginated when in contact with the penetration peg. No change in electron density or erosion of cuticle was observed. It is believed that the cuticle is penetrated mechanically. The spine projection and wall thickening at the base of the appressoria would provide necessary anchorage and sufficient force for the invagination and puncturing of the cuticle.

Evidence presented indicated that the host wall was chemically altered. Electron micrographs showed that the host cellulose wall changed in electron density at the penetrating area and around the penetration site. The electron-dense host wall around the penetration peg was in the same position as the 'halo' observed by means of the light microscope. Although the cellulose wall did not show any obvious sign of swelling, the fibrillar structure was more obvious and the peg hole through the host wall was wider at the center than at both ends. The

presence of the densely-stained materials at the penetration peg near the penetration site of the appressorium is significant. The densely-stained material resulted due to the penetration.

Although the author believes that the host penetration was mainly accomplished by chemical means, the possibility of a mechanical thrust through the host wall is not excluded.

Evidence for supporting the chemical penetration of the host is increasing. Smith (1900) reported the possibility of chemical degradation in the penetration of powdery mildew infection pegs into geranium epidermal cells. Recently, as reported by Edwards and Allen (1970) several investigators provided some experimental support of the enzymatic mechanism (Heinen and Linskens, 1960; Hess 1969; Kunoh and Akai, 1969) and others demonstrated that pathogens were capable of producing the necessary enzymes for cuticle and cell wall digestion (Albersheim et al., 1969; English and Albersheim, 1969; Heinen and Linskens, 1960). Histochemical techniques have been used to show that chemical changes occur in the host cell wall in the area of penetration (Akai et al. 1966, 1968 a,b; Brown and Harvey, 1927; Edwards and Allen, 1970; Kunoh and Akai, 1969; McKeen and Bhattacharya, 1969). Despite these the problem of the mechanism of the penetration process of pathogenic fungi into healthy host cells is still not resolved, primarily because the process has not

been analyzed in a systematic, chronological sequence. Edwards and Allen (1970) claimed that there was a possibility that the cuticle might be chemically penetrated. The evidence presented here did not indicate such possibility. Further investigation is necessary.

Edwards and Allen (1970) suggested that the penetration process be divided into 2 steps. The first step was the chemical penetration of the cellulose wall. The second step involved the mechanical push through the collar. Unfortunately they did not obtain any evidence or electron micrographs to substantiate this point, although they tried to make a chronological and systematic study of the penetration processes during primary infection. They believed collar passage to be mechanical and rapid. In their study, the penetration may have already been completed due to the fact they used material 48-72 hr after inoculation. Penetration normally occurs between 9-14 hr after inoculation or not more than 30 hr (Masri and Ellingboe, 1966 a,b; McKeen and Bhattacharya, 1970).

Electron micrographs showed that the bathing haustorial initial was surrounded by a layer of densely-stained material. The collar always appeared to be divided into 2 regions. The inner region which differed from the fibrous outer region, appeared granular and more electron transparent and usually was permeated with darkly stained granules. The inner region is believed

to be the area in which the fungus enzymes acted. The fact, that the haustorial initial lacked cytoplasmic organelles and contained many densely-stained granules in its cytoplasm indicated that synthesis had not been initiated and that nutrients were being supplied from the appressorium.

The nature of the collar has been variously interpretated as swellings of the subcuticular layer (Dey, 1919), accumulation and aggregation of cytoplasm (Aronescu, 1934), deposition of callose (Fraymouth, 1956), cellulose-like material (Western, 1936), lomasomal material (Ehrlich et al.), polysaccharide callose (Edwards and Allen, 1970), debris from cell-wall decomposition, secretions from the invading fungus, products of synthesis by the invaded host cell or from some combination of these possibilities (Stavely et al. 1969). The formation of a collar in response to infection-peg formation is a common phenomenon in many host-parasite combinations (Hess, 1969; McKeen and Bhattacharya, 1969; Temmink, 1969), but its origin, nature and function are not well understood. Additional research is necessary.

McKeen and Bhattacharya (1969) reported a chemical and morphological alteration of the epidermal wall of several hosts which had been attacked by species of powdery mildews. Their electron micrographs and cytochemical tests suggested that the cellulose wall around the infection peg was degraded by an enzyme or enzymes, such as cellulase.

The attempted infection of E. polygoni hyphae by its own mycelium (Leong et al., 1970) showed that penetration pegs developed from hyphal strands at points of contact with other E. polygoni hyphae. These pegs penetrated the adjacent hyphae. In some instances the penetration peg enlarged after penetration to about 4 times the diameter of the pore-like openings. The penetration process resembled that which occurred when E. polygoni attacked the clover plant host, and entrance seemed to be gained by dissolution of the cell wall and not by mechanical means. If this is so, then E. polygoni is able to produce an enzyme(s) which is effective against different host walls. In the penetration of the fungal wall, a chitinase was probably produced. The attempted self-parasitism by E. polygoni, an obligate parasite, is unique in its mode of parasitism. Intimate connections between related and unrelated fungi have frequently been reported. In some instances parasitism results, and in others compatibility occurs. For example, a species of Papulospora or Trichoderma lignorum may penetrate and destroy Rhizoctonia solani mycelium (Warren, 1948; Weindling, 1932). When anastomosis of somatic hyphae and plasmogamy occurs before the parasexual or sexual cycle, a compatible reactions occurs. When the gametangia are formed the walls between them dissolve and the contents mix (Alexopoulos, 1962). This phenomenon of anastomosis has also been reported to occur in E. graminis (Hirata 1967).

But in E. polygoni, interaction between the hyphae was observed. It is difficult to understand why the reaction is unilateral and how an incompatible reaction arises between colonies of the same strain of powdery mildew.

Recently Griffiths and Campbell, (1970 a,b) reported the self-penetrating pheonomenon in hyphae of Verticillium dahliae and also reached the conclusion that penetration resulted after chemical dissolution of the invaded cell wall. They found that the hyphae of Verticillium had an incompatible reaction. They thought that the autoparasitism in Verticillium was presumably associated with the development of dead microsclerotial cells whose function was probably protection of living storage cells which are capable of germination (Nadakavukaran, 1963).

However, in E. polygoni, the invaded mycelium did not cease to grow or show any sign of dying and the penetration peg did not contact the host plasma membrane. The fact that E. polygoni was able to attempt self-infection on its own mycelium suggested that it probably possessed an ability to invade any potential host wall. It will be interesting to know if other members of the powdery mildew possess such ability. Hirata (1969) reported that Erysiphe had the largest host range among the powdery mildew genera. Whether or not this is in any way related to the behavior or character of E. polygoni is not known.

The general structure of the haustoria in E. polygoni was similar to that described by McKeen et al. (1966) in E. cichoracearum and by Bracker (1968) in E. graminis. The haustorium in E. polygoni resembled more closely E. cichoracearum haustoria than E. graminis haustoria. The haustorium was an oval or elongated ellipsoidal body with tortuous branches. In E. polygoni the branches emerged at first from both ends and wove back over the body as in E. cichoracearum. Later they developed from any spot on the surface of the central body. The branches seldom came into contact with each other or the body of the haustorium or the surrounding membrane.

Hirata (1967) in his light microscope observations divided the haustoria of ectoparasitic powdery mildew fungi into 2 types. The Erysiphe graminis-type and the Sphaerotheca fuliginea-type. The E. polygoni and E. cichoracearum belong to the later type. Hirata found that these 2 types of haustoria might appear quite different but they were fundamentally similar in structure when their developmental stages were traced and compared. The long axis of

the oval body of the initial haustorium of S. fuliginea, did not become parallel to the host leaf surface as does E. graminis, and the lobes grew out of each end of the body. As the lobes elongate, they wind and cover the oval body, giving a granular appearance. McKeen et al (1966) suggested 2 possible reasons for their presence. First the branch hyphae were vestigial and have lost their ability to extend straight away, like fingers, from the body of the haustorium in which position they were surrounded individually or in groups by the host plasma membrane, as in E. graminis. Second, the hyphae were not vestigial but have evolved in a woven fashion around the central body. This increased the surface area several times greater than that of the membrane which surrounded the haustorium body. This indicated that the membrane surrounding the S. fuliginea-type haustorium had a greater capacity for transfer of nutrients than did the membrane, surrounding the fungus protoplasm. The second postulation is more likely true. The difference in appearance, may be due to the adaptations of the powdery mildews to different conditions in the host cells. As pointed out by Hirata (1967) the shape and size of epidermal cells may have influenced the

development of the 2 types of powdery mildew haustoria. The epidermal cells of grasses and cereals, parasitized by E. graminis-type haustoria species were rectangular, whereas the epidermal cells of dicotyledonous plants parasitized by powdery mildew fungi other than E. graminis were in most cases irregularly shaped and not so spacious. Hirata thought that there was insufficient space for the haustorial lobes to straighten in epidermal cells of leaves of dicotyledons, and therefore, the hyphae wove back and forth as in S. fuliginea. If this was so, it is equally valid to assume that finger-like appendages in E. graminis were evolved from the S. fuliginea-type of haustoria and adapted to the more spacious cells of the monocotyledonous leaves. In both cases they appeared to occupy the host cytoplasm to the fullest extent and have the maximum capacity for absorbing host nutrient. However, E. cichoracearum haustoria in sunflower cells are of the Sphaerotheca fuliginea-type but the host cells of the stem are elongated like the grass cells which contain E. graminis-type haustoria. To explain this phenomenon, the suggestion by McKeen et al. (1966) may be applied. The branch hyphae of the E. cichoracearum haustorium have already

lost their ability to extend straight away like fingers.

The membrane which surrounded the haustorium in E. polygoni appeared to originate from the host plasmalemma and was invaginated by the haustorium. Unfortunately no definite connection of the plasmalemma with the haustorial sheath membrane was observed. This difficulty in obtaining such evidence may be due to the extremely rare chances of getting thin sections which show all the haustorial features such as the neck, host plasmalemma and the young haustorium on one plane. Also the young haustorial neck usually changed course immediately after penetration.

Recently Littlefield and Bracker (1970) showed the continuity of host plasma membrane around haustoria of Melampsora lini in electron micrographs. Their observations regarding the invagination of host protoplasts confirmed the conclusions reached in earlier studies by Thatcher (1943) who used plasmolytic methods and studies by Allen (1923) and Rice (1927) who used light microscopy and Manocha and Shaw (1967) and Van Dyke and Hooker (1969) who used electron microscopy. The sheath membrane was regarded as a regional invagination of host plasma membrane which

might be specialized due to its association with haustoria. Such continuity has been observed also in electron microscopy of phycomycetous infections, (Berlin and Bowen, 1964; Ehrlich and Ehrlich, 1963a; Peyton and Bowen, 1963). With powdery mildew infections, it has yet to be demonstrated conclusively. Several investigators (Ehrlich and Ehrlich 1963b; McKeen et al., 1966; Bracker, 1968) have expressed difficulty in tracing the plasma membrane around the haustorium in powdery mildews. The present author believes that it is more difficult to trace the membrane in older infections because the sheath membrane is highly invaginated.

Ehrlich et al. (1963b) considered that the haustorial sheath was neither part of the host nor the fungus. Bracker (1964, 1968), McKeen et al. (1966) thought the sheath was continuous with the host ectoplast. Hirata (1967) had the same view and said the haustorium in its youngest stage must be surrounded by the ectoplast of the host protoplasm. As the haustorium grew older, the ectoplast gradually increased in thickness and turned into a distinct structure which he called a sac. The present study supports Hirata's view. According to Hirata, sac separation is due to the repeated

shrinking and swelling of the haustorium within the elastic sheath membrane. The gap formed is infiltrated with sap from either the host or haustorium or both. This in turn causes higher osmotic pressure, and the enlargement of the sac. Hirata (1967) believed that calcium was responsible for the thickening of the sheath membrane and promoted its development.

Conclusions can be drawn from the differences and characteristics of the young and mature or old haustoria. The haustoria obtained before 14 hr after inoculation were considered young, 3-5 days as mature and there after old haustoria. The organelles and morphology of the haustorium changed as it aged. On the basis of these differences a criterion for the young, mature or old haustoria can be established. This can be probably applied to most of the powdery mildews. These criteria may also serve to identify the stage of infection , and in certain cases may also help to clarify certain interpretations of the haustorial features in previous studies.

First of all, the infection did not seem to involve the rapid migration of large amounts of material from the appressorium into the haustorium as have been observed in rust (Manocha and

Shaw, 1967) and Phycomycete (Temmink, 1969) infections. Probably in powdery mildew, the appressorium was more active in secretion during the early stage of penetration. Later, materials or organelles were then slowly translocated into the haustorial initial. Perhaps many of the cytoplasmic organelles were synthesized within the young haustorium after the nucleus migrated into it, since very few cell organelles were observed before the nucleus appeared in the haustorium. The nucleus was present only after the haustorium had passed through the collar.

Manocha and Shaw (1967) reported that in rust, during the early stage of penetration, the wall of haustorial mother cells thickened, and the young haustoria appeared without a zone of encapsulation. Later, the haustoria was separated from host cytoplasm by encapsulation. These observations were similar to that observed in the present study of E. polygoni. If this is so, the encapsulation of rust and the sheath matrix of powdery mildew and the zone of apposition of Phycomycetes are homologous structures. Chou (1970) claimed however that the zone of apposition was not a structure separate from the haustorium wall but

was an integral part of it. The expansion of the space in the sheath matrix in powdery mildew may be partly due to the formation and extension of the haustorial branches. However in the rust fungus and downy mildew no haustorial branches are formed in any stage of development. McKeen et al. (1966) considered the haustorial branches might serve to increase the absorbing surface area. If this is so, the haustoria of powdery mildew are highly evolved with specialized organs for parasitism.

No direct connections were observed by the author between the haustorium and the host cytoplasm. Vesicular secretory structures were found inside and outside the sheath membrane of the young haustorium. This indicated that the sheath was a dynamic structure, actively engaged in the exchange of materials between the host and parasite. Similar structures such as secretory bodies (Peyton and Bowen 1963), vesicles (Ehrlich and Ehrlich, 1963a; Shaw and Manocha, 1965), secretory tubules (Berlin and Bowen,1964) have also been reported at the interface between the host and fungus in various fungi. All these bodies may play a role in the uptake of nutrients or secretory activity of the host. The sheath ma-

trix may serve as a food reservoir for the fungus besides accommodating the haustorial branches. It may also serve as a 'buffer zone' between the host and the parasite in which the excretion from the fungus and host may accumulate. McKeen et al. (1966) thought that the structureless, irregularly scattered encapsulation material along the host membrane was waste product.

The basic ultrastructure of the haustorial apparatus of all the organisms reported to date is essentially the same (Ehrlich and Ehrlich, 1966). It consists of an expanded haustorial head surrounded by the fungal wall and in turn is enclosed in an encapsulation of unknown material surrounded by a membrane.

The first staining of the infected epidermal cell with Azure B occurred at the ninth hour. At 20°C, penetration of the host epidermal wall may begin 8 hr after inoculation. Thus the infection peg, within 1 hr after it begins to form, stimulate the host cell either mechanically or chemically. The latter is more probable (McKeen and Bhattacharya, 1969).

In the susceptible cell an apparent recovery is indicated because Azure B usually does not stain the infected cell for a few days after the immediate

reaction. In the resistant cell, however, there is a gradual increase in coloration until death occurs.

The shade of color of the susceptible and resistant cell also differs after staining with Azure B, a metachromatic dye. The orthochromatic shade, blue-green, is more characteristic in the resistant cell and the metachromatic shade is more characteristic in the susceptible cell. This indicates according to Jensen (1962) that different high-molecular-weight substances having free anionic groups are present in the susceptible and resistant cell.

The yellow-brown coloration of the infected and adjoining cells after treatment with Benedict's solution shows that reducing compounds appear soon after infection occurs. These reducing substances become much more abundant in the resistant clover cells and may be responsible directly or indirectly for resistance. Obviously the redox potential of the cells changes as more reducing compounds form in cells. Benada (1966b; 1968a,b) has reported that distinct limits of redox potential in host tissues were needed for successful infection in barley and wheat powdery mildew. Perhaps a particular redox potential range is necessary for a

compatible mildew clover relationship.

Because there is little or no response when barley and sunflower infected cells are stained with Azure B or boiled in Benedict's solution it is evident that each host responds in a specific fashion. One might have expected an increase in reducing compounds in infected sunflower cells because large quantities of simple carbohydrates must be used in the formation of the collar around the infection peg (McKeen et al., 1966).

Yarwood (1955), Shaw (1954) and Shaw and Samborski (1956) have shown previously that chemicals and radioactive substances accumulate in diseased host tissues but this work reveals that alterations occur in the infected cell and sometimes in the neighbouring cells. It is apparent that cells rather than tissues need to be studied.

Edwards (1970) found a substance in infected cell of E. graminis which stained with basic stains. He found that the spread of this substance correlated with the expression of resistance in the host cells but the nature of this substance was not known. Whether or not this is due to a mechanism of resistance which is related to the redox potential of the host cell is not known. Additional investigation is needed.

It appeared that the penetration process was similar in both the susceptible and resistant hosts. The failure of the fungus to establish a congenial relationship with the resistant host may be mainly due to lack of response of the resistant cells and the early degeneration of these cells. Electron micrographs revealed that the collar of the resistant host was much shallower than in the susceptible cell.

Hirata (1967) observed the hypersensitive reaction in the leaves of highly resistant barley, and found that the penetration peg of powdery mildew often degenerated within 10 hr after inoculation and the germ tube which produced the penetration hypha also appeared dead. However he was not able to make an accurate assessment because he could not see the ultrastructure as he made only light microscope observations. In the present study, the penetration peg did not seem to die immediately after entering the resistant cell, and it did not expand quickly into a full sized haustorium as it did in the susceptible cell. The appressorium of the penetrated peg in the resistant cell appeared normal and full of cytoplasmic organelles. It seems that the inability of the penetration peg to develop into a normal haustorium resulted from the

prompt death of the resistant host cell which caused the death of the infection peg.

Stavely and Hanson (1966c, 1969) observed that haustoria of E. polygoni in resistant clover cells reached maximum size, 20 hr after inoculation. Differences in fine structures of infected resistant and susceptible host cells also became apparent. Stavely and Hanson (1969) found degeneration of the cell organelles in the resistant host soon after infection. In infected resistant cells they observed that not much of the sheath membrane was destroyed within 20 hr after inoculation. They thought that destruction of the host plasmalemma resulted in elimination of a membrane barrier between the host cytoplasm and sheath matrix and thus caused the death of the host and the fungus. Membrane destruction in resistant cells might be an injury response of the host that resulted from inability to respond or to cope with fungal infection. Stavely and Hanson (1966c) also found that different chemicals would induce varying degrees of resistance in susceptible clovers. They suggested that for susceptibility to occur, host metabolism must respond in certain specific ways to the invading fungus. A lack of response in resistant host cells might result in fungal pro-

duction of metabolites or conditions that resulted in selective host-membrane degradation and death of the invaded cell.

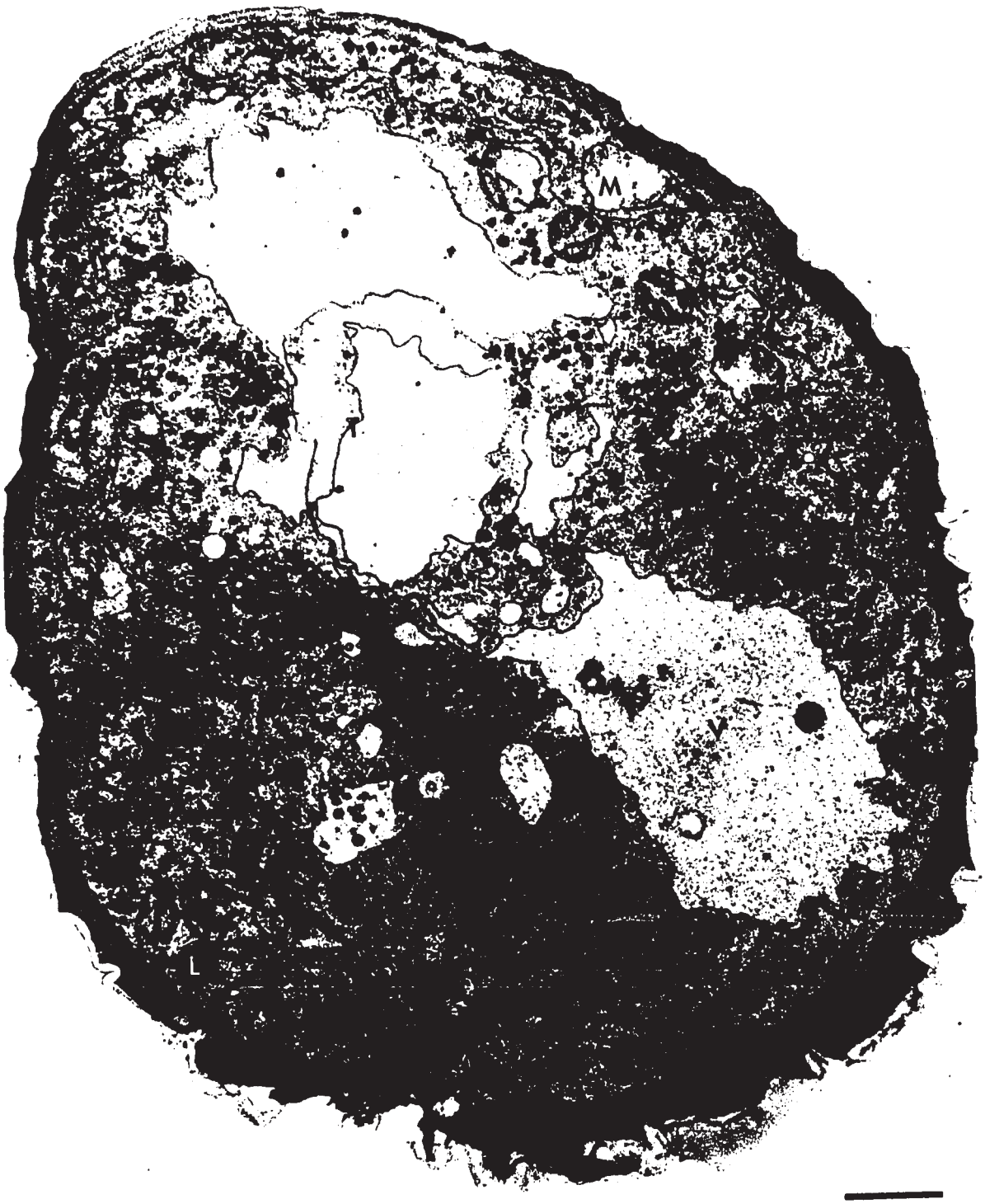
Shaw and Manocha (1965) reported that in wheat resistant to rust Puccinia graminis, resistance was associated with a breakdown of encapsulation, plasmalemma and other subcellular host membranes and organelles in 6-7 days in invaded resistant cells versus 15-20 days in susceptible cells. Van Dyke and Hooker (1969) in a study of interreaction of Zea mays and Puccinia sorghi, observed that in invaded incompatible host cells, the sheath membrane of the haustoria was broken down. Whereas the death of the haustoria of Albugo candida was associated with the walled-off material. No such material was observed in the present study.

PHOTOMICROGRAPHS

Figure 1 Cross section of a mature conidium.

A typical electron micrograph of a cross section of a mature conidium, fixed in glutaraldehyde and osmium tetroxide. It shows all the components of a conidium. Observe the various well preserved cell organelles especially the vacuoles and their contents; rosettes of darkly-stained glycogen particles and less darkly-stained smaller ribosomal particles. Note also the distinct mitochondria, plasma membrane, endoplasmic reticulum, lipid bodies, spore wall and the gelatinous layer.

Uranium and lead stained. 14,000x.



1

Figure 2 Section of a portion of a mature conidium, fixed in osmium tetroxide, and showing different cytoplasmic organelles. Note the irregular-shaped nucleus with nucleolus and double-membraned nuclear envelope. In contrast to Figure 1, fewer cell organelles are present in the cytoplasm, the membrane structure of various organelles are clearly shown. The individual units of each glycogen rosette is not discernible.

Lead stained. 35,000x.

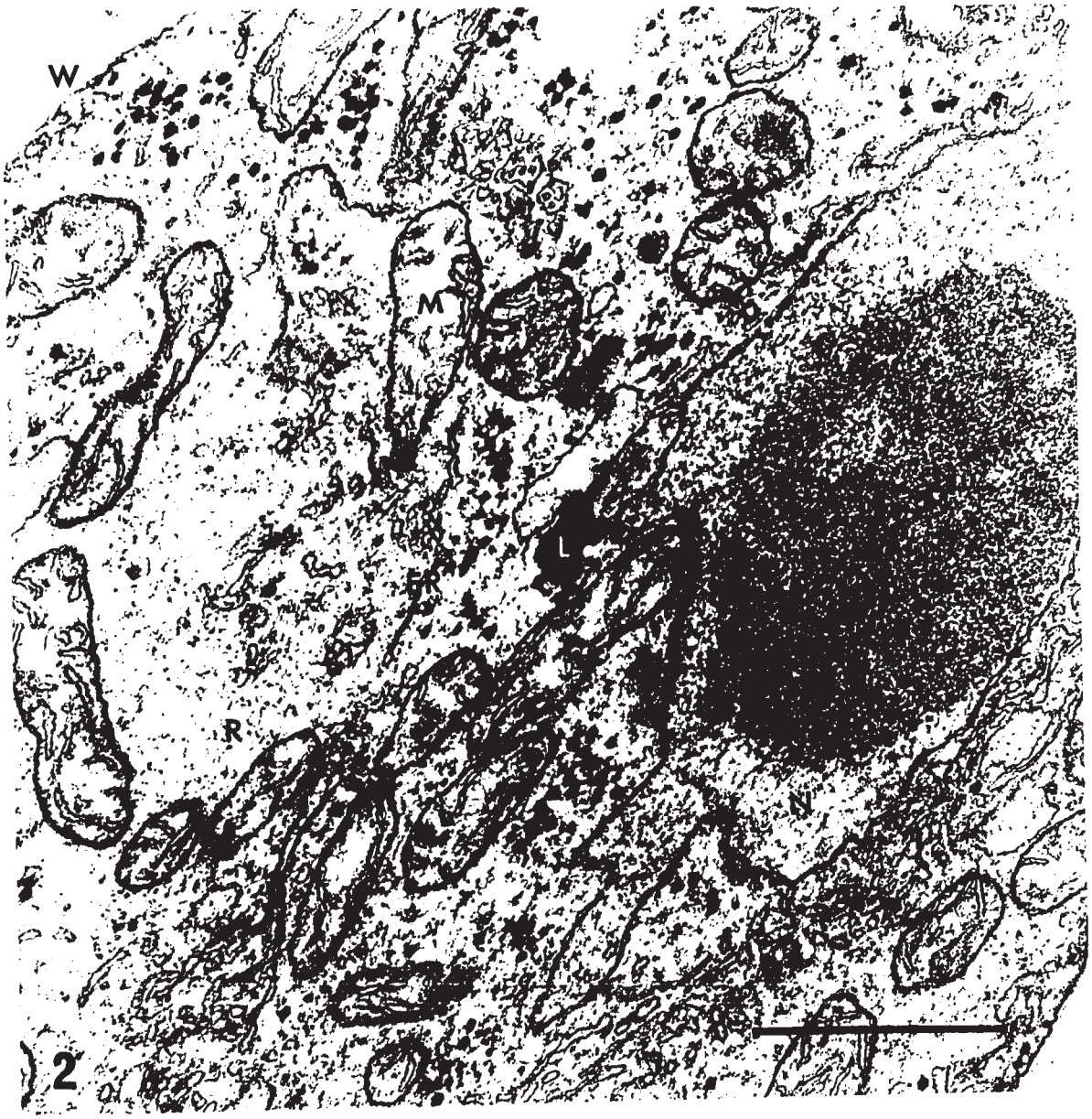


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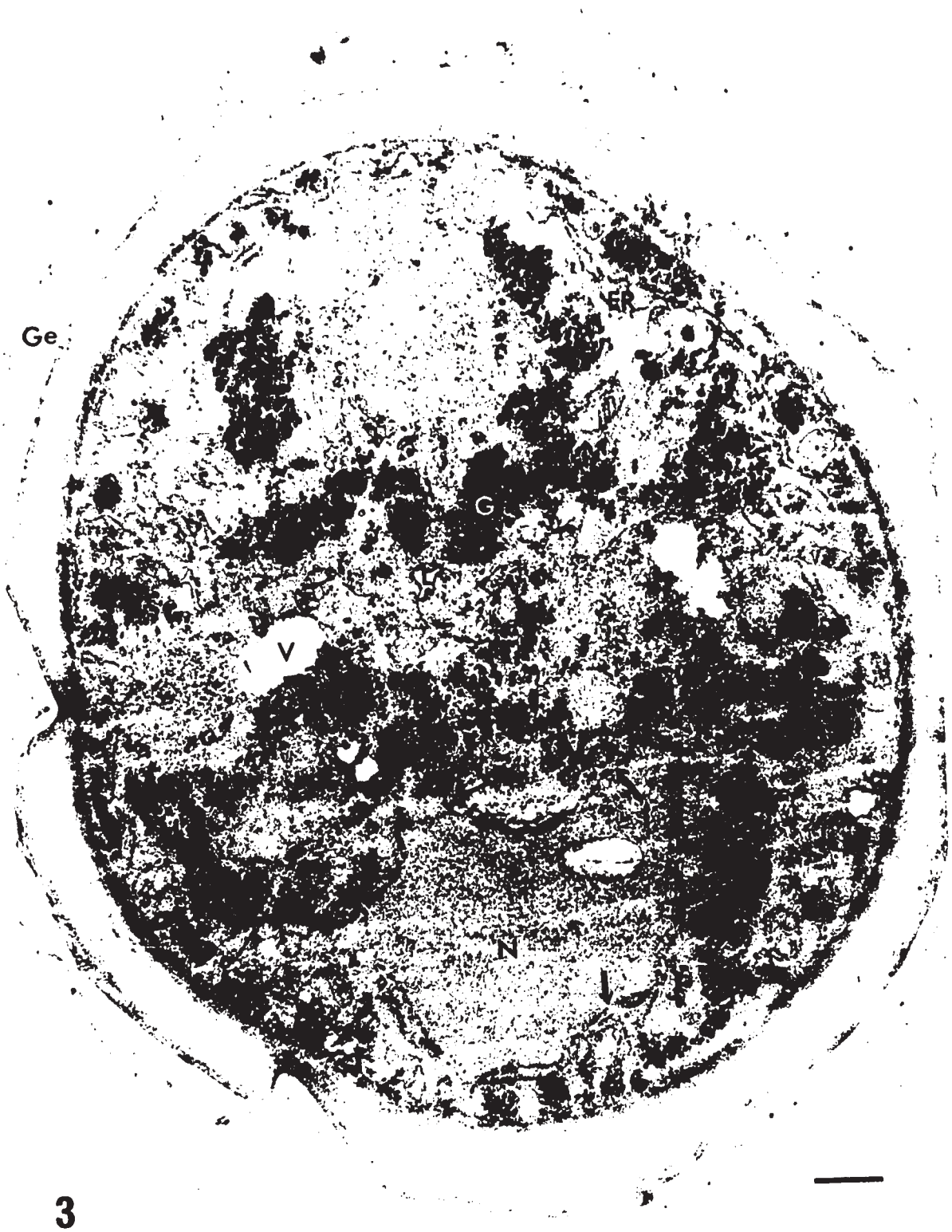
Figure 3 Cross section of a conidium, fixed in potassium permanganate. Note the ribosomal particles are destroyed; the vacuoles are poorly preserved; membranes are broken, e.g. the plasma membrane, E.R., and mitochondrial membranes. Lipid bodies appear as electron-transparent areas with darkly stained outlines. Note the distinct nuclear membrane with nuclear pores (arrows).

Uranium and lead stained; 11,000x.



3

Figure 4 Section of the tip of a conidium with a papilla, a plug-like structure, at the abscission end. Note the long E.R. which is parallel to the wall. Lead stained; 16,000x.

Figure 5 Cross section of part of a conidium which shows the wall structure and the mucilaginous layer. Note the outer and inner wall, the spine-like and the irregular protrusion of the wall. Glutaraldehyde and osmium fixed; uranium and lead stained. 30,000x.

Figure 6 Cross section of part of a mature conidium showing the vesicular-like invagination of the plasma membrane.
Glutaraldehyde and osmium fixed; uranyl acetate stained. 57,000x.

Figure 7 Cross section of part of a mature conidium showing a tube-like invagination. Note a vesicle is formed at the side of the invagination.
Glutaraldehyde and osmium fixed; uranyl acetate stained. 57,000x.

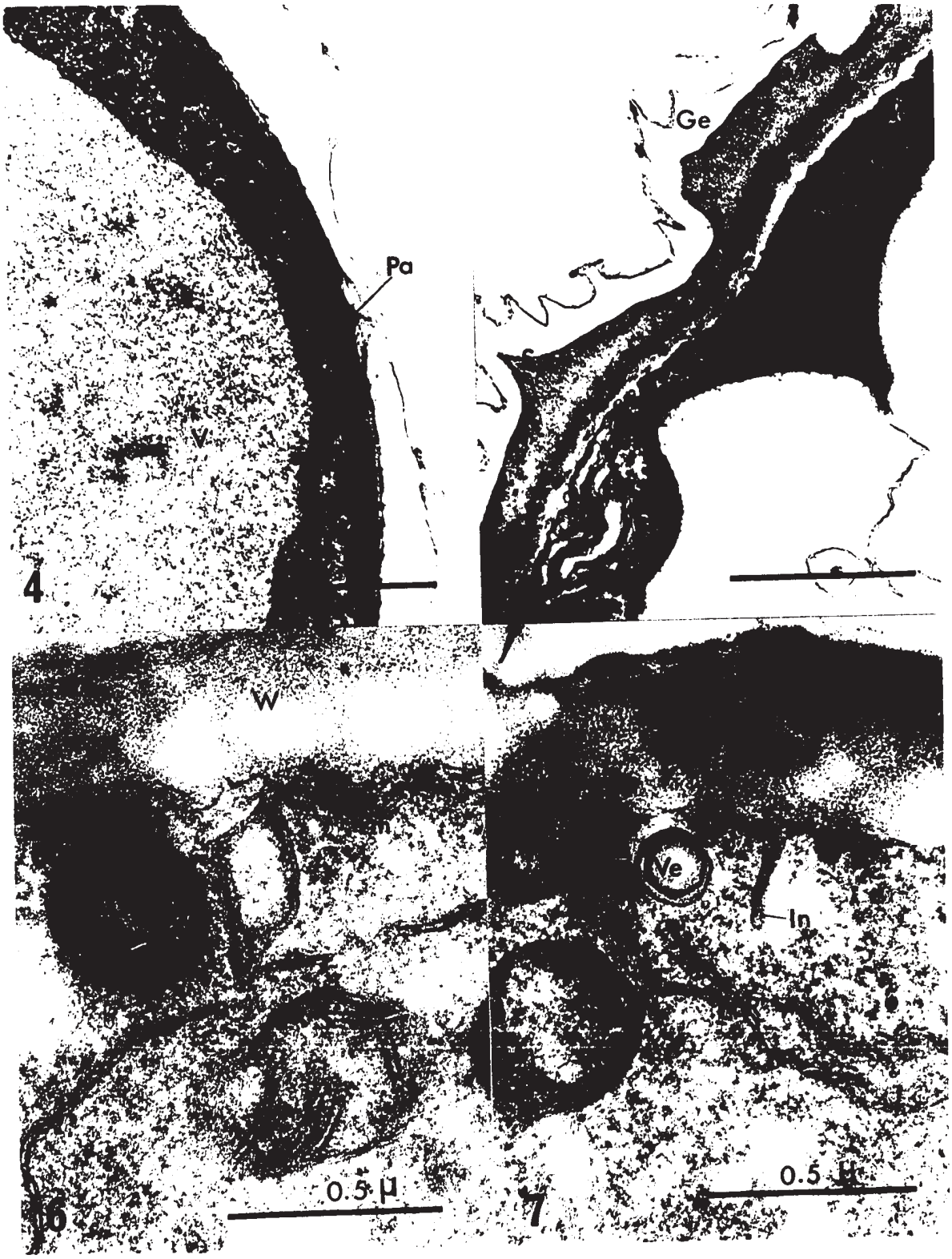

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Figure 8 Cross section of part of a mature conidium showing a myelinoid body in the cytoplasm. Glutaraldehyde and osmium fixed; uranium and lead stained. 48,000x.

Figure 9 Cross section of part of a conidium showing a lomasome-like body and the invaginated plasma membrane.
Potassium permanganate fixed; uranium and lead stained. 32,000x.

Figure 10 Cross section of part of the tip of a conidium showing a myelinoid body in a vacuole with one end connected with the tonoplast.
Glutaraldehyde and osmium fixed; lead stained. 18,000x.

Figure 11 Cross section of part of a mature conidium showing numerous membrane structures and vesicles in the cytoplasm. Note also the glycogen particles. Glutaraldehyde and osmium fixed; uranium and lead stained. 36,000x.

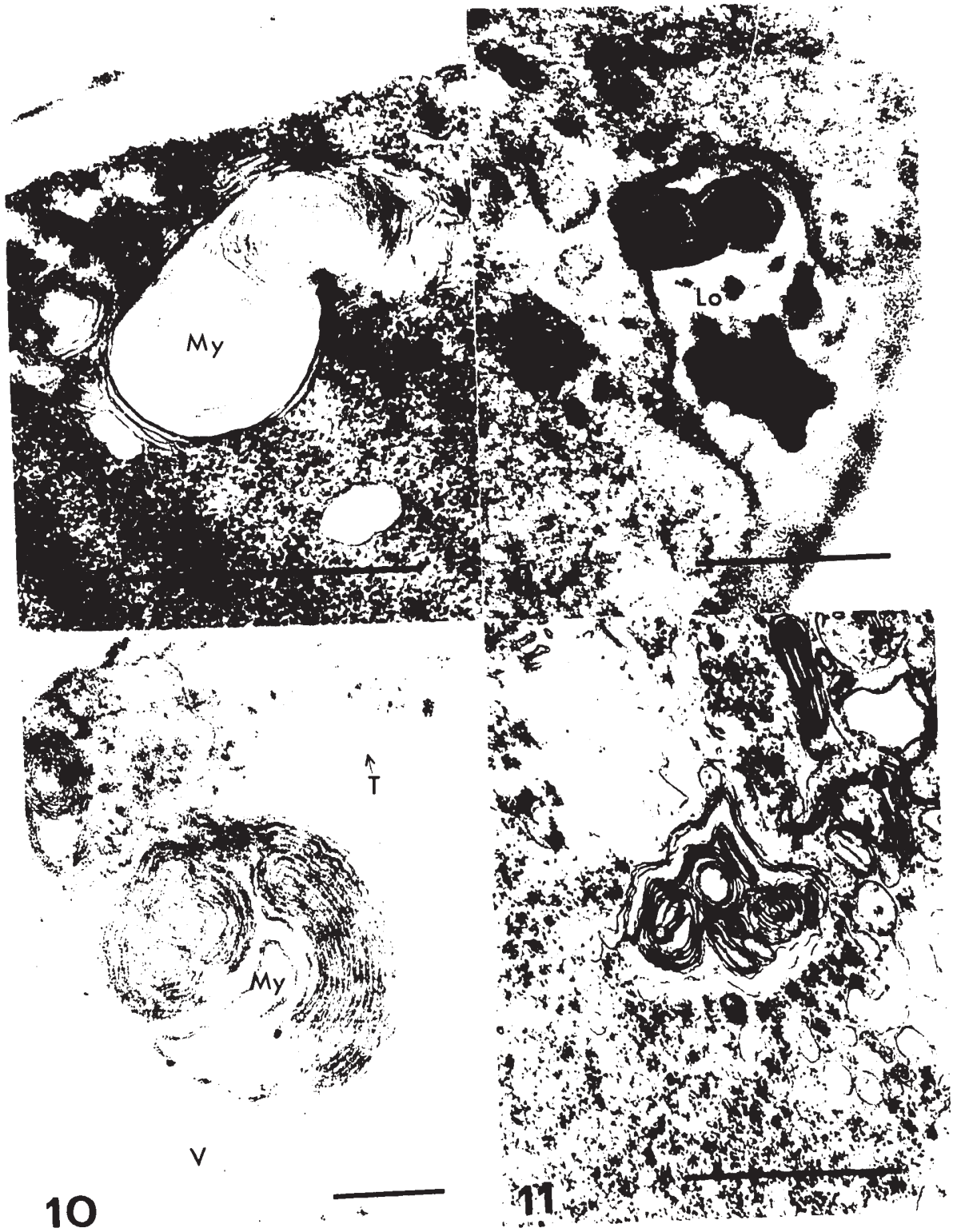
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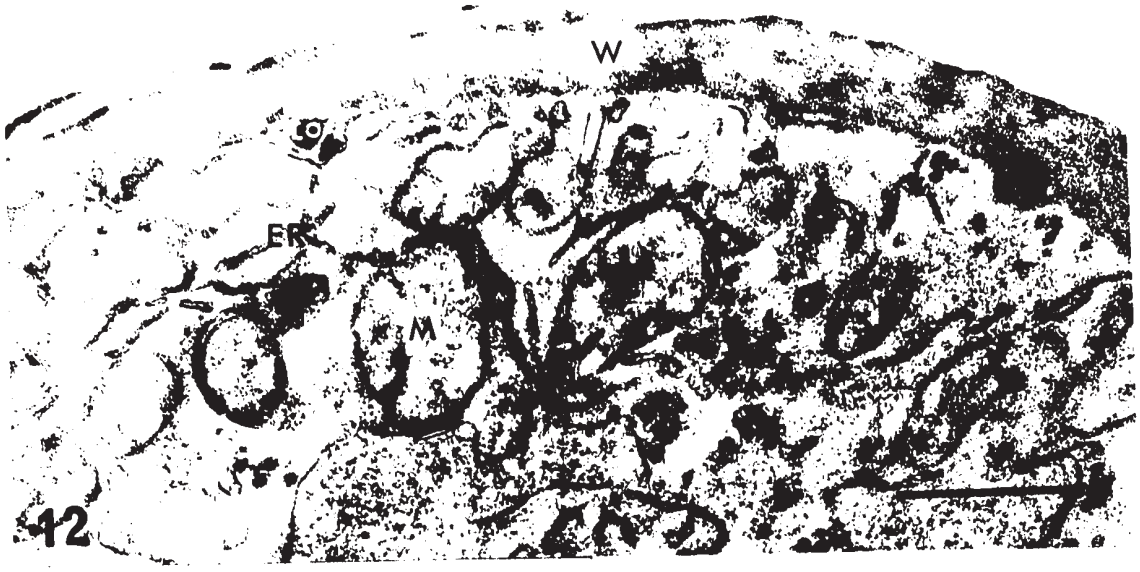
Figure 12 Cross section of part of a mature conidium showing lomasomes and other cell organelles. Potassium permanganate fixed; uranium and lead stained. 25,000x.

Figure 13 Cross section of part of a germinated (12 hr) conidium showing many lomasomes along the wall. Note the numerous mitochondria and E.R. Potassium permanganate fixed; uranium and lead stained. 16,000x.

Figure 14 A serial cross section adjacent to the one shown in Figure 13. Note the slightly different appearance of the lomasomes and the identical mitochondria. 13,000x.

Figure 15 A cross section of part of a germinated conidium. Different-sized vesicular structures in the lomasome are shown. Note also the mitochondria, glycogen and the plasma membrane. Potassium permanganate fixed; uranium and lead stained. 49,000x.

Figure 16 A serial cross section adjacent to the one shown in Figure 15. The lomasomes, mitochondria, E.R., and glycogen are shown. Potassium permanganate fixed; uranium and lead stained. 21,000x.



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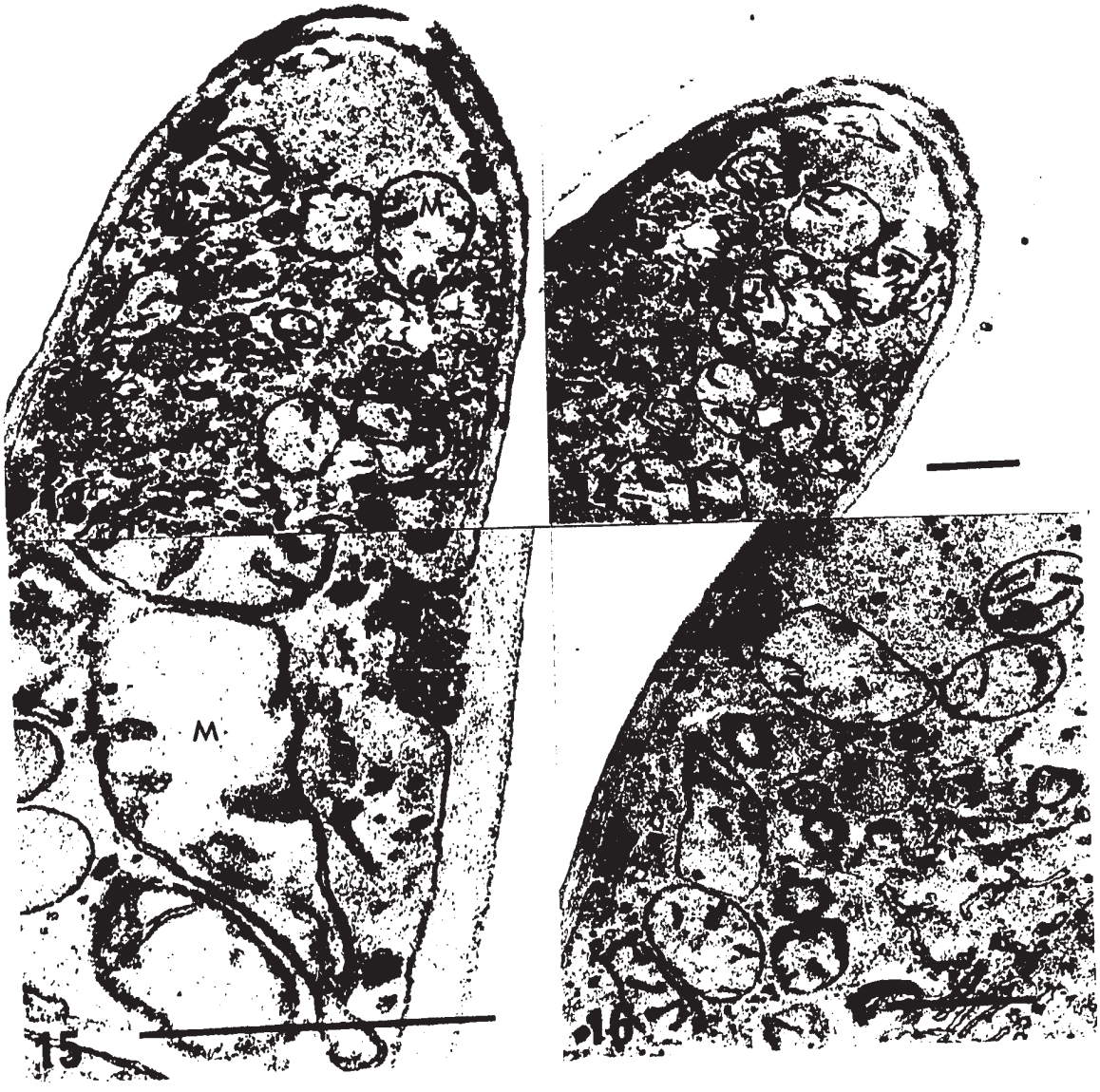

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Figure 17 Longitudinal section of a mature non-germinated conidium. Note the numerous interlocking vacuoles which occupy most of the space in the cytoplasm. Glutaraldehyde and osmium fixed; uranium and lead stained. 3800x.

Figure 18 Portion of a section of a mature conidium showing net-like heterogenous materials in the vacuoles. Note the nucleus with nucleolus at the center of the conidium.
Glutaraldehyde and osmium fixed; uranium and lead stained. 9100x.

Figure 19 Cross section of a germinated conidium (24 hr). Note the large interlocked vacuoles and the nucleus with nuclear pores; no glycogen particles are observed.
Glutaraldehyde and potassium permanganate fixed; uranium and lead stained. 7400x.

Figure 20 Longitudinal section of a germinated conidium (36 hr) showing the large vacuoles and the relatively small amount of cytoplasm. No glycogen is observed.
Glutaraldehyde and osmium fixed; uranium and lead stained. 6400x.

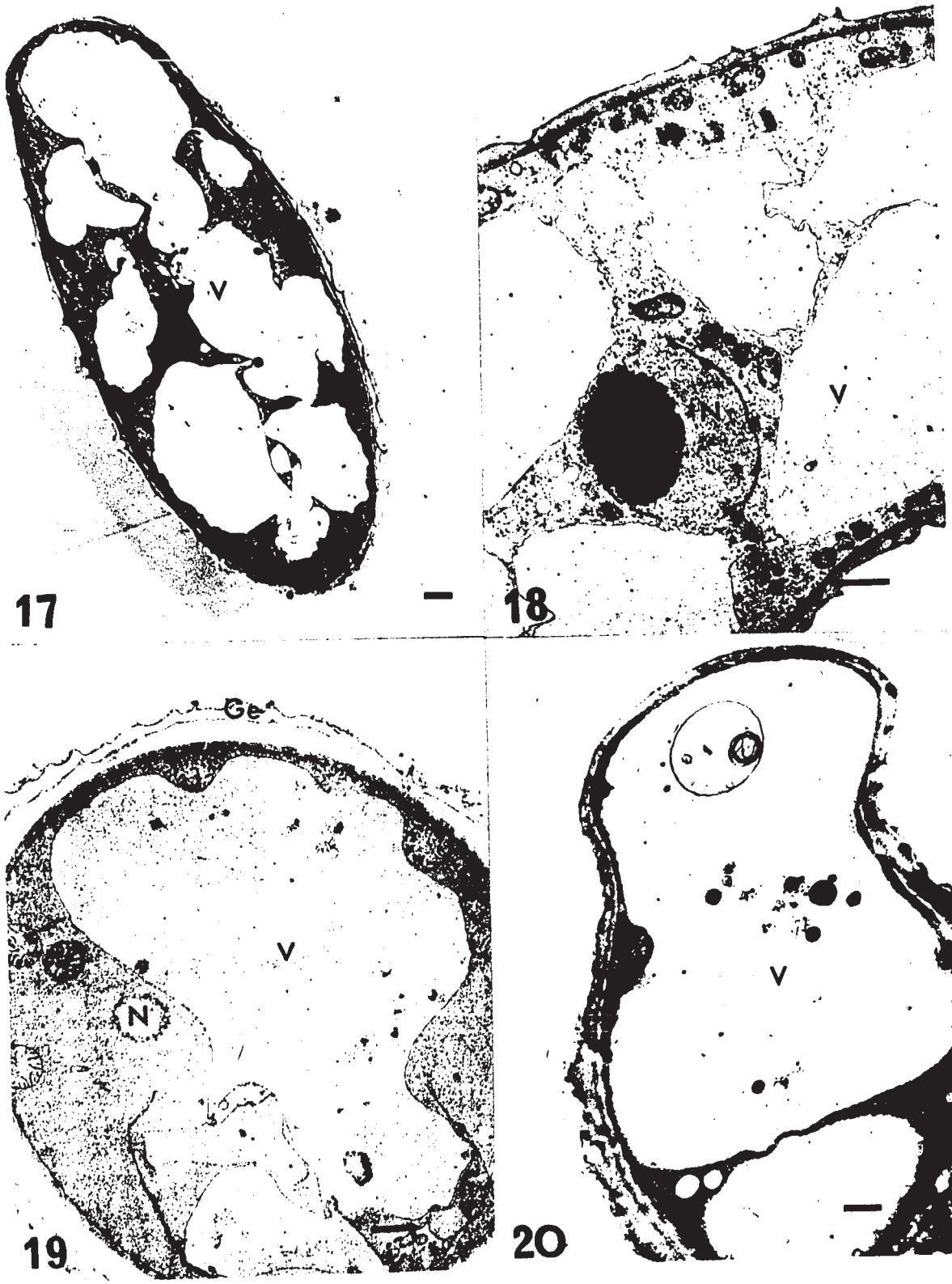
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Figure 21 Portion of a section of a non-germinated conidium showing the myelinoid structure in the vacuole. The whorled membranes are connected with the cytoplasm. Glycogen and the lipid bodies are present in the cytoplasm.
Glutaraldehyde and osmium fixed; uranium and lead stained. 24,000x.

Figure 22 Section of part of a non-germinated conidium. The laminated membranous structure in the vacuole is connected at one end to the cytoplasm.
Glutaraldehyde and osmium fixed; uranium and lead stained. 36,000x.

Figure 23 Section of a part of a mature conidium showing the vacuole with various inclusions -- lipid bodies, vesicles and the heterogenous material. Glycogen is present in the cytoplasm.
Glutaraldehyde and osmium fixed; uranium and lead stained. 20,000x.

Figure 24 Section of the tip of a germinated conidium (36 hr) showing expanded membranous structures inside the vacuole. Note the myelinoid body is enclosed in another membraned body.
Glutaraldehyde and osmium fixed; uranium and lead stained. 25,000x.

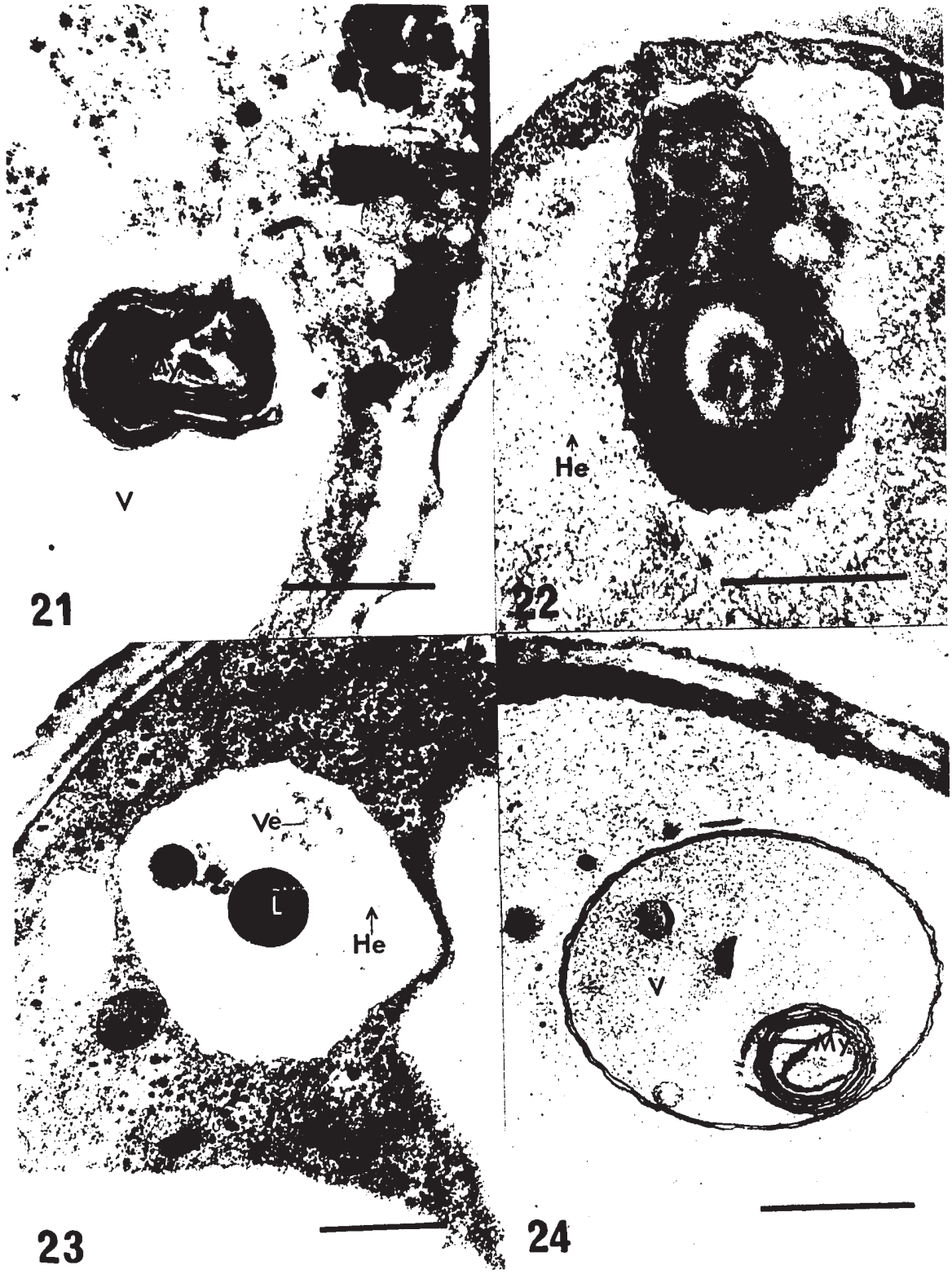
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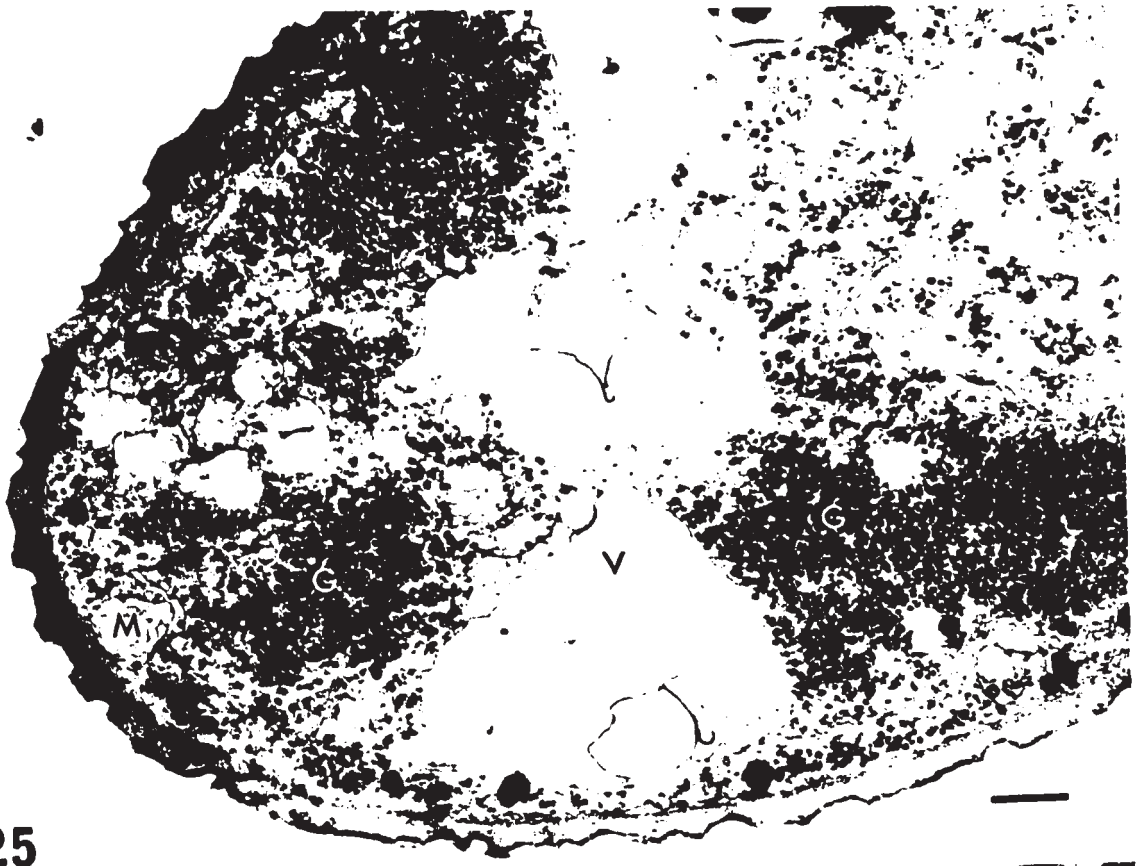
Figure 25 Section of part of a non-germinated conidium showing compact groups of glycogen particles which are dispersed throughout the cytoplasm. Glutaraldehyde and osmium fixed; uranium and lead stained. 10,000x.

Figure 26 Portion of Figure 25 at a higher magnification. Rosettes of glycogen particles, each consists of 6-10 individual units.
90,000x.



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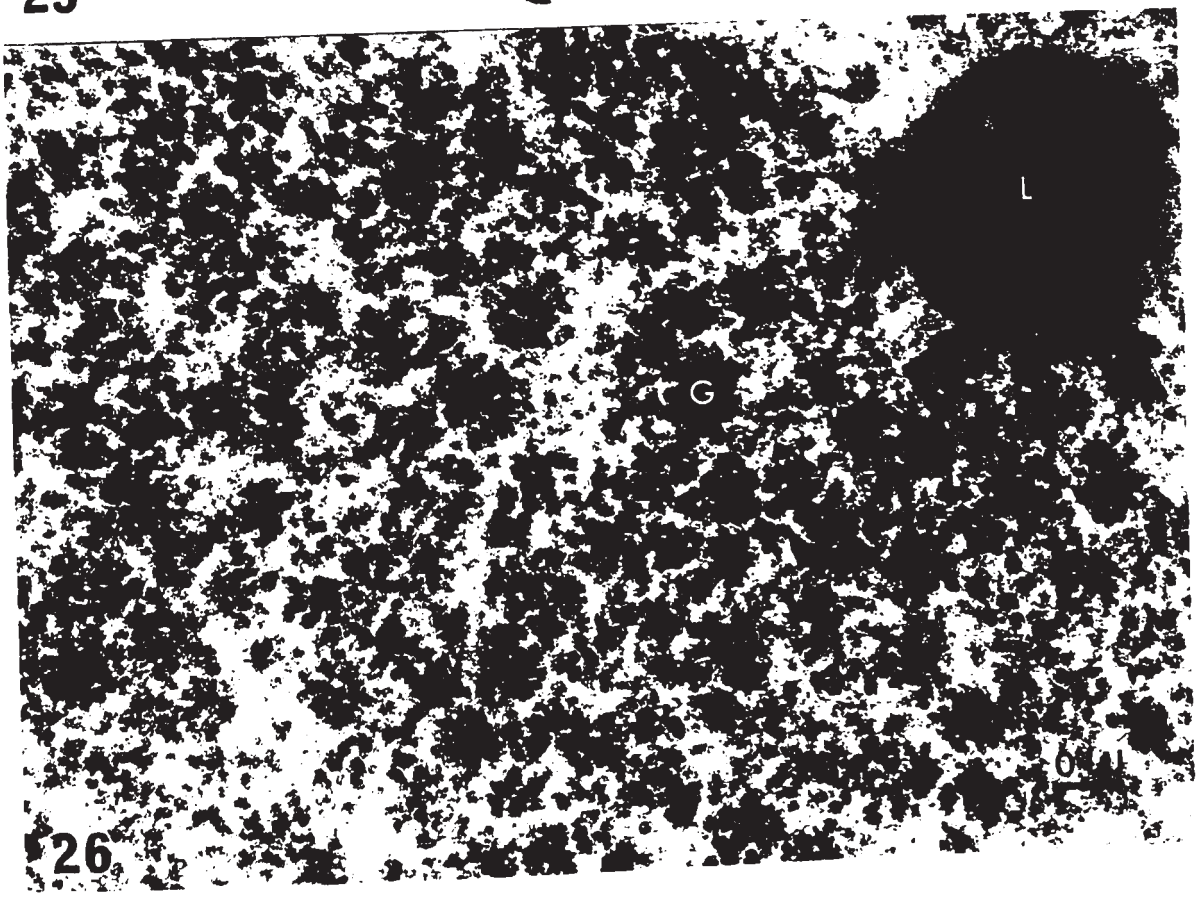

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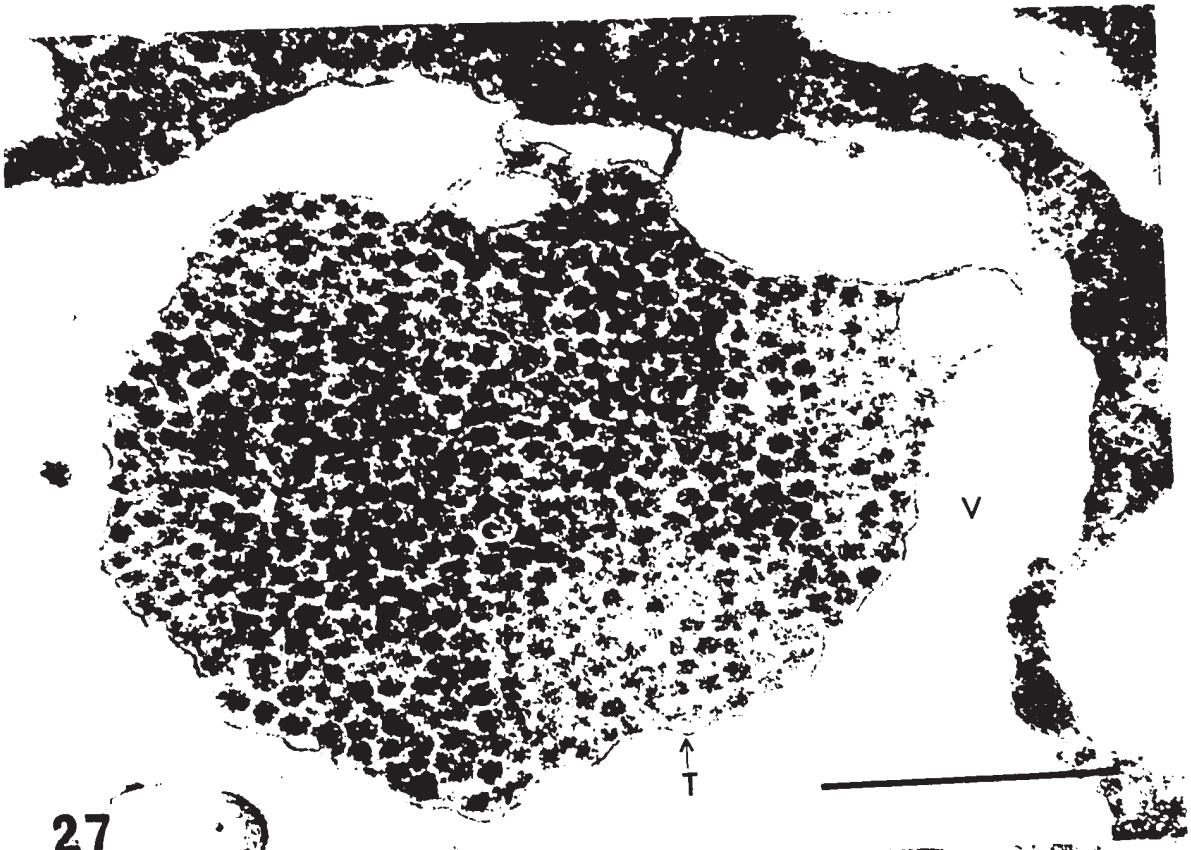
Figure 27 A section of part of a non-germinated conidium which contains a compact package of glycogen particles bounded by a membrane, inside a vacuole. Dense glycogen particles are present in the cytoplasm also.
Glutaraldehyde and osmium fixed; uranium and lead stained. 39,000x.

Figure 28 Section of part of a non-germinated conidium containing a group of glycogen rosettes in the cytoplasm. Note the invaginated plasma membrane. Due to permanganate fixation the sub-unit of each rosette of glycogen is not discernible.
Potassium permanganate fixed; uranium and lead stained. 30,000x.



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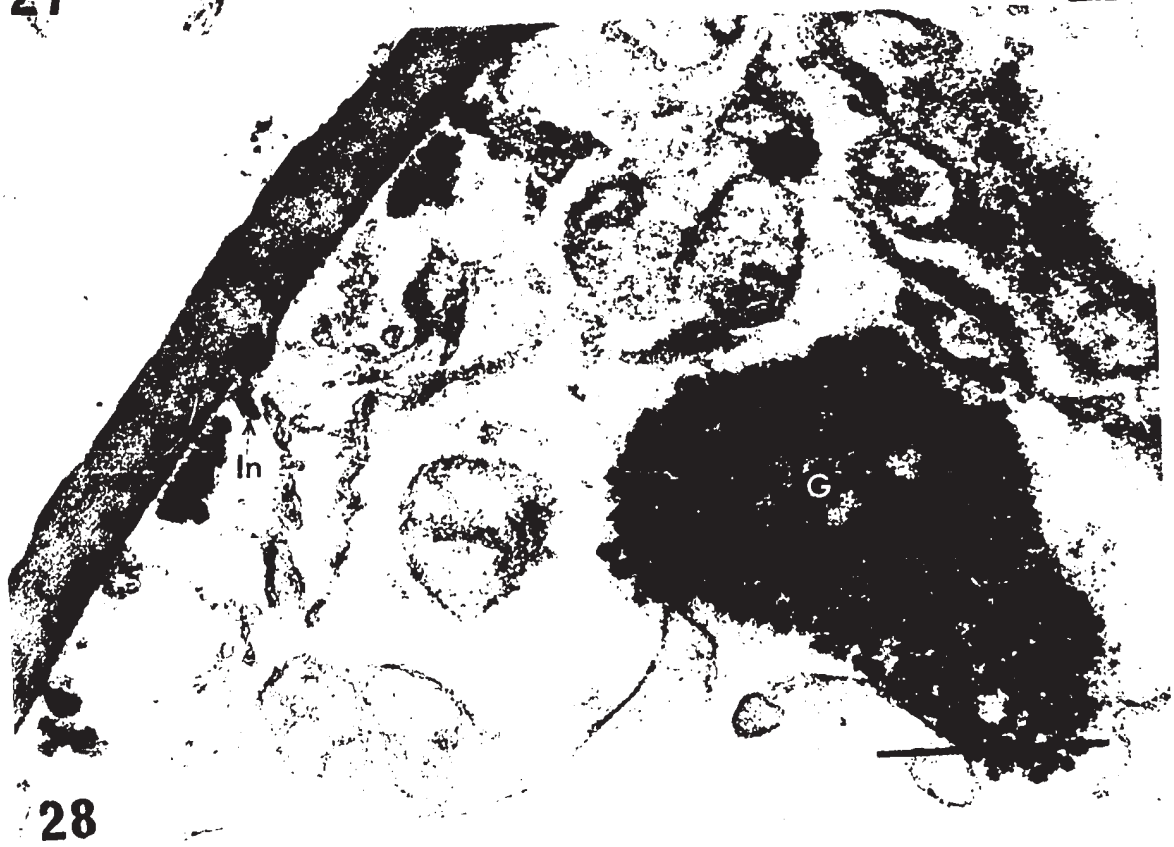

28

Figure 29 Light micrograph of a whole mature conidium treated with Bauer-Fuelgen solution to show presence of glycogen. Each of the glycogen groups is identical to that shown in Figure 25, 28 and 30. Note the glycogen occupies much of the space in the cell. 3400x.

Figure 30 Section of a mature conidium negatively stained with aqueous uranyl acetate solution. Each white spot represents a rosette of glycogen which is identical to that shown in Figure 26. Note many lipid bodies, mitochondria and E.R. at the periphery of the conidium.

Glutaraldehyde and osmium fixed; stained with 0.5% of uranyl acetate solution alone prior to the embedding. 4000x.

Figure 31 Section of part of a mature conidium treated with the enzyme, ∝-amylase and then stained with lead citrate solution. Most of the glycogen particles are removed leaving many blank spots in the cytoplasm. Mitochondria and lipid bodies are still discernible.

Glutaraldehyde and osmium fixed. 12,000x.

Figure 32 Serial section adjacent to the section shown in Figure 31 which was not treated with ∝-amylase. The glycogen particles are grouped and electron dense.

Glutaraldehyde and osmium fixed; lead stained. 12,000x.

Figure 33 Section of a part of a mature conidium treated with ∝-amylase for 5 hr and then stained with lead. The blank spots represent the location of the rosettes of glycogen particles which were completely digested. Note the spore wall and mitochondria are still discernible.

Glutaraldehyde and osmium fixed; Araldite embedded. 14,000x.

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Figure 34 Electron micrograph of the isolated glycogen suspension stained with lead citrate. It shows some glycogen rosettes. Note they are darkly stained and some sub-units are still discernible. Their appearance in thin section may be compared to those in Figure 26. 89,000x.

Figure 35 Electron micrograph of glycogen suspension after phosphotungstic acid staining. The glycogen particles appear negatively stained. They may be compared with those in Figure 34. 50,000x.

Figure 36 Electron micrograph of glycogen suspension after α-amylase treatment and staining in lead citrate solution. The light spots are the locations of the glycogen particles which have been dissolved away due to enzyme treatment. Figure 34 and Figure 35 may be compared with Figure 36. 29,000x.

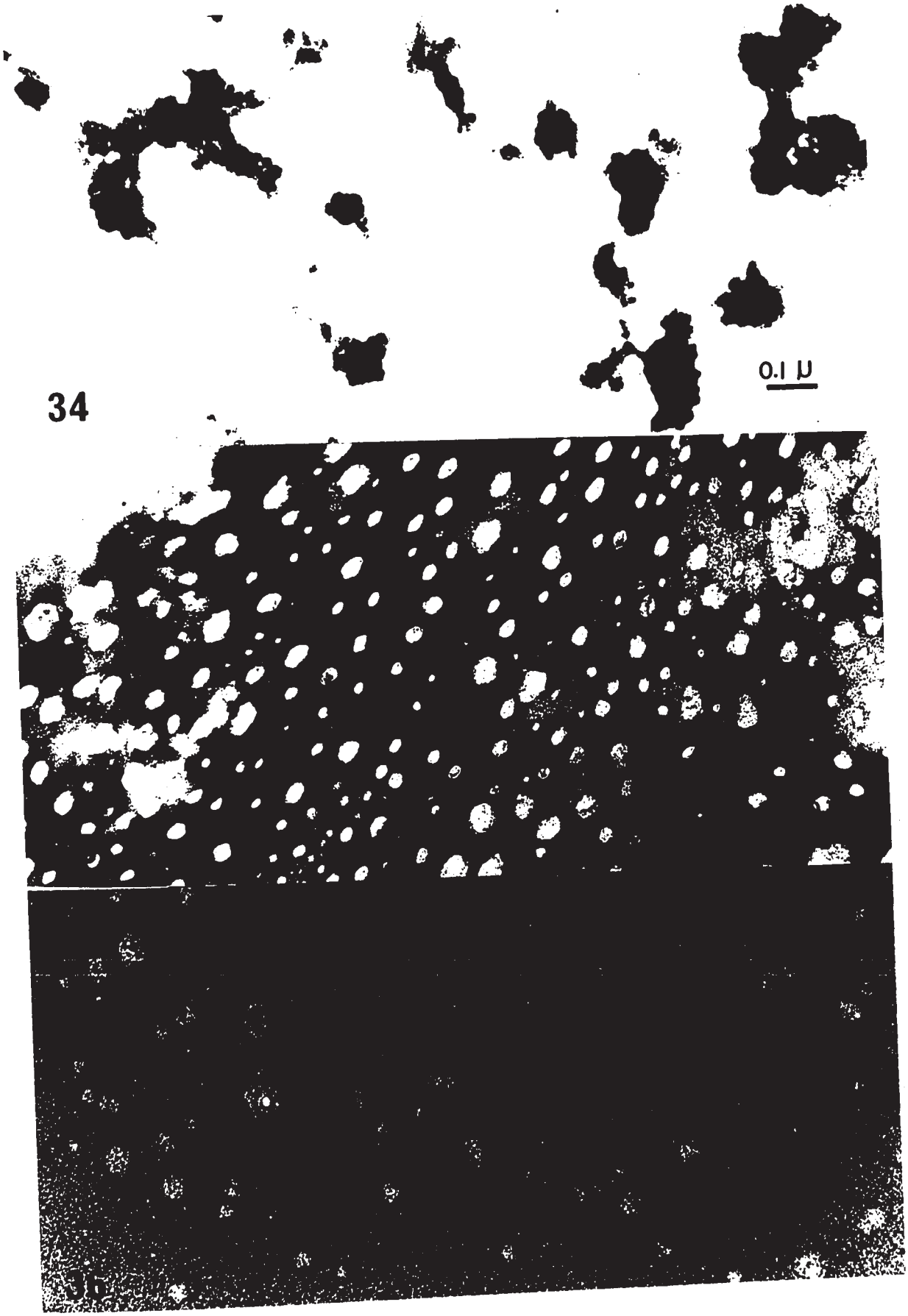
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Figure 37 Section of part of a mature conidium showing different cell organelles: the plasma membrane, endoplasmic reticulum, mitochondria with sparse cristae and the oval-shaped lipid body. The glycogen particles are evenly distributed in the cytoplasm.

Glutaraldehyde and osmium fixed; uranium and lead stained. 30,000x.

Figure 38 Section of a portion of a mature conidium showing part of the plasmalemma unit membrane and part of a mitochondrion. The inner membrane of the mitochondrion invaginates to form the cristae. Note the glycogen is in close association with the mitochondrion.

Glutaraldehyde and osmium fixed; uranium and lead stained. 118,000x.

Figure 39 Section of a portion of a mature conidium. A rosette of glycogen is observed within the mitochondrion (arrow). The glycogen is also closely associated with the endoplasmic reticulum.

Glutaraldehyde and osmium fixed; uranium and lead stained. 42,000x.

Figure 40 Section of part of a mature conidium showing a nucleus and a dumb-bell shaped mitochondrion.

Glutaraldehyde and osmium fixed; uranium and lead stained. 14,000x.

Figure 41 Portion of mature conidium showing a v-shaped mitochondrion with a narrow neck at the centre.

Osmium tetroxide fixed; lead stained. 34,000x.

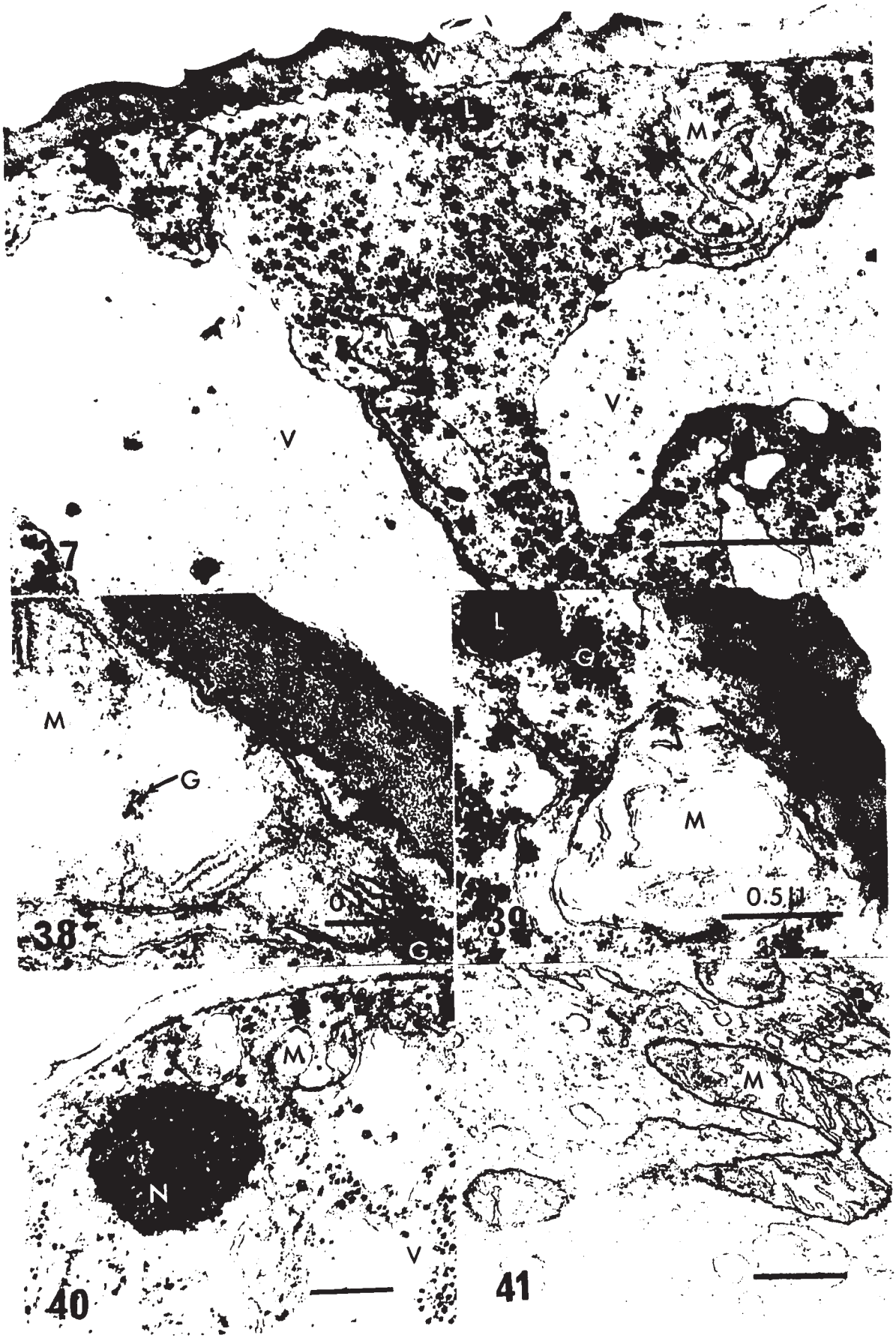
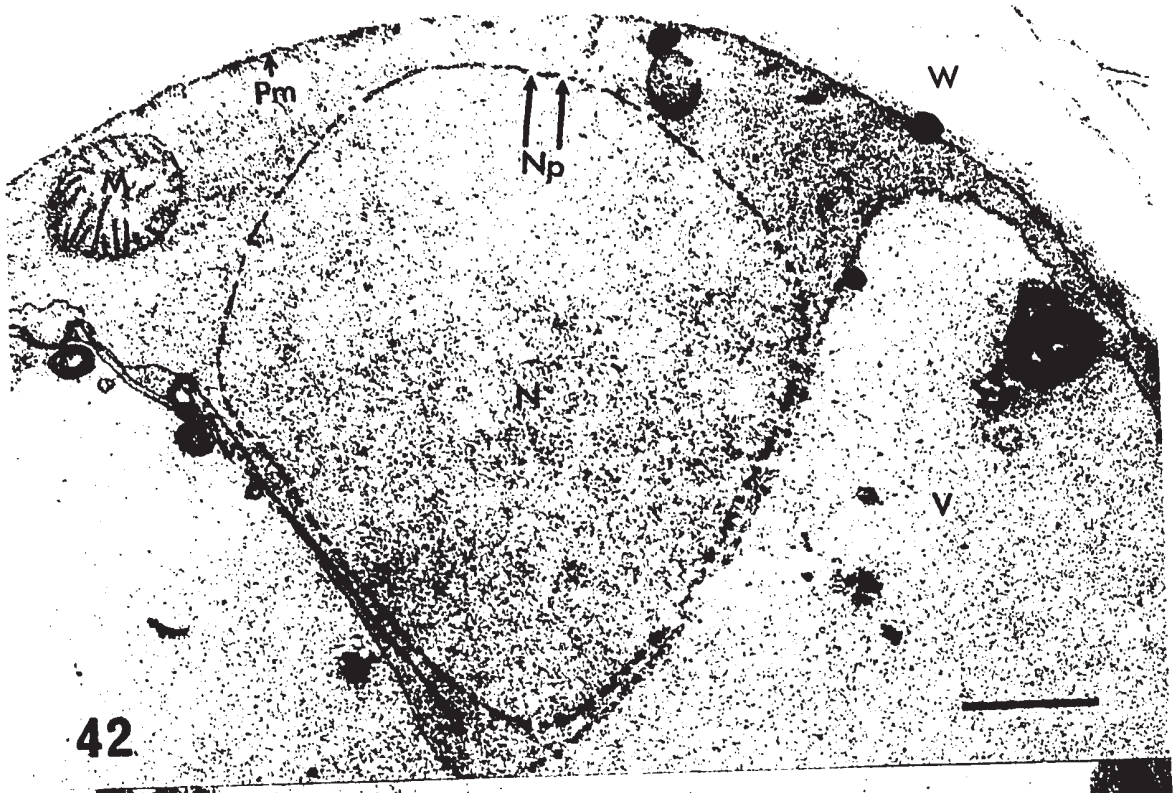

V
L
M
V
V
7
L
G
M
M
G
G
38
39
0.5 μ
M
M
N
V
40
41

Figure 42 Section of part of a germinated conidium (24 hr) showing some cell organelles. Note the nucleus with nuclear pores and the distinct tube-like cristae of the mitochondrion. Compare figures 25, 28, and 40.

Glutaraldehyde and potassium permanganate fixed; uranium and lead stained. 18,000x.

Figure 43 Section of part of a conidium, germinated 36 hr, showing the netted heterogenous materials in vacuoles and lack of glycogen in the cytoplasm. Glutaraldehyde and osmium fixed; uranium and lead stained. 20,000x.



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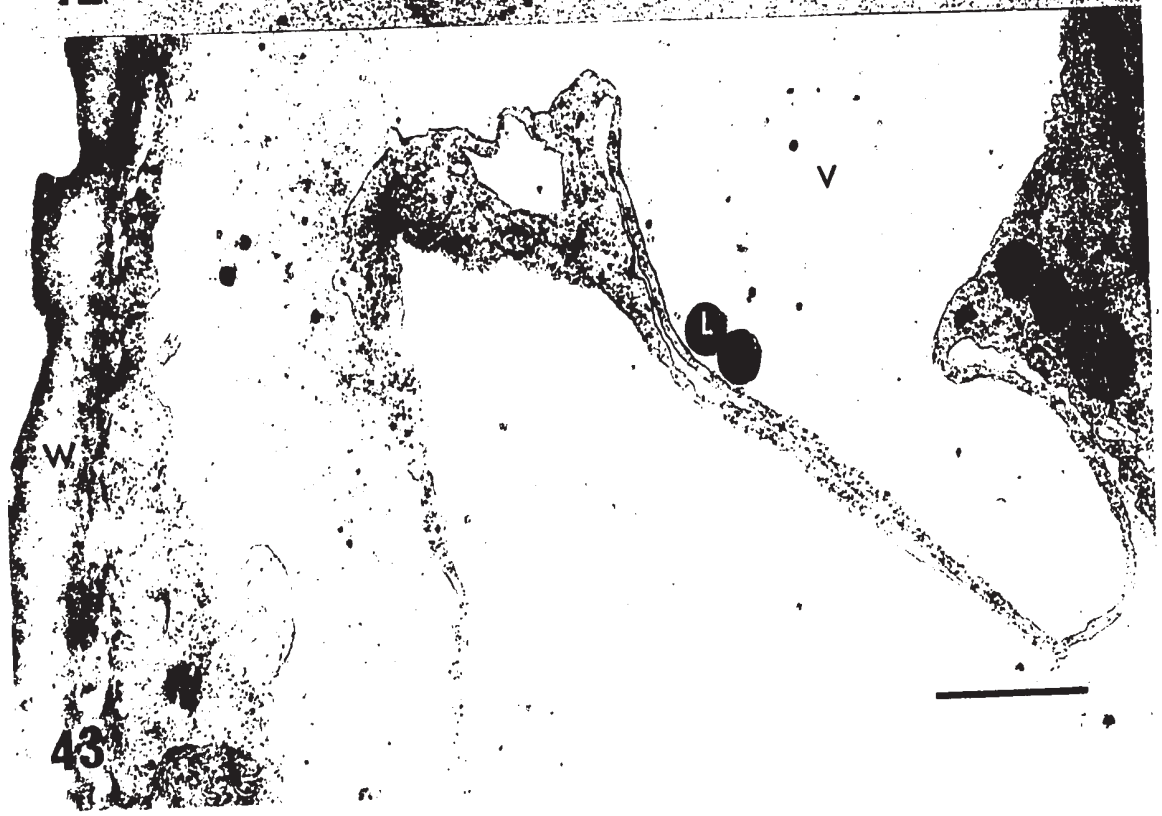


43

Figure 44 Section of part of a germinated conidium (18 hr) showing mitochondria, endoplasmic reticulum, lipid and some glycogen particles. Osmium tetroxide fixed; lead stained. 20,000x.

Figure 45 Section of part of germinated conidium (36 hr) showing a nucleus, vacuole with lipid inclusion. No glycogen particles are present. Glutaraldehyde and osmium fixed; uranium and lead stained. 17,000x.

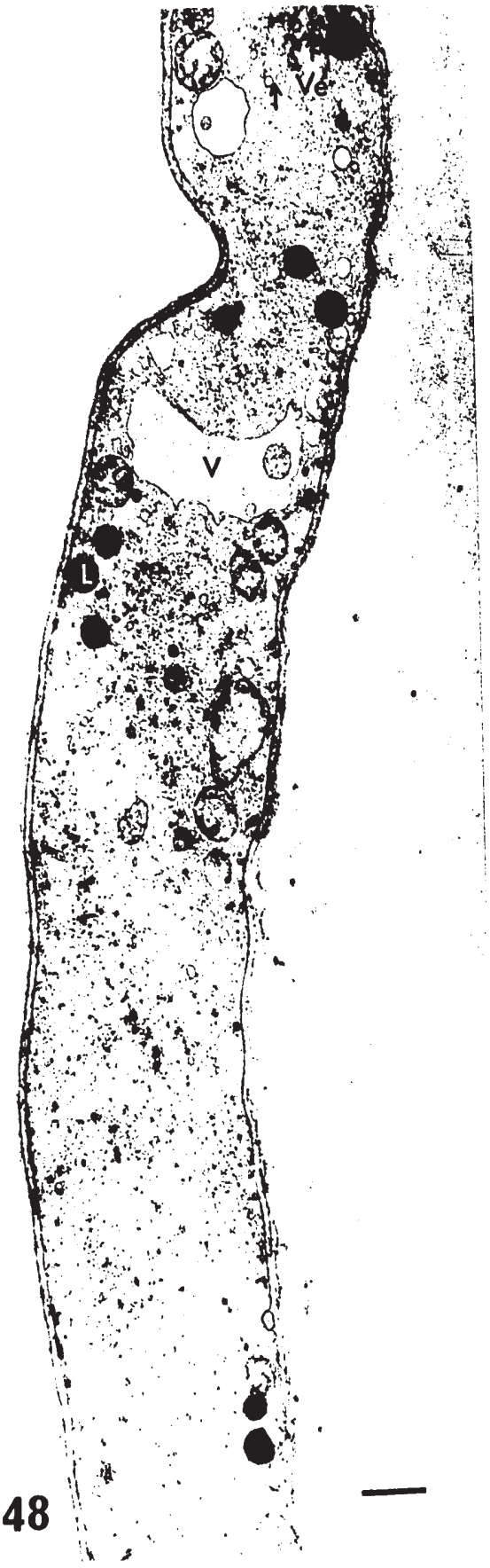
Figure 46 Cross section of a germ tube produced by a conidium, 18 hr after germination, showing prominent nucleus with nucleolus, oval-shaped mitochondria and the E.R. around the nucleus. Potassium permanganate fixed; uranium and lead stained. 13,000x.

Figure 47 Cross section of a germ tube produced by a conidium, 36 hr after germination, showing vacuoles, oval-shaped mitochondria, lipid bodies and numerous ribosomal particles. Glutaraldehyde and osmium fixed; uranium and lead stained. 16,000x

Figure 48 Longitudinal section of part of a germ tube, 36 hr after germination, produced by a conidium. Note the mitochondria and lipid bodies and the many vesicles situated near the tip of the germ tube. The ribosomal particles are evenly distributed. Glutaraldehyde and osmium fixed; uranium and lead stained. 10,000x.

Figure 49 Section of a germ tube showing a portion of the tip area. Note numerous vesicles of different sizes at the apex, intermixed with some endoplasmic reticulum. Mitochondria are not observed in the terminal 2 microns of the germ tube but are abundant in other regions.
Potassium permanganate fixed; uranium and lead stained. 16,000x.

Figure 50 A longitudinal section of a part of a hypha showing a protrusion of a side branch (arrow). Osmium tetroxide fixed; lead stained. 18,000x.



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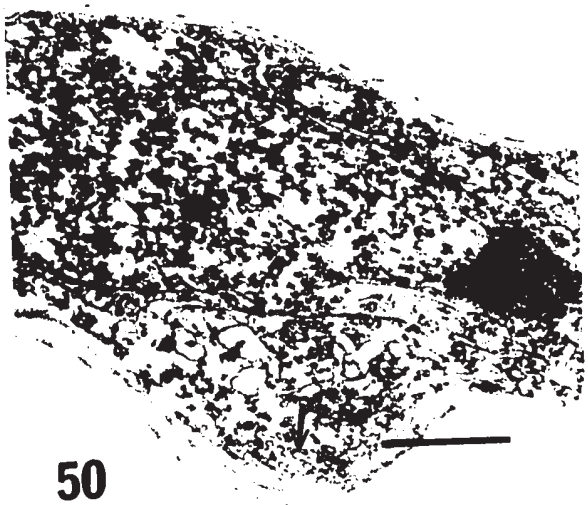


50

Figure 51 Longitudinal section of a portion of a long germ tube from a 36 hr germinated conidium showing the cytoplasm and large vacuole. Glutaraldehyde and osmium fixed; uranium and lead stained. 51,000x.

Figure 52 Portion of the germ tube in Figure 51, showing the vacuole with inclusions, tonoplast, lipid bodies, ribosomes and a few glycogen rosettes. 32,000x.

Figure 53 Section of a conidium, 36 hr after germination showing an elongated nucleus situated near the conidial wall. Note the nucleolus and the chromatin bodies. A mitochondrion is sandwiched between the plasma membrane and the nuclear envelope.

Glutaraldehyde and osmium fixed; uranium and lead stained. 13,000x.

51

53

Figure 54 Longitudinal section of a germinated conidium, 36 hr after germination showing a septum in the germ tube at some distance away from the conidium.
Glutaraldehyde and osmium fixed; uranium and lead stained. 3100x.

Figure 55 A magnified portion of Figure 54 showing the centripetal growth of the septum. Observe also that a Woronin body is present at the septal pore and a few other similar bodies are at the side of the cell. 26,000x.

Figure 56 Section of a germ tube showing the septum with 2 Woronin bodies situated opposite each other, one of which appears to be plugging the septal pore.
Glutaraldehyde and osmium fixed; uranium and lead stained. 28,000x.


Se
Se
Wn
V
Vi
55
V
V
C
10 μ
54
56

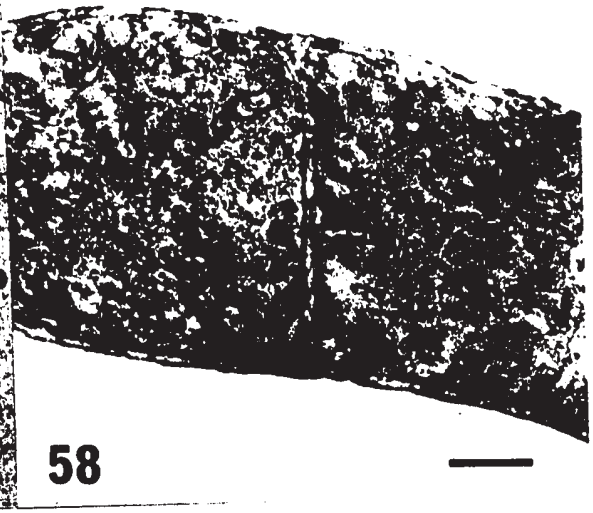
Figure 57 Section of part of a mature conidium and a conidiophore. The septum is dumb-bell shaped. Glutaraldehyde and osmium fixed; uranium and lead stained. 8400x.

Figure 58 Part of a longitudinal section of a hypha showing a septum with Woronin bodies at both sides near the center.
Potassium permanganate fixed; uranium and lead stained. 11,000x.

Figure 59 Part of a longitudinal section of hypha showing 2 adjoining cells with a septum. Observe that the darkly stained Woronin body appears different from the lipid bodies which have only a dark outline with a blank center. No such difference is observed in the glutaraldehyde fixed materials (Figs. 55, 56, and 57).
Potassium permanganate fixed; uranium and lead stained. 13,000x.

Figure 60 Cross section of 2 hyphae showing vacuoles, oval-shaped mitochondria and other cell organelles. Note the nucleolus with a lightly-stained area on left.
Glutaraldehyde and osmium fixed; uranium and lead stained. 24,000x.

57



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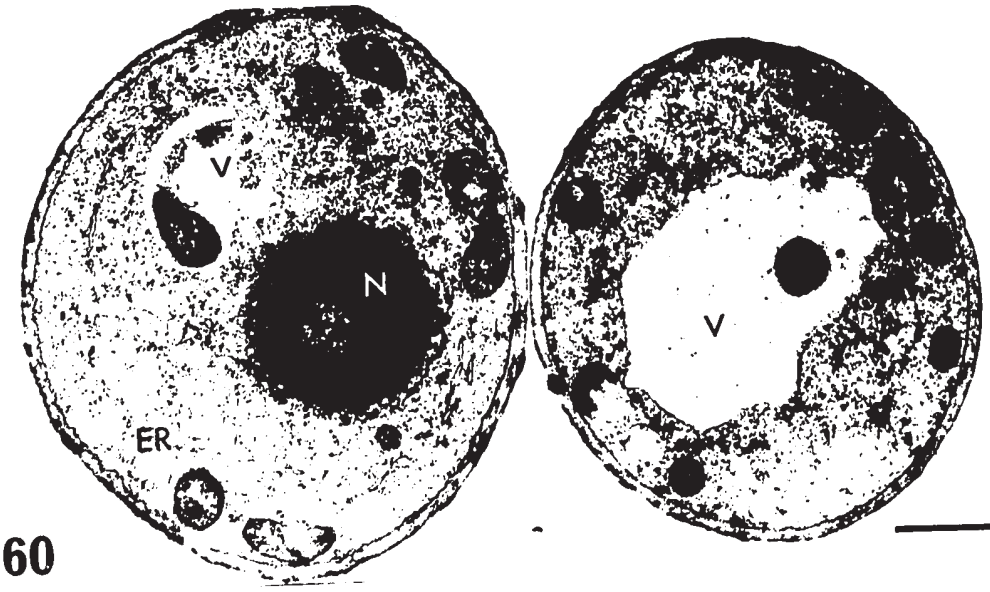
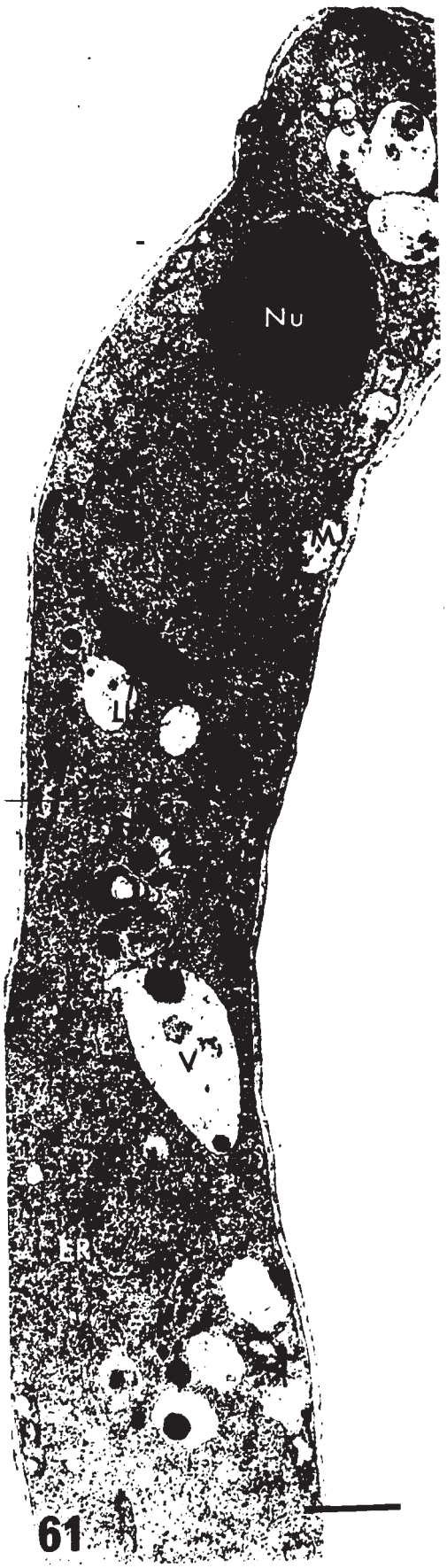


60

Figure 61 Section of a hypha showing the dense cytoplasm. Note the parallel endoplasmic reticulum, elongated mitochondria, myelinoid body and a prominent nucleus. A lateral or peripheral body is observed at one end of the nucleus (arrow). It is closely associated with the nuclear envelope.
Glutaraldehyde and osmium fixed; uranium and lead stained. 14,000x.

Figure 62 Portion of a hypha showing various cytoplasmic organelles. Note the numerous lipid inclusions in the vacuoles.
Glutaraldehyde and osmium fixed; uranium and lead stained. 14,000x.



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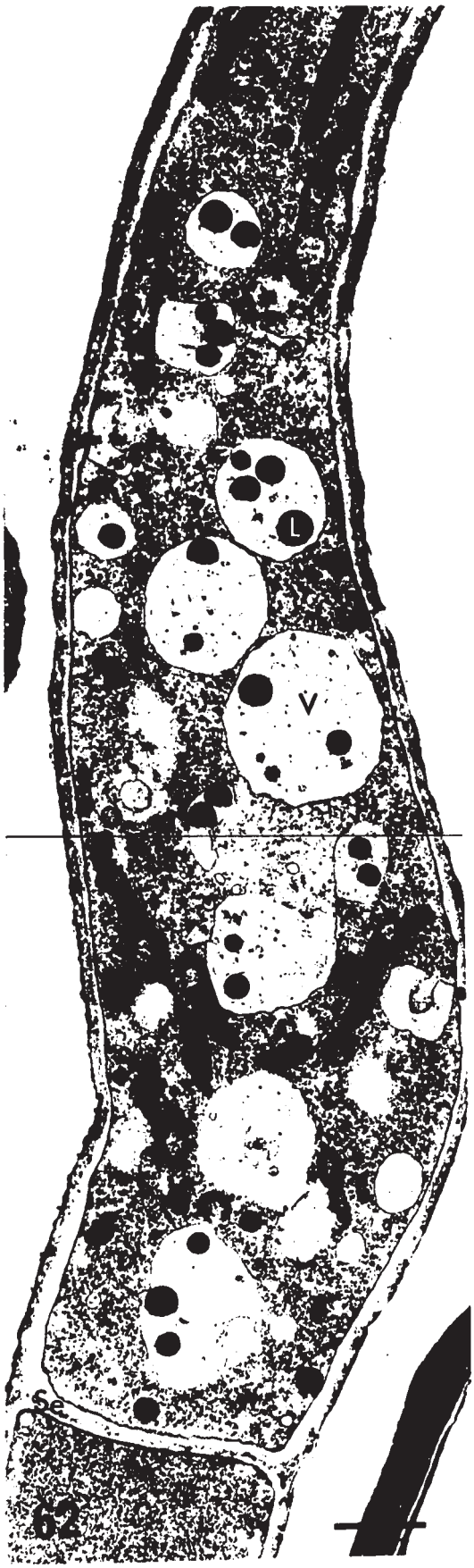


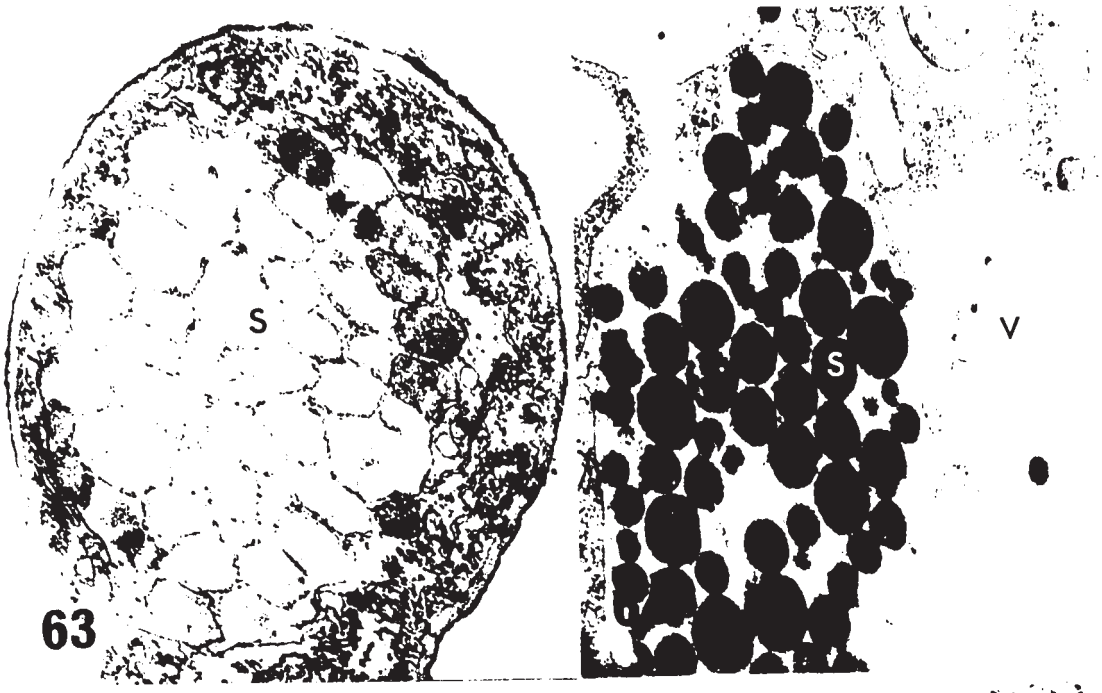
62

Figure 63 Section of a hypha showing a large group of spherosome-like bodies, bounded by a membrane. Each granule is in turn surrounded by its own membrane. Note the different degrees of staining. Some of the outer bodies are deeply stained.
Osmium fixed; lead stained. 36,000x.

Figure 64 Section of a hypha showing a large group of spherosome-like bodies surrounded by a membrane which appears similar to the tonoplast. The bodies are electron dense and appear oval in shape.
Glutaraldehyde and osmium fixed; uranium and lead stained. 18,000x.

Figure 65-67 Section of a part of hypha fixed with potassium permanganate. Note the granules are of 2 sizes in Figure 65. In Figure 66, they are tightly packed in a group. In Figure 67, they appear to be part of the inclusions in the vacuole and are separated.

Figure 65 - 8,000x.
Figure 66-- 15,000x.
Figure 67 - 18,000x.



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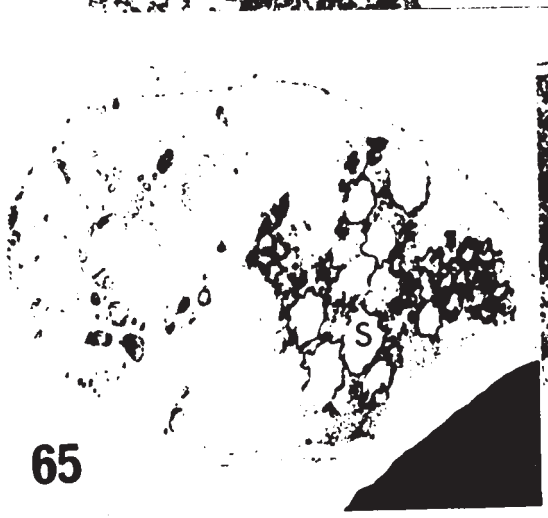

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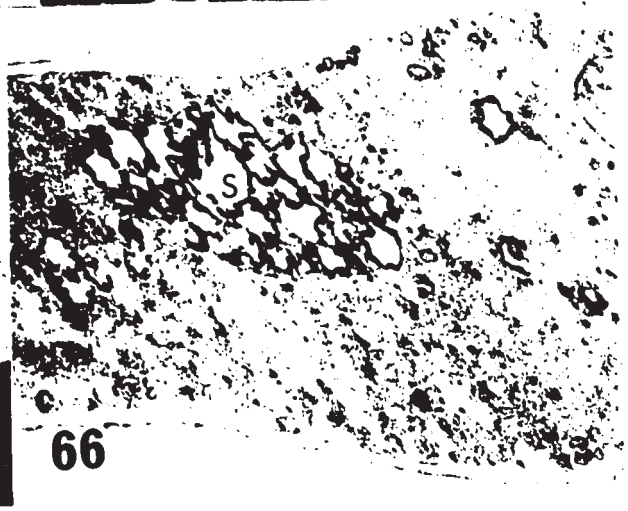

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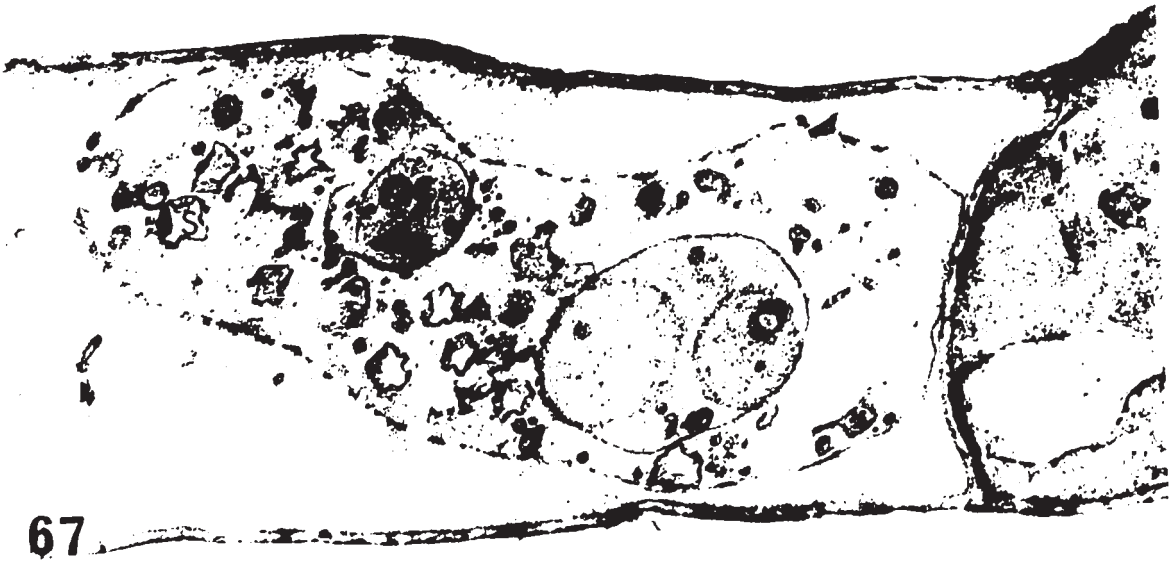

67

Figure 68 Section of a conidiophore. Note the concave septum at the top and the convex septum at the bottom. A nucleus is seen in each of the cells. Potassium permanganate fixed; lead stained. 4600x.

Figure 69 A highly magnified portion of the conidiophore tip shown in Figure 68. Note the nucleus, numerous vacuoles and mitochondria. Note also the dumb-bell shaped septum. Potassium permanganate fixed; lead stained. 12,000x.

68

69

Figure 70 Section of a conidiophore showing a terminal developing conidium. Note the dense cytoplasm with numerous oval and elongated mitochondria, endoplasmic reticulum and vacuoles.
Potassium permanganate fixed; lead stained.
7000x.

Figure 71 Section of a maturing conidium showing a nucleus and numerous vacuoles and mitochondria. Note the cell is slightly expanded at centre. Compare Figures 68 and 69.
Potassium permanganate fixed; lead stained.
6000x.

Figure 72 Section of a fully developed conidium attached to a developing conidium. Note the highly expanded spore with a mucilaginous layer. The cresent septum becomes the conidial wall of the adjoining conidia. Groups of glycogen particles are present in the cytoplasm along with much endoplasmic reticulum.
Potassium permanganate fixed; lead stained.
8000x.

Figure 73 Longitudinal section of a fully developed conidium showing a nucleus at one side, groups of glycogen particles, E.R., vesicles, mitochondria and the freshly detached abscission end (de) (arrow) of the conidium.
Potassium permanganate fixed; uranium and lead stained. 6000x.

70

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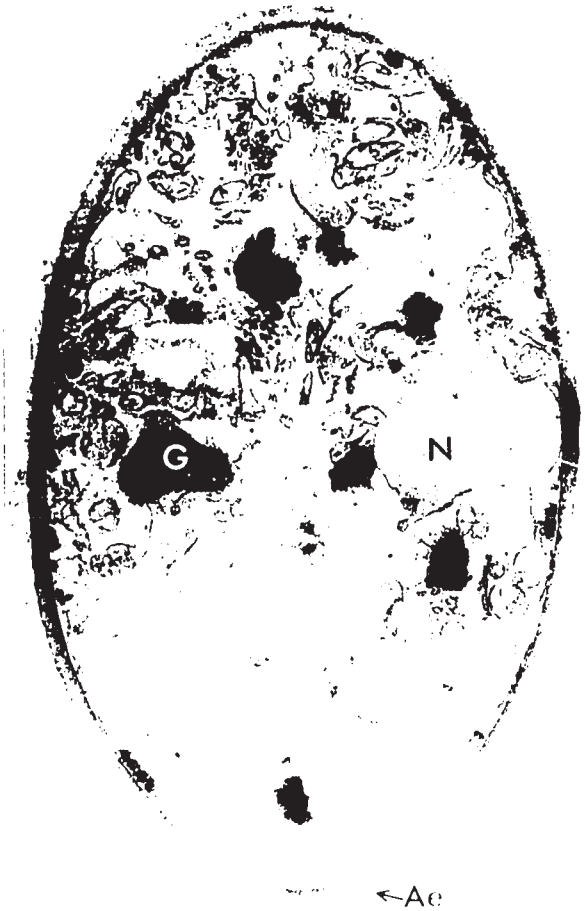


73

Figure 74 Electron micrograph of a chain of conidia of Erysiphe graminis showing several maturing conidia. Note the top 3 spores contain many glycogen particles similar to that shown in Figures 72 and 73. Glycogen particles are not present in the 4th and 5th cell. A number of lipid bodies are observed in the bottom cell but not in the more terminal cells. Note also the dumb-bell shaped septum (arrow) between conidia.
Glutaraldehyde and osmium fixed; lead stained. 5000x.


G
G
N
N
G

Figure 75-83 Light micrographs of a few epidermal cells from susceptible and resistant red clover leaves which have been infected with Erysiphe polygoni.

Figure 75 Epidermal cells from a susceptible red clover leaf upon which a conidium of E. polygoni is resting. The central cell inoculated 18 hr previously shows an appressorium and is stained light blue with Azure B. The noninfected cells are not stained. 760x.

Figure 76 A susceptible infected epidermal red clover cell surrounded by healthy cells. The cell containing the haustorium is deeply stained with Azure B; the healthy cells are unstained. The epidermal cells had been infected for 2 weeks. 900x.

Figure 77 A group of susceptible epidermal red clover cells one has been infected for 7 days. The infected and adjoining cells are stained with Azure B, but the more distant cells are unstained. 470x.

Figure 78 Resistant red clover epidermal cells stained with Azure B, 2 days after inoculation. The central cell is infected and is stained blue-green around its margin. 650x.

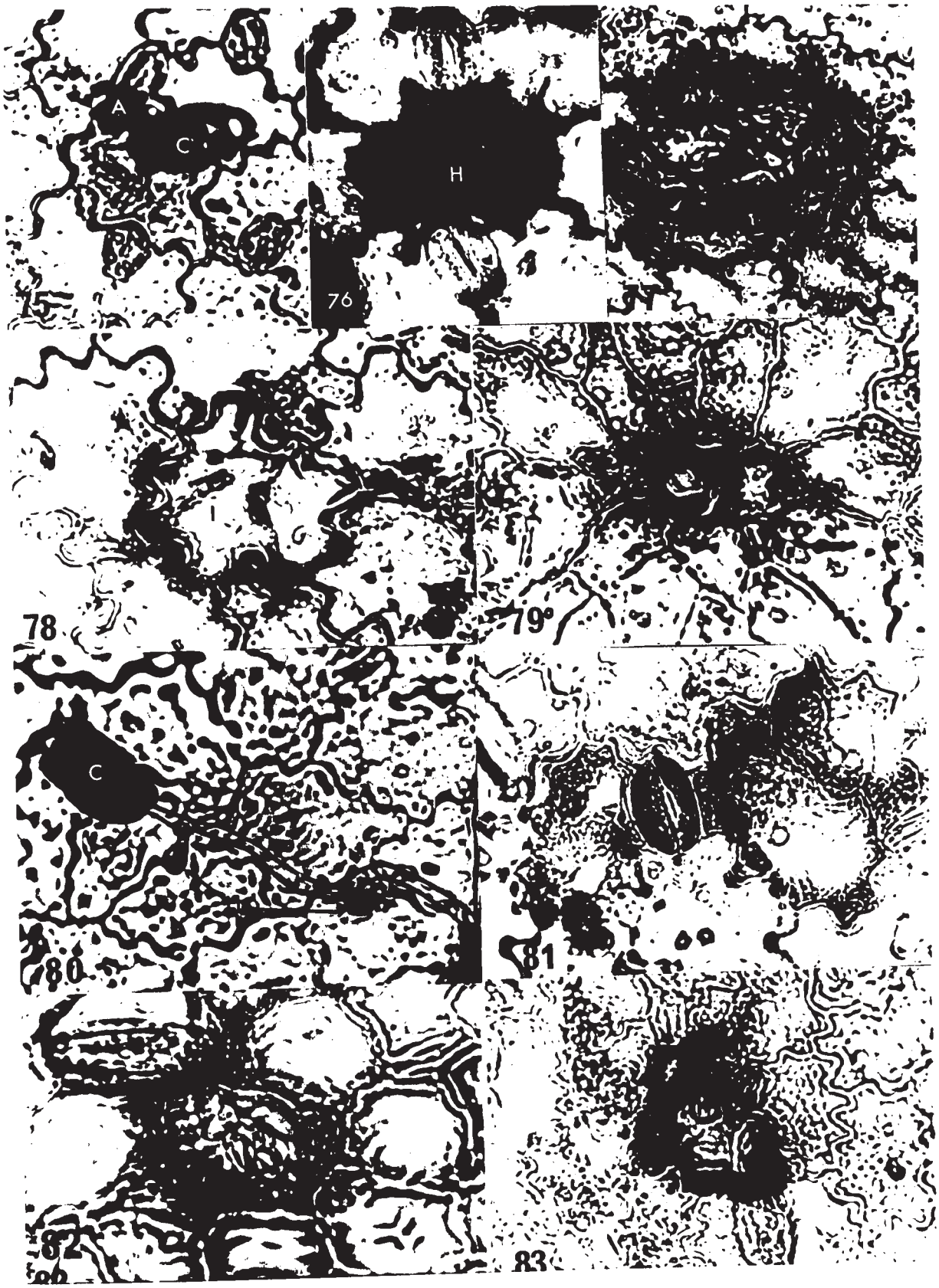
Figure 79 Resistant red clover epidermal cells stained with Azure B, 5 days after inoculation. The dead central cell was infected and stained deep blue, while the neighboring cells are only slightly stained adjacent to the infected cell. 570x.

Figure 80 Susceptible red clover epidermal cells treated with Benedict's solution 3 days after inoculation. Two of the cells contain a haustorium each, but all cells are uncolored. 590x.

Figure 81 A few susceptible red clover epidermal cells which have been treated with Benedict's solution. The 2 central cells have been infected for 3 weeks and are an orange color. All cells appear to have been living. 980x.

Figure 82 A group of resistant red clover epidermal cells treated with Benedict's solution. The central cell contains a haustorium, and was inoculated 2 days prior to treatment with Benedict's solution. It is a yellowish brown color. 1080x.

Figure 83 A group of resistant red clover epidermal cells treated with Benedict's solution, 5 days after inoculation. The central cell contains a haustorium and is a deep orange color and was dead when fixed. 610x.


A
C
H
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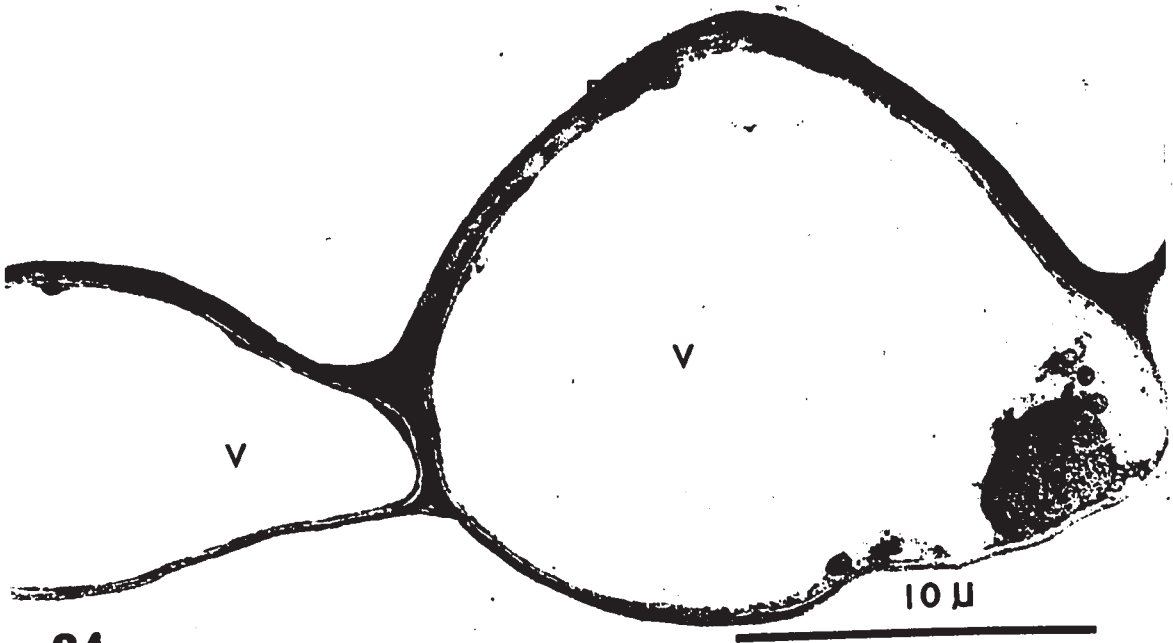
83

Figure 84 Electron micrograph of a transverse section of 2 susceptible red clover epidermal cells showing the fine structures of the normal uninfected cells. Note the thin layer of protoplasm around the cells with a large vacuole occupying the center. The plasma membrane adpresses closely to the wall. The outer wall is about twice the thickness of the inner wall. The nucleus is situated at a side near the lower wall. Glutaraldehyde and osmium fixed; uranium and lead stained. 5000x.

Figure 85 Tranverse section of a portion of a normal red clover epidermal cell showing the outer thin cuticular layer and the fibrillar wall. Between the plasmalemma and tonoplast is a thin layer of cytoplasm which contains a golgi apparatus. Glutaraldehyde and osmium fixed; uranium and lead stained. 49,000x.

Figure 86 A surface section of part of a clover epidermal cell showing the microfibrillar structures of the wall and some cell organelles. Note the laminated chloroplast with starch granules, long rough E.R., mitochondrion, vesicles and the ribosomal particles.
Glutaraldehyde and osmium fixed; uranium and lead stained. 14,000x.



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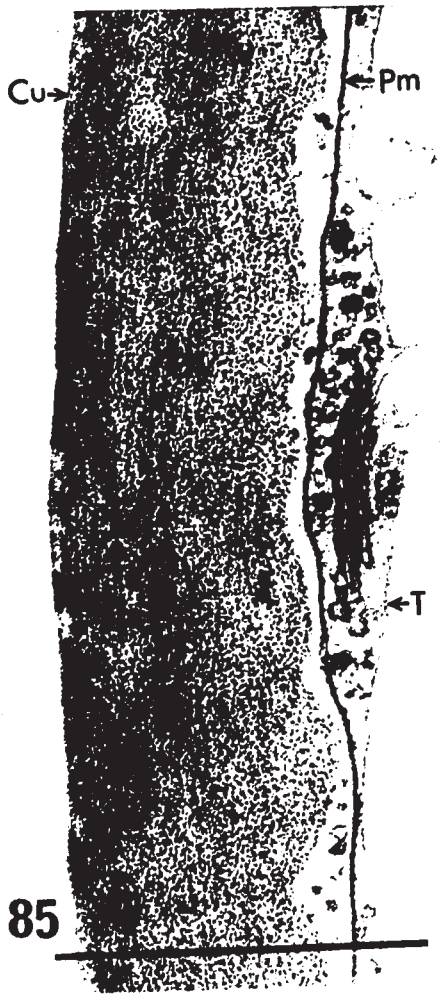

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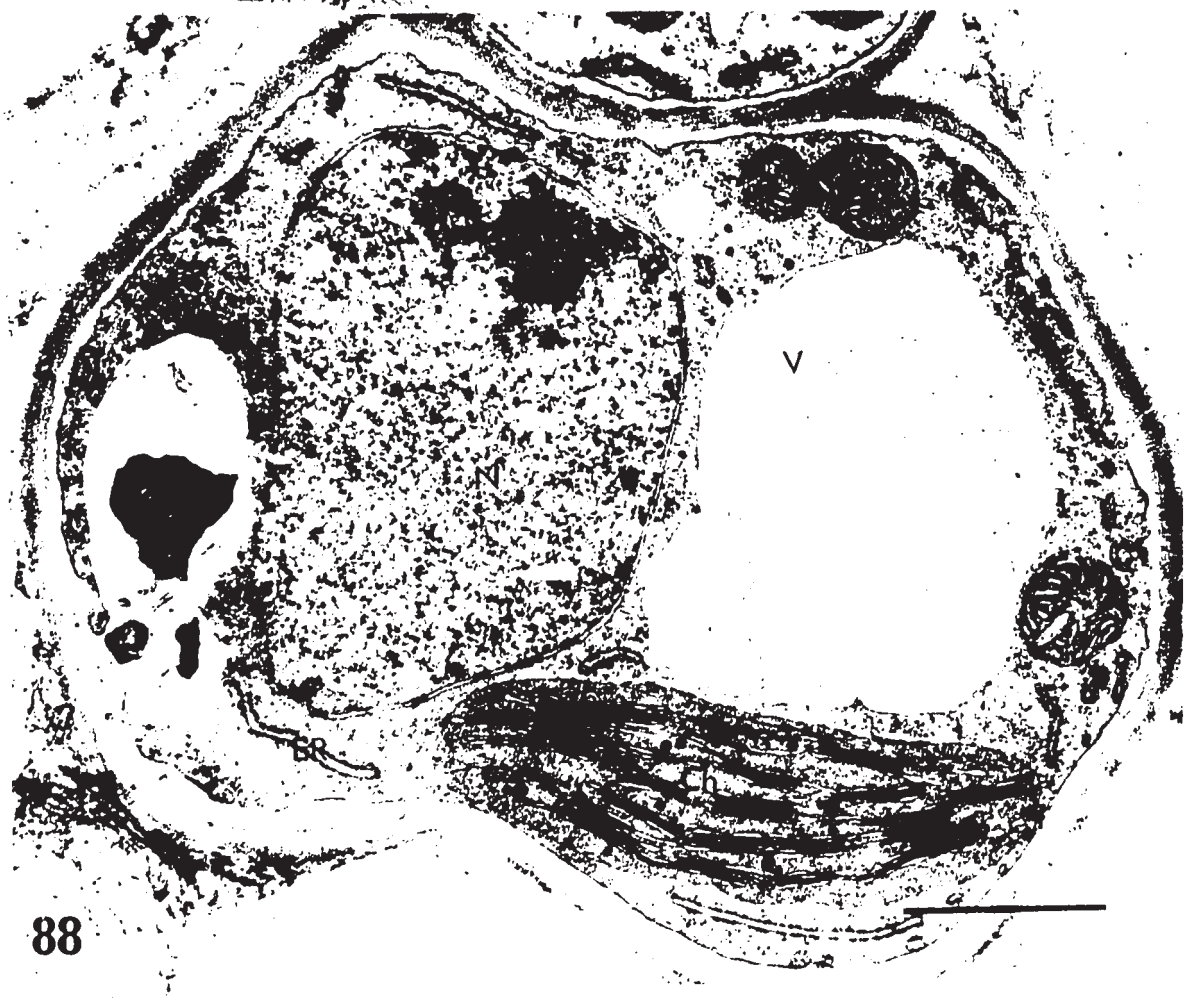
86

Figure 87 Portion of a healthy clover epidermal cell showing a normal chloroplast situated near the lower wall of the cell. It contains grana, frets, plastoglobuli (Pg) and much stroma.
Glutaraldehyde and osmium fixed; uranium and lead stained. 29,000x.

Figure 88 Section of a healthy resistant clover mesophyll cell showing the normal appearance of the cell organelles. Note the nucleus with clearly shown nuclear envelope, chloroplast, rough E.R., mitochondria and vacuoles.
Glutaraldehyde and osmium fixed; uranium and lead stained. 27,000x.

87



88

Figure 89 Section of a lobed appressorium and the narrow neck (arrow) of the germ tube on a clover leaf 14 hr after inoculation. Note nucleus, a group of vesicles and numerous randomly arranged mitochondria.

Glutaraldehyde and osmium fixed; uranium and lead stained. 9,000x.

Figure 90 Serial section adjacent to the one shown in Figure 89. Note the large nucleus, several vacuoles with various inclusions, abundant smooth E.R. and numerous various-shaped mitochondria interspersed in the dense ribosomal particles.

Glutaraldehyde and osmium fixed; uranium and lead stained. 11,000x.

Figure 91 Section of the tip of a germ tube from a 36 hr germinated conidium showing the hooked-tip with a large nucleus in it.

Glutaraldehyde and osmium fixed; uranium and lead stained. 7,000x.

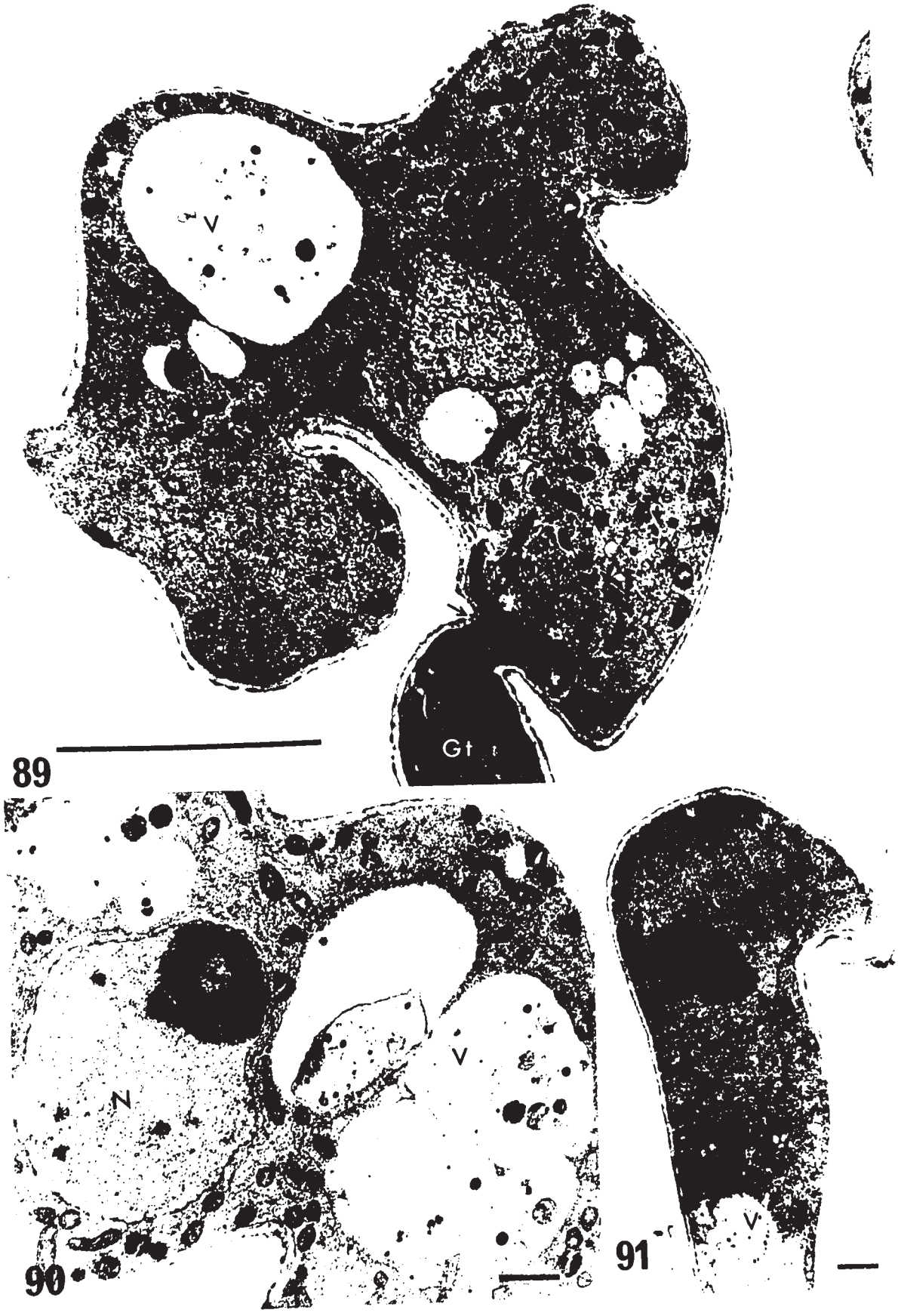

V
Gt
89
N
V
90
V
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Figure 92 Section of an appressorium on an infected susceptible clover cell, 14 hr after inoculation. Note the densely-stained material at the penetration peg area as well as in the collar. Two protrusions are observed on the appressorium at the side of the peg region.

Glutaraldehyde and osmium fixed; uranium and lead stained. 12,000x.

Figure 93 Section of part of secondary appressorium showing extracellular secretion at the point of penetration (arrow). Note also a group of spherosome-like bodies in the appressorium.

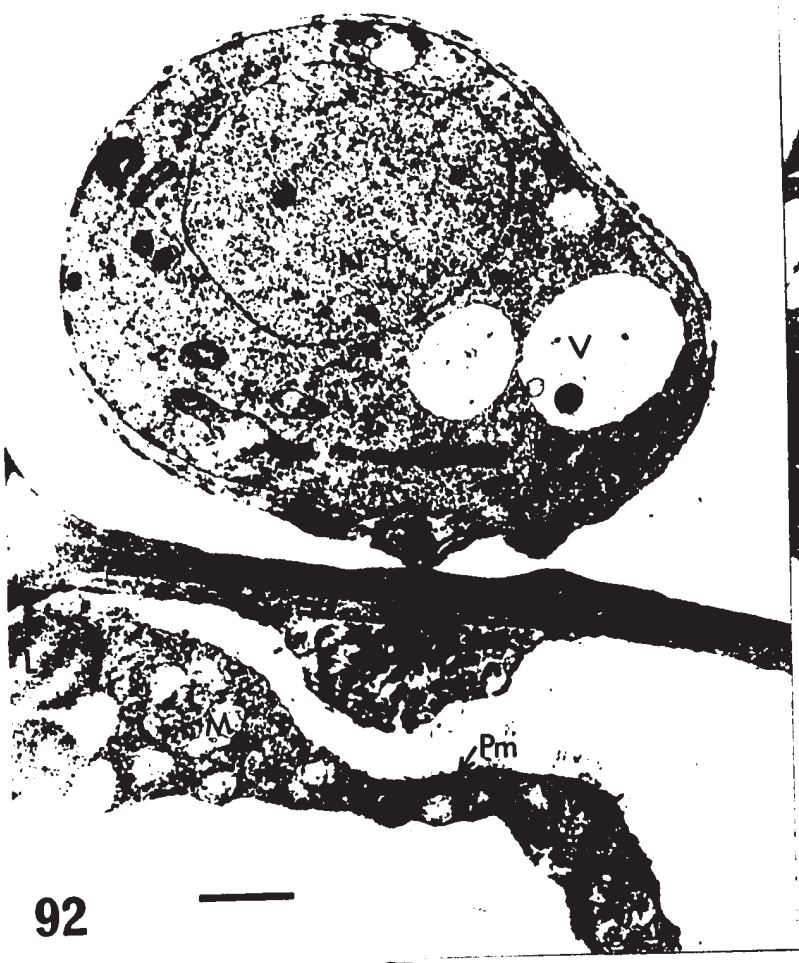
Osmium tetroxide fixed; lead stained. 14,000x.

Figure 94 Secondary appressorium with its penetration peg. Note the change of wall structure beside the peg area. A large nucleus is present inside the appressorium. Numerous mitochondria and E.R. are also observed.

Potassium permanganate fixed; uranium and lead stained. 8,000x.

Figure 95 Section of secondary appressorium, 5 days after inoculation. Note the torn host wall beside the peg region. Lipid inclusions are present inside the vacuole in the appressorium.

Glutaraldehyde and osmium fixed; uranium and lead stained. 6,000x.



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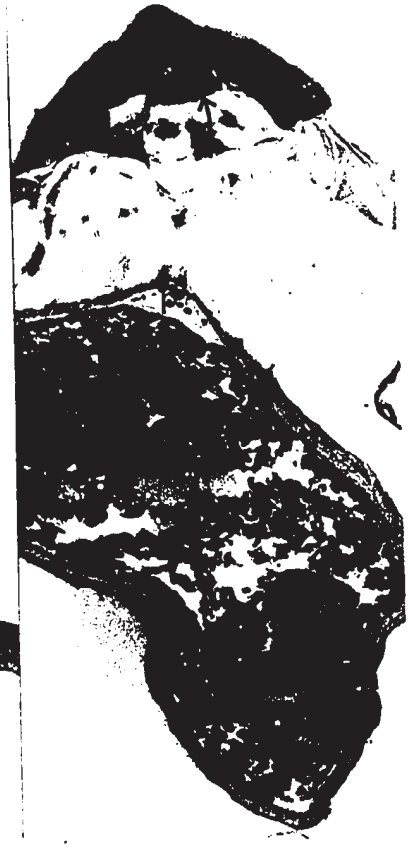

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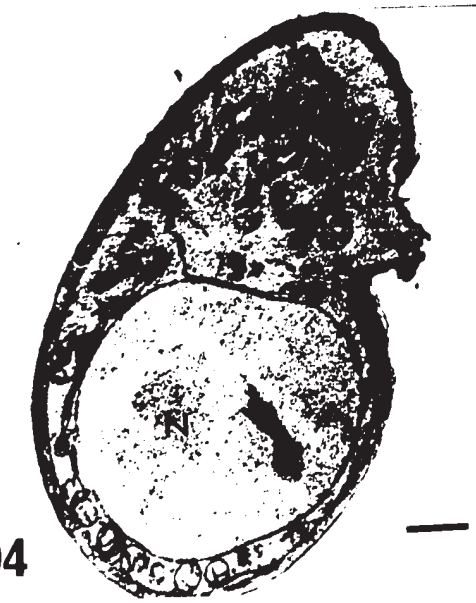

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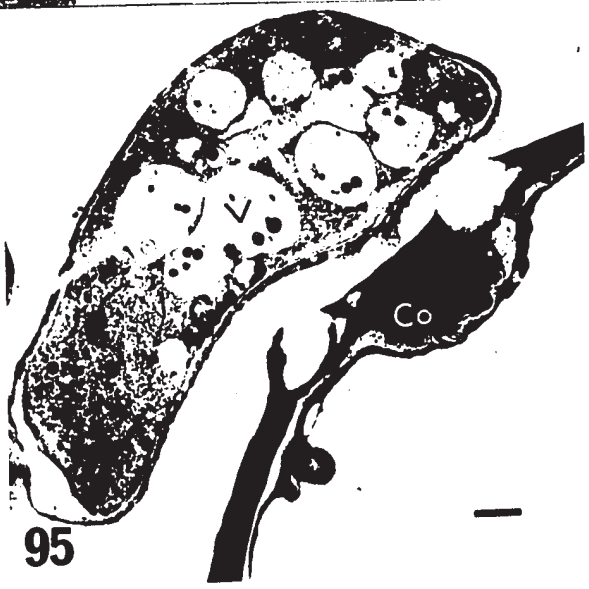
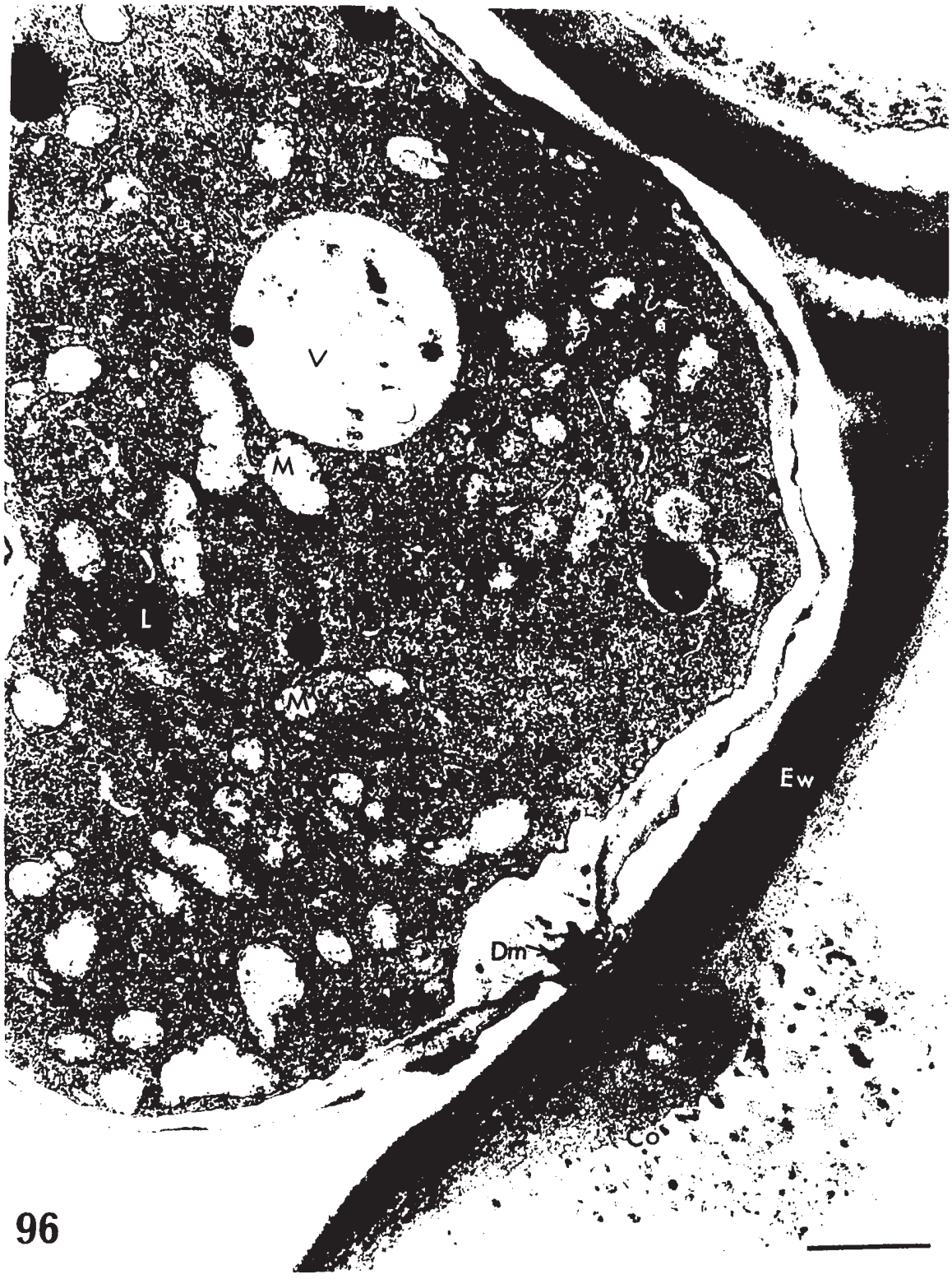


95

Figure 96 Section through an infection site, 14 hr after inoculation. Note the dumb-bell shaped mitochondria in the appressorium, the darkly-stained material near the penetration peg area and in the collar. A large amount of electron-transparent material is present at the mouth of the penetration peg.

Glutaraldehyde and osmium fixed; uranium and lead stained. 20,000x.



96

Figure 97 An enlarged portion of Figure 96 showing the area of interaction. The section is slightly to one side of the peg. The cuticle is invaginated, the wall immediately below appears slightly degraded (arrows). The microfibrillar structures below are bent toward the collar. The wall and the collar are permeated with the darkly-stained material or granules, some of which appear to be membrane bounded. 27,000x.

Figure 98 A serial section adjacent to the section shown in Figure 97. The spine-like expansion of the appressorial wall near the penetration area is shown. The plasma membrane is slightly withdrawn and accommodates the elaborated newly-formed wall-like material. 46,000x.

Figure 99 A portion of the infection area showing the spine-like projections of the appressorial wall, one of which is attached to the wall (arrows).

Glutaraldehyde and osmium fixed; uranium and lead stained. 30,000x.

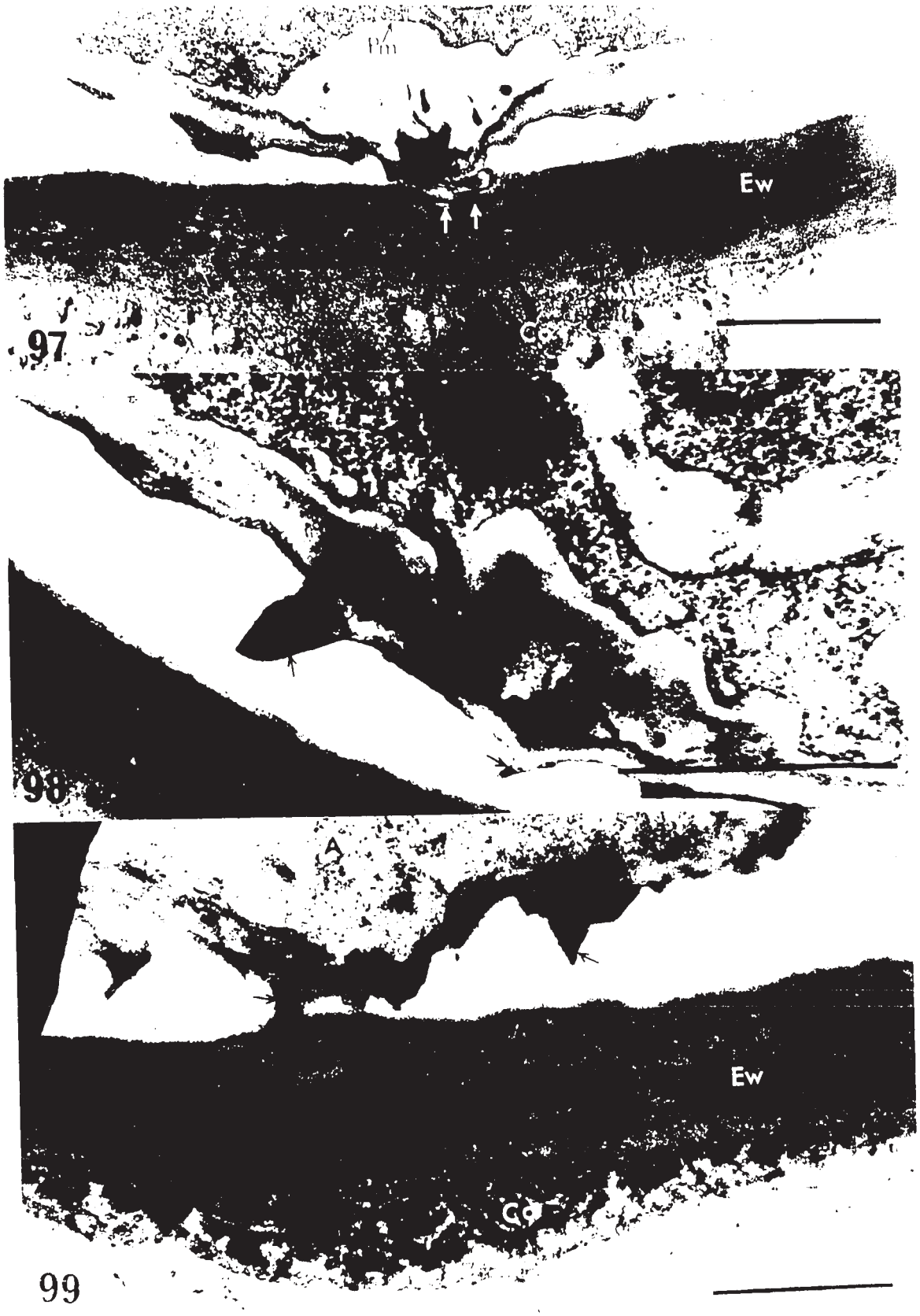
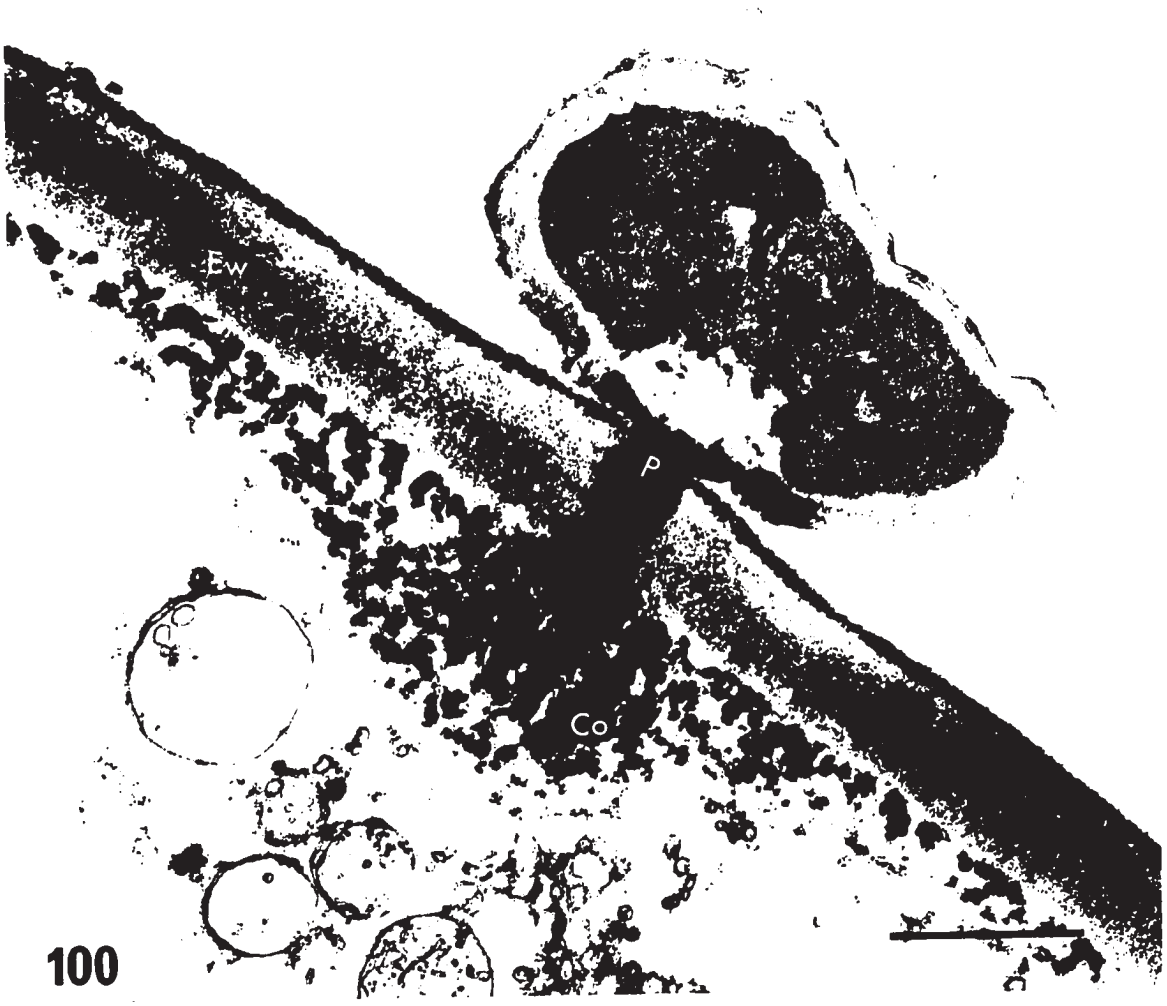

Pm
Ew
Cg
97
98
A
Ew
Cg
99

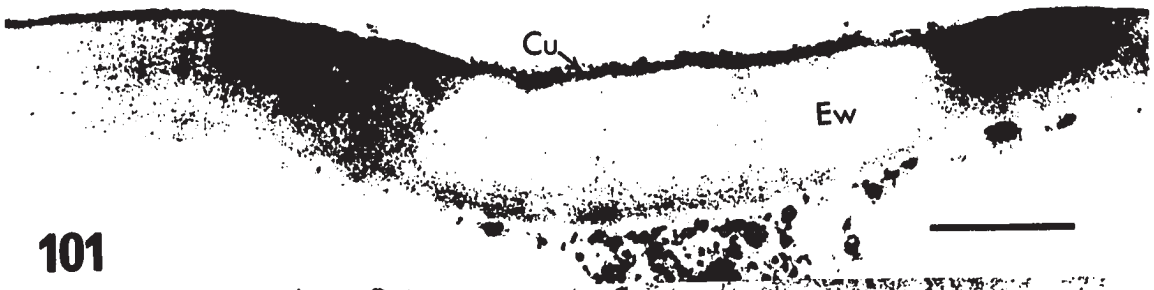
Figure 100 Section of part of an infected susceptible cell and an attached appressorium. The penetrated wall is wider at the point of penetration.
Potassium permanganate fixed; unstained.
26,000x.

Figure 101 Serial section of the wall of an infected cell adjacent to that shown in Figure 100. Two electron-dense areas are present. They correspond to the ring around the halo which is observed in the light microscope.
19,000x.

Figure 102 Section of a penetrated clover leaf wall. Note the wall has been degraded at 2 areas near the peg (arrows).
Potassium permanganate fixed; lead stained.
22,000x.



100

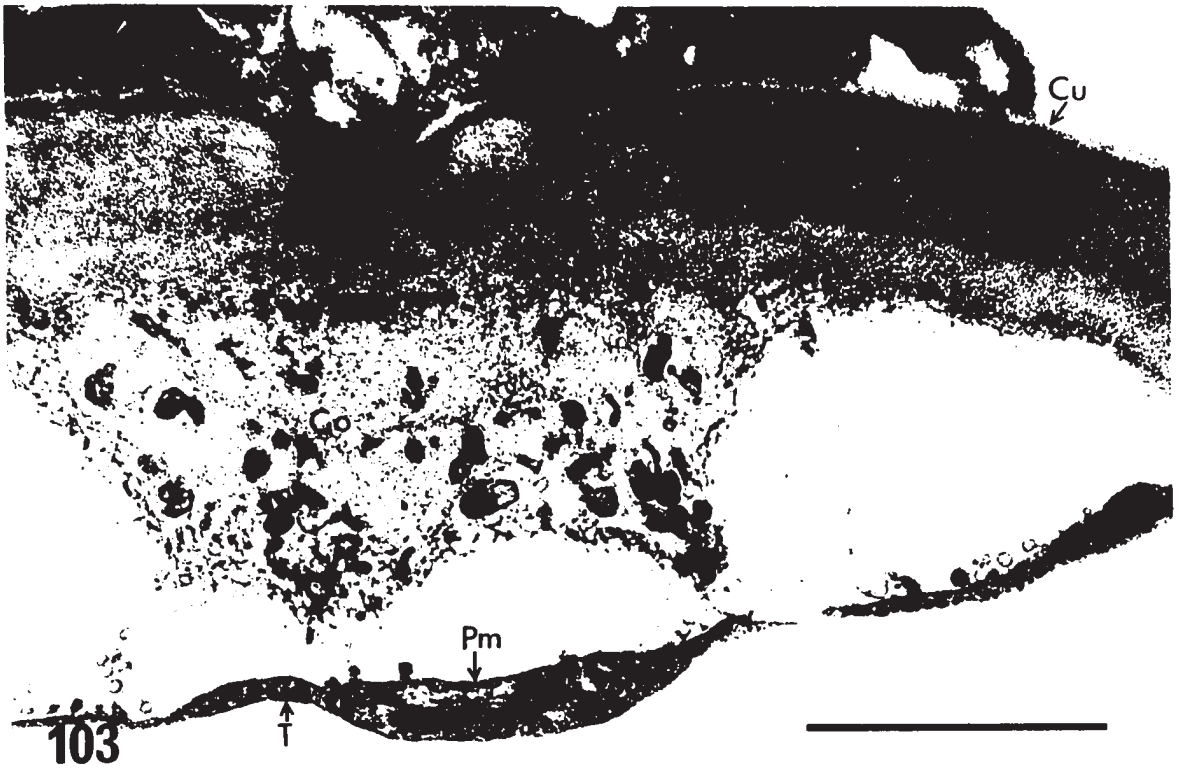


101

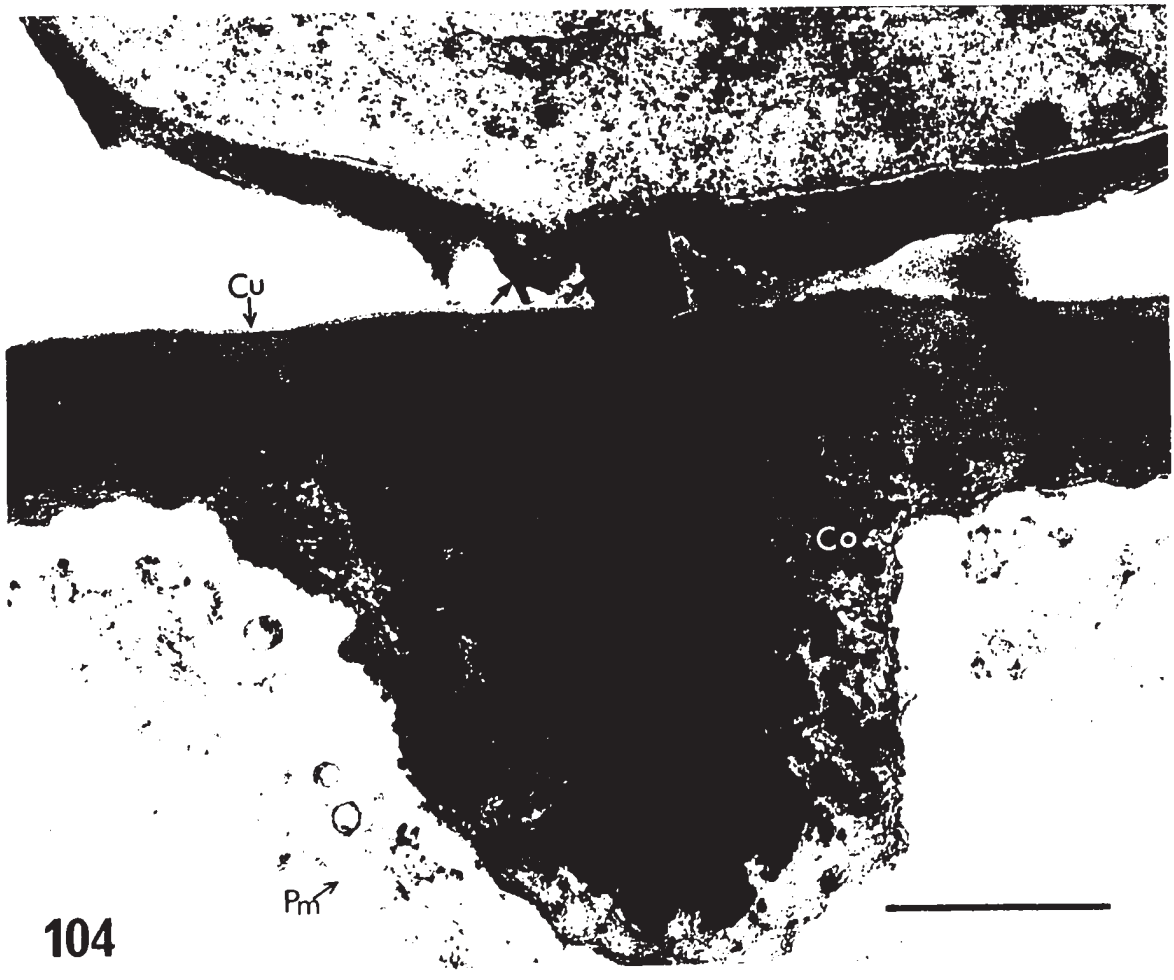
102

Figure 103 Section of part of an appressorium and an infected susceptible host cell, 14 hr after inoculation. The appressorium is closely adpressed to the epidermal wall. Densely-stained material and granules are observed in the host cuticle and wall below the bent cuticle. Note that the microfibrillar structures are bent toward the collar (arrows).
Glutaraldehyde and osmium fixed; uranium and lead stained. 39,000x.

Figure 104 Section of part of an appressorium and part of an infected susceptible host cell, 14 hr after inoculation. Note the anchoring of the spine-like wall of the appressorium to the host epidermal wall. The collar contains many electron-dense bodies. Note the secretion on the appressorial wall and the invaginated host plasma membrane.
Glutaraldehyde and osmium fixed; uranium and lead stained. 29,000x.



103

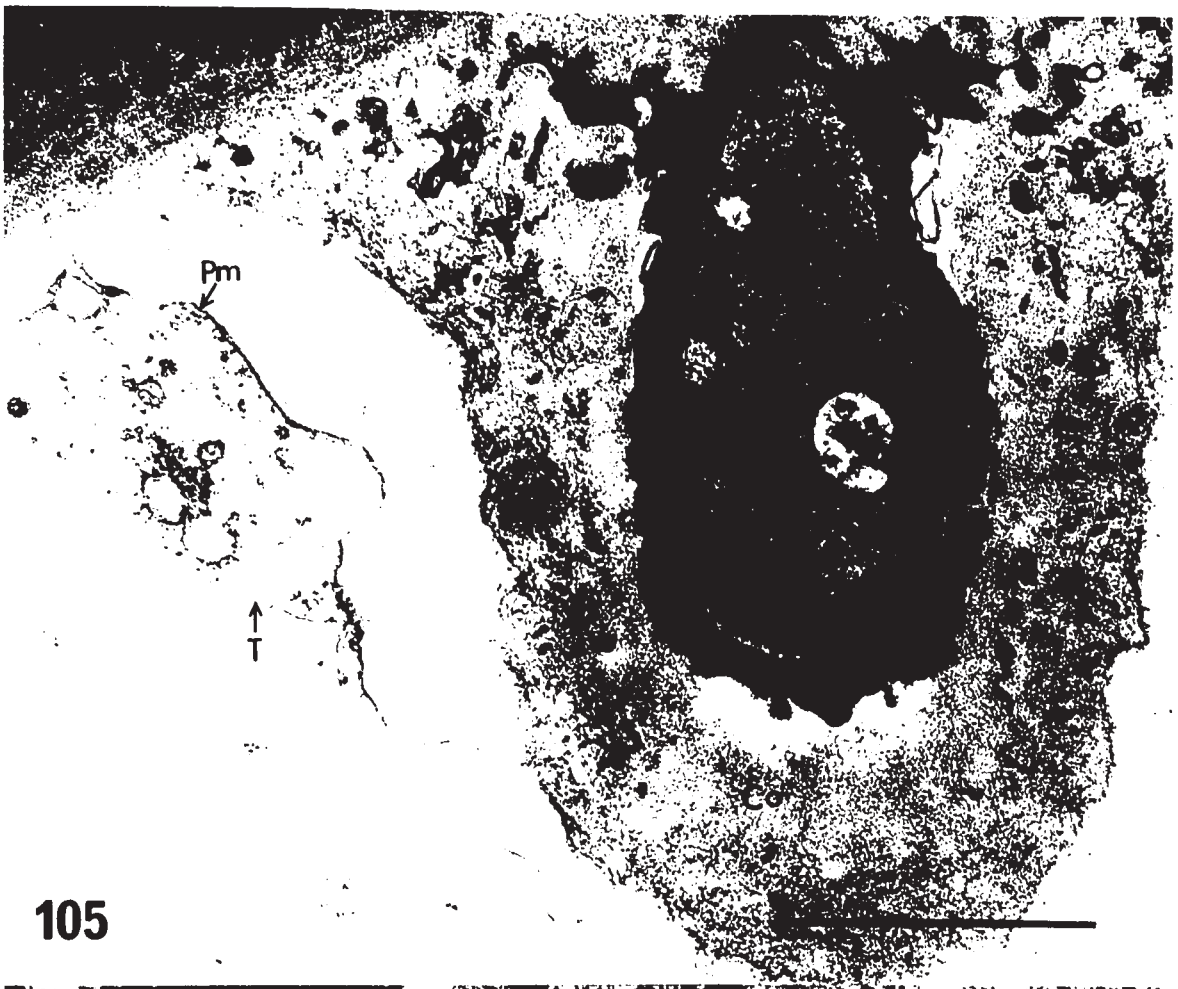


104

Figure 105 Section of part of an infected susceptible cell, 14 hr after inoculation showing a longitudinal section of an infection peg in the collar. Note a vacuole is present in the young haustorium. The peg is surrounded by a thick darkly-stained covering which opens near the neck area. The peg's plasma membrane is darker and thicker than the invaginated host plasma membrane. Observe the dark granules in the collar, some of which appear membrane bounded. Glutaraldehyde and osmium fixed; uranium and lead stained. 40,000x.

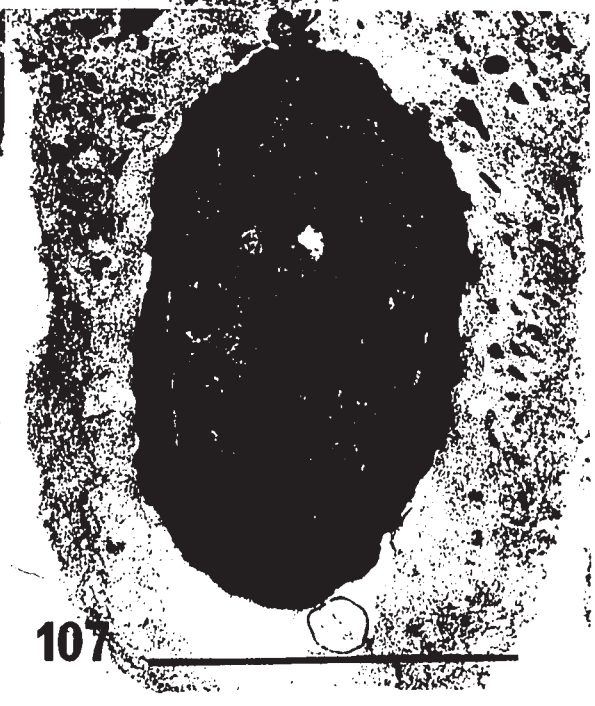
Figure 106 Serial section of part of an infected cell adjacent to the section shown in Figure 105. The narrow neck and the thick covering of the peg are shown. 48,000x.

Figure 107 Another serial section of part of an infected cell adjacent to the section shown in Figure 105 and 106, showing the similar features and the dense ribosomes in the infection peg. 48,000x.



105

106



107

Figure 108& 109 Light micrographs of a conidium infecting a resistant clover host (14 hr). The appressorium is closely adpressed to the host wall. Note the penetrated loop-like haustorial initial. The cytoplasm in the infected cell appears to be coagulated. Pictures were taken of material in an Epon block.
Glutaraldehyde and osmium fixed. 1,000x.

Figure 110 Electron micrograph of a thin section from same material as that shown in Figures 108 and 109. Note the infection peg is not not completely covered by a thick darkly-stained material. At the distal end of the peg, the wall appears to have been broken. The plasma membrane of the host cell has been broken and the cytoplasm has coagulated. Glutaraldehyde and osmium fixed; uranium and lead stained. 12,000x.

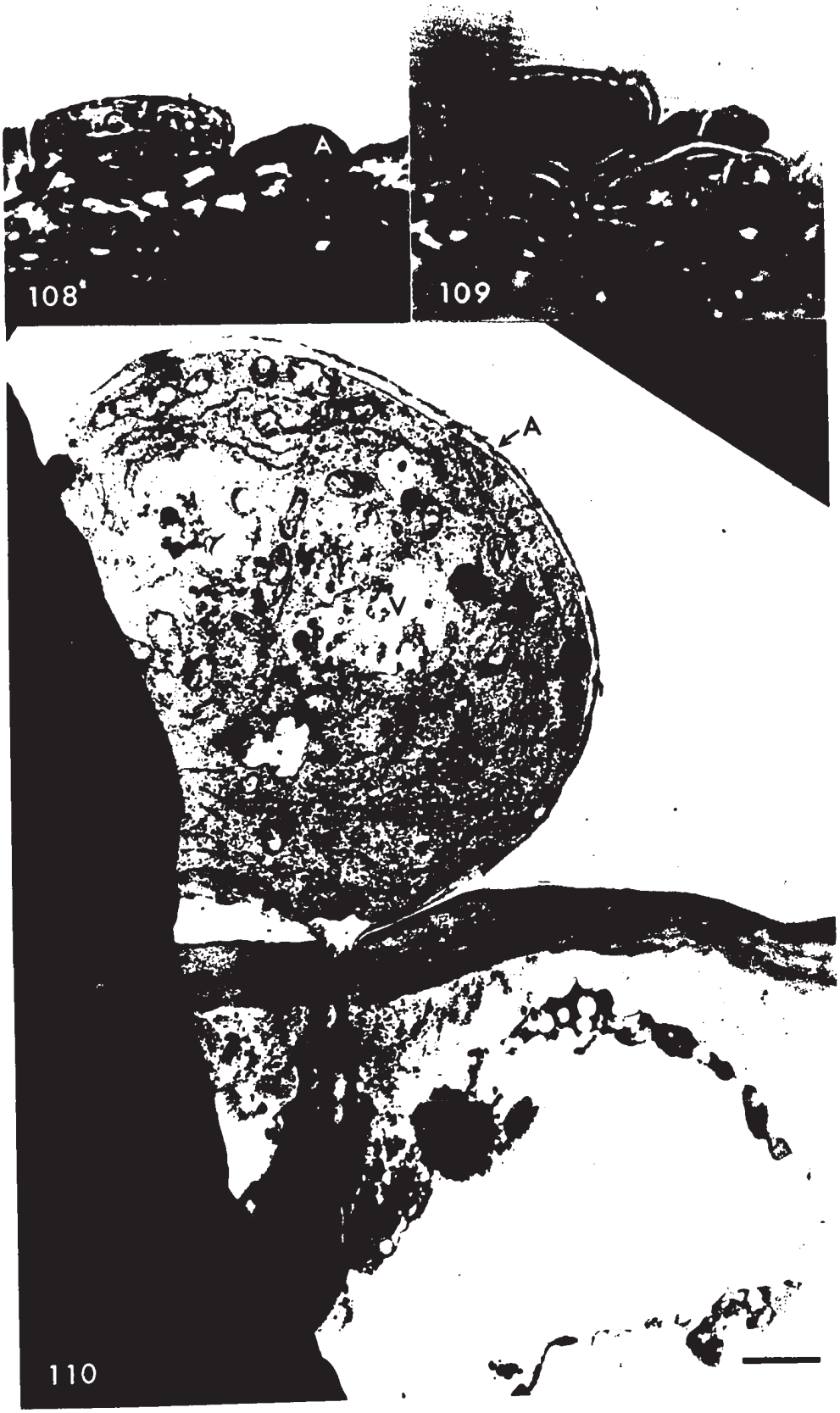

A
108
109
A
V
110

Figure 111 Portion of Figure 110 at a higher magnification showing the penetration region. Note the densely-stained granules or materials in the host wall, collar and inside and around the peg. The orientation of the microfibrillar structure of the epidermal wall near the vicinity of penetration is changed. Note the material on the appressorial outer wall at both sides of the peg and the membrane structure inside the peg. Electron-transparent material is present at the mouth of the penetration peg. 46,000x.

Figure 112 A section of an appressorium and an infected resistant clover cell, 14 hr after inoculation. The host cell appears to be degenerating and the appressorium appears to be dead. Glutaraldehyde and osmium fixed; uranium and lead stained. 7800x.

Figure 113 Section of an infected resistant clover cell 5 days after inoculation showing the dead haustorium and the electron-dense coagulated host cytoplasm. Compare with light microscope Figure 83.
Glutaraldehyde and osmium fixed; uranium and lead stained. 5000x.

111

112

N

113

Figure 114& 115 Light micrographs of an infected susceptible clover cell, 14 hr after inoculation showing a young haustorium at different depths of focus. Note the V-shaped invaginated host plasma membrane, nucleus in the center of the haustorium above which a haustorial branch is seen. Pictures taken from an Epon embedded block. Glutaraldehyde and osmium fixed. 1,000x.

Figure 116 Electron micrograph of a thin section of an appressorium and an infected cell. It was cut from an Epon embedded block observed in the light micrograph (Figure 114 & 115). Note the edge of the collar, the V-shaped invaginated plasma membrane and host cytoplasm, the young haustorium with a nucleus in the center, a branch at the tip and an expanding wavy haustorial sheath surrounded by a thin layer of host cytoplasm. Glutaraldehyde and osmium fixed; lead stained. 11,000x.

Figure 117 A magnified portion of part of the material shown in Figure 116, which shows the epidermal wall, host plasma membrane and cytoplasm, Golgi apparatus, mitochondria, lipid body, host tonoplast and the haustorial sheath. 54,000x.


Ch
114
115
Ew
Ca
Pm
T
M
L
N
T
Sh
0.1 μ
116
117

Figure 118 A high magnification of the young haustorium which was shown in Figure 116. Note the vacuoles with membranes and vesicular inclusion. The haustorial sac or sheath matrix appears empty except for the presence of several vesicles (arrows) which are in close association with the sheath membrane or the haustorial wall. The plasma membrane of the haustorium is highly convoluted. 19,000x.

Figure 119 A highly magnified portion of the haustorium which was shown in Figure 118. A vacuole with numerous vesicular structures of about equal size are shown. Note also the laminated inclusion with its open end facing towards the cytoplasm. 85,000x.

Figure 120 A highly magnified part of the wavy or convoluted haustorial sheath which was shown in Figure 118. Vesicles are present inside the sheath (in the sheath matrix) and outside the sheath (in the host cytoplasm). 85,000x.

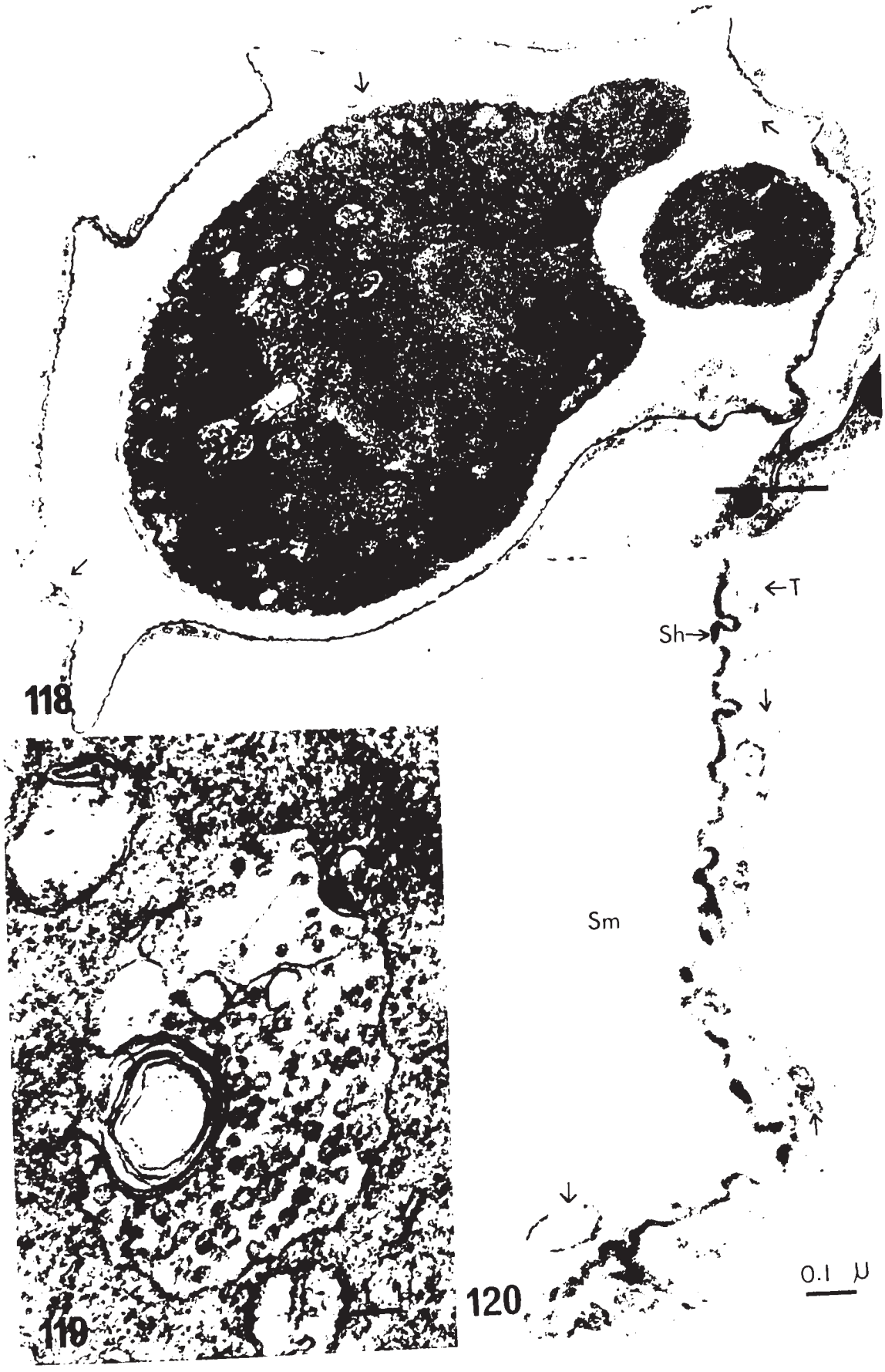

118
119
120
←T
Sh→
Sm
0.1 μ

Figure 121 Serial section of an appressorium and an infected cell adjacent to a section shown in Figure 116. The nucleus appears to have just passed through the neck. 10,000x.

Figure 122 Portion of the material shown in Figure 121. Some inclusions in the sheath matrix, including the membranous and amorphous structures are shown. 60,000x.

Figure 123 Highly magnified haustorial neck which was shown in Figure 121. Note that part of the flask-shaped nucleus is constricted between the 2 mitochondria in the haustorial neck. 33,000x.

Figure 124 A highly magnified portion of the appressorium shown in Figure 124. Note the vacuole with vesicular inclusions, irregular-shaped mitochondria and long parallel E.R. 36,000x.

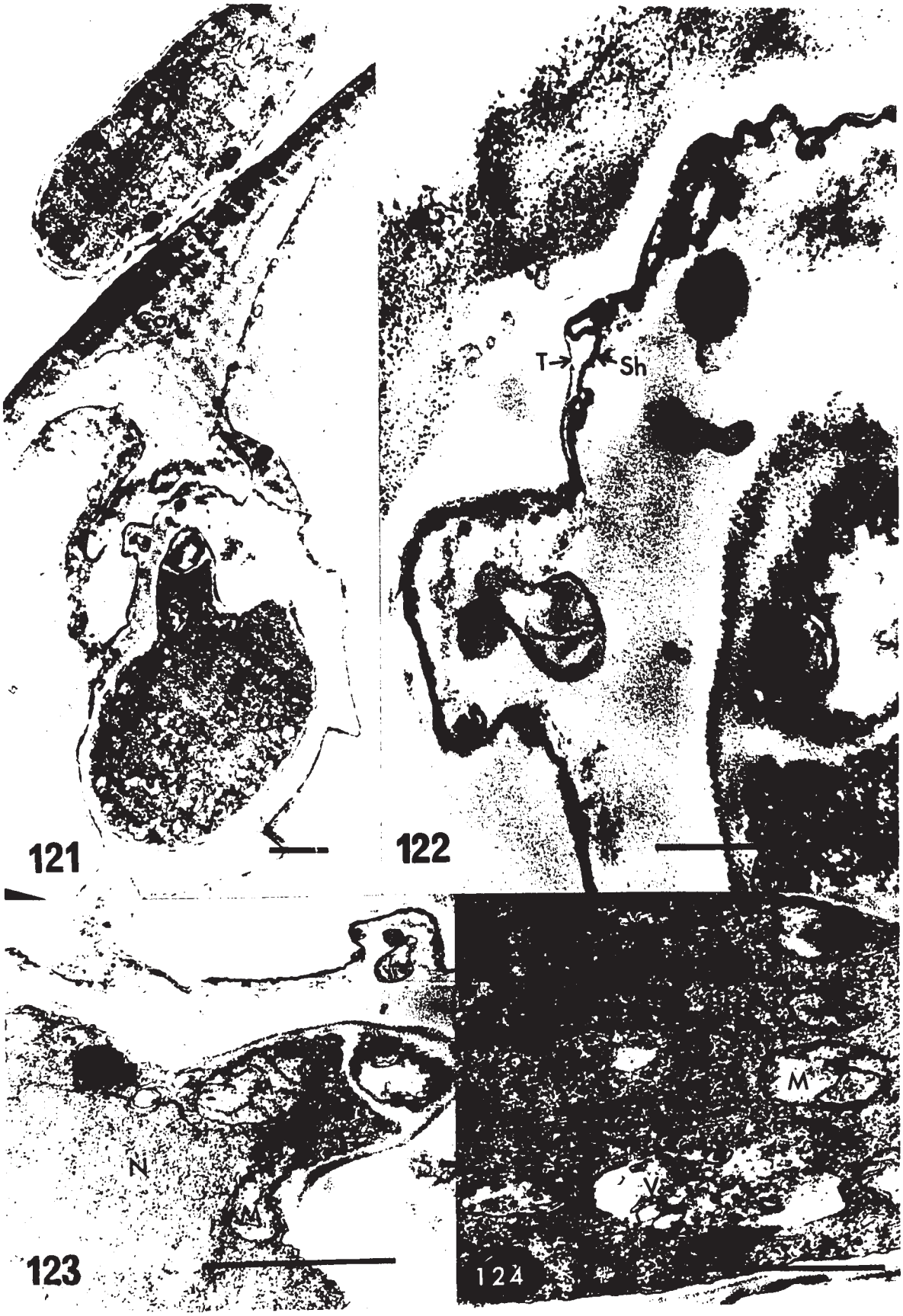
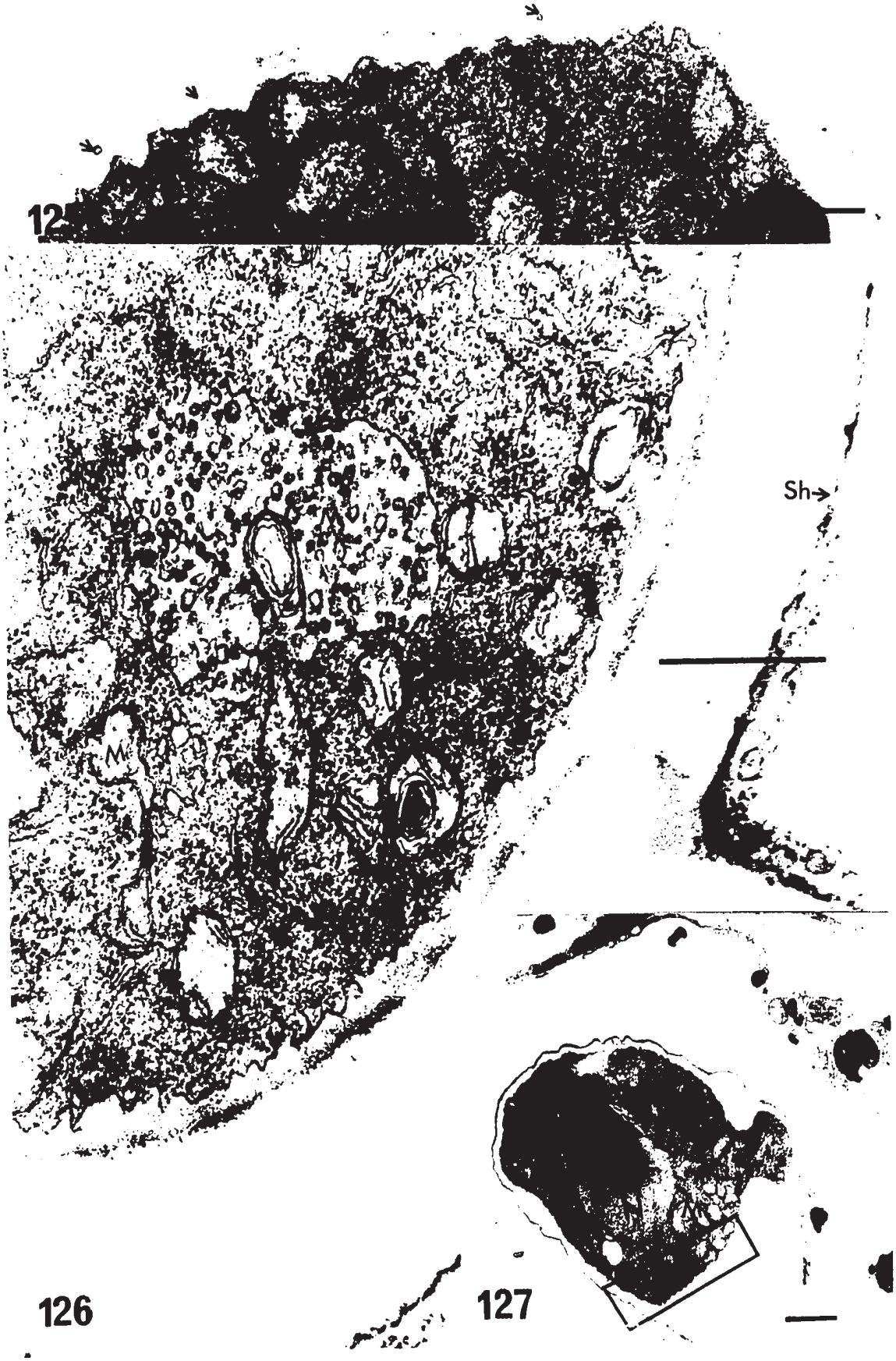

121
122
T
Sh
123
N
M
124
M
V

Figure 125 Part of a young haustorium shown in Figure 127. Observe the highly convoluted plasma membrane and many vesicles in the haustorial wall (arrows).
Glutaraldehyde and osmium fixed; lead stained. 46,000x.

Figure 126 A highly magnified part of the young haustorium shown in Figure 121. Note the multi-vesicular structures in the vacuole, dense ribosomes and the highly convoluted haustorial plasma membrane. 56,000x.

Figure 127 Section of a young haustorium, 14 hr after inoculation, which shows a nucleus and numerous irregular-shaped mitochondria. Compare with the highly magnified portion in Figure 125. 8,500x.



126

127

Figure 128 Part of the distal end of a young haustorium, 14 hr after inoculation. Note the membranous and vesicular contents in the vacuole, the wavy haustorial sheath and the highly convoluted haustorial plasma membrane. Glutaraldehyde and osmium fixed; uranium and lead stained. 46,000x.

Figure 129 Part of an appressorium showing a sausage-shaped mitochondrion and the vesicular inclusions in the vacuole. Glutaraldehyde and osmium fixed; uranium and lead stained. 25,000x.

Figure 130 Part of a haustorium shown in Figure 128. Note the haustorial sheath appears to be double in certain areas (arrows). 65,000x.

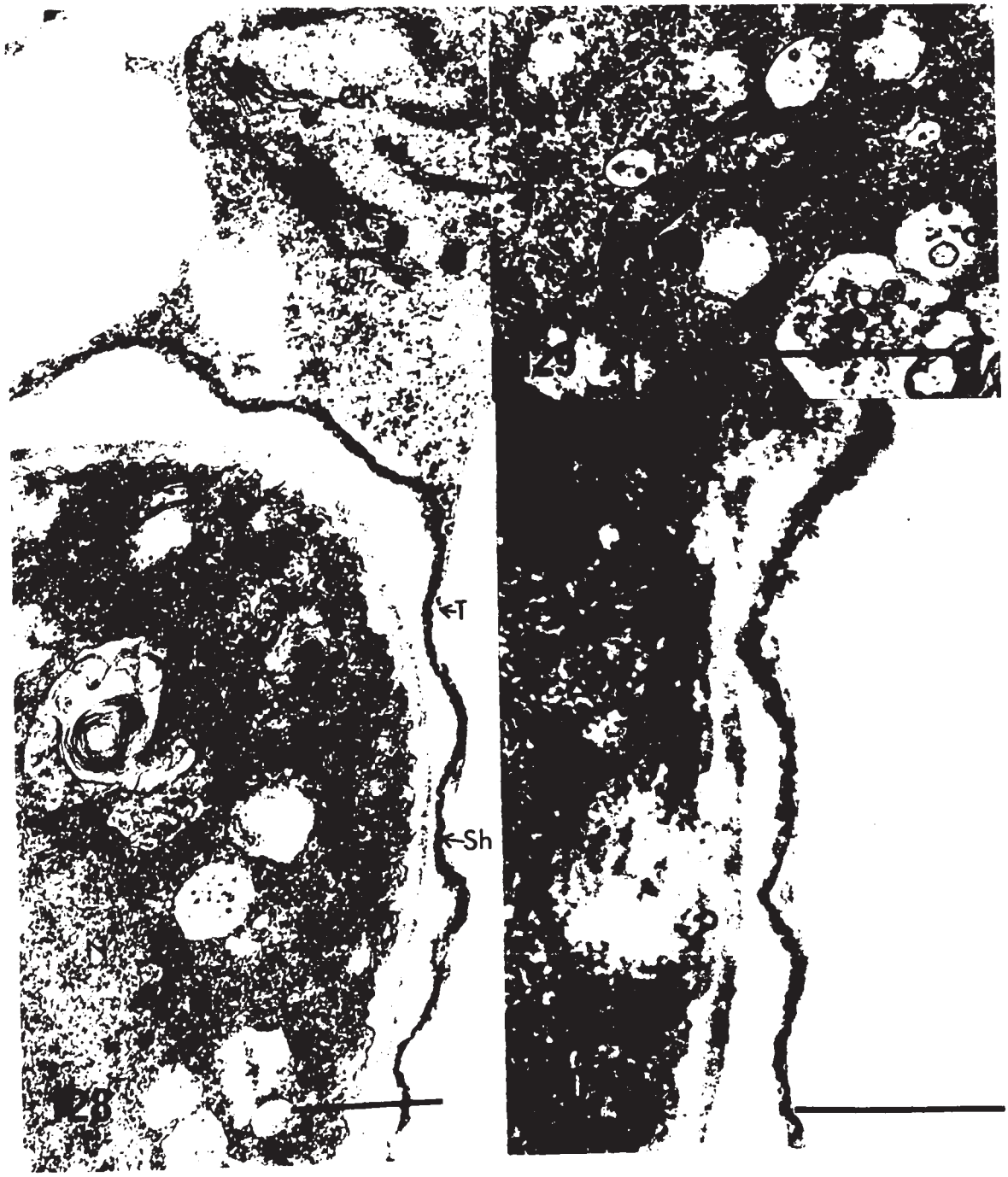

CR
T
Sh
28
29

Figure 131 Electron micrograph of a haustorium, 18 hr after inoculation showing the anterior and the distal haustorial branches. Glutaraldehyde and osmium fixed; uranium and lead stained. 7,000x.

Figure 132 Tangential section of a portion of a haustorial sheath, 24 hr after inoculation, showing the vesicular structures at both sides of the sheath membrane. Glutaraldehyde and osmium fixed; uranium and lead stained. 40,000x.

Figure 133 Section of an infected susceptible host cell 5 days after inoculation, containing a fully developed haustorium. Note numerous convoluted haustorial branches originate from all parts of the central body and twist about in the sheath matrix. Many of the branches are situated close to the sheath membrane. Glutaraldehyde and osmium fixed; uranium and lead stained. 6,200x.

Figure 134 A highly magnified portion of the epidermal wall which was shown in Figure 133. Observe the collar and a thin layer of host cytoplasm with a mitochondrion and lipid body. 25,000x.

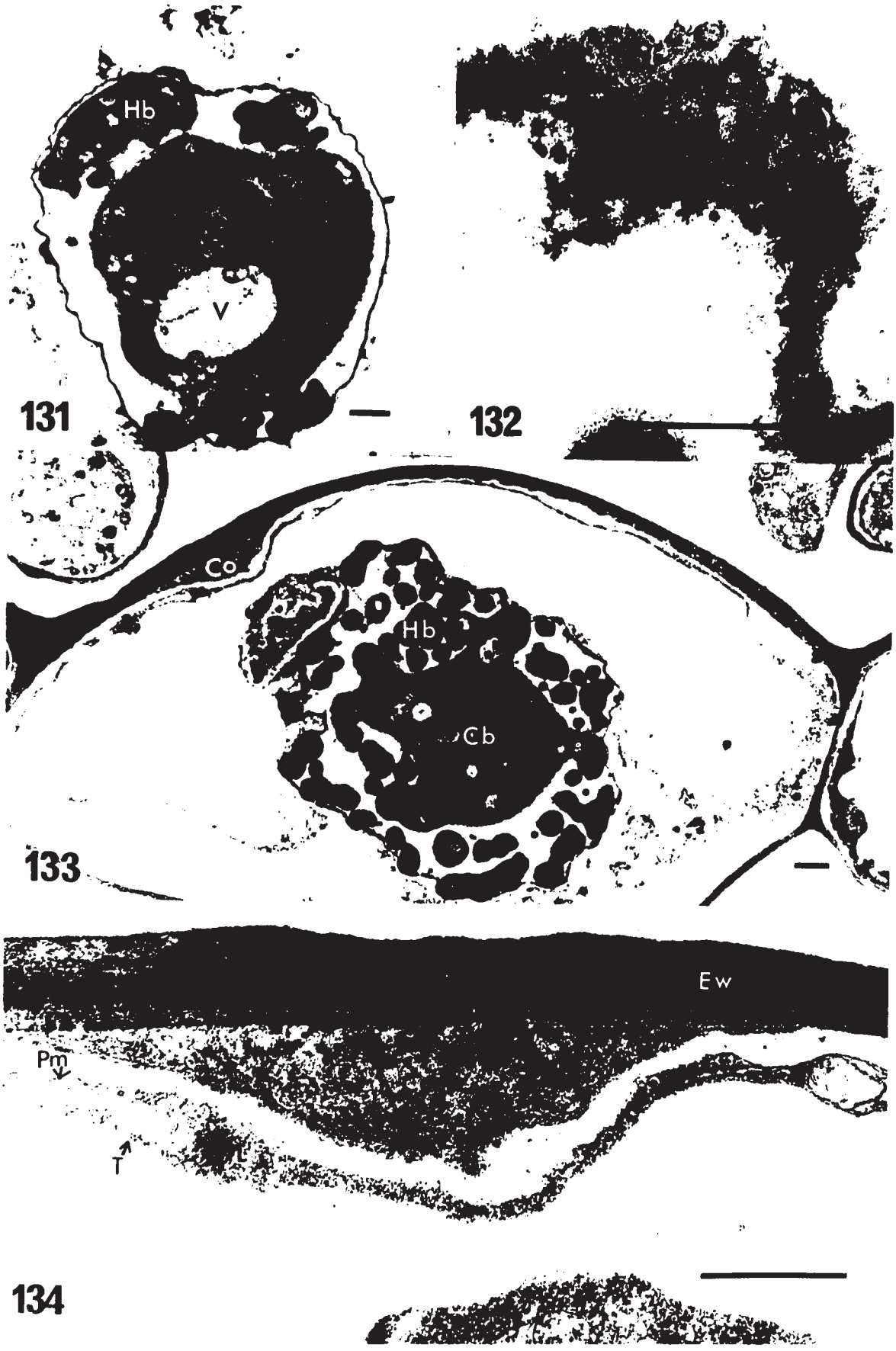

Hb
V
131
132
Co
Hb
Cb
133
Ew
Pm
T
134

Figure 135 Oblique section of an infected clover epidermal wall showing the cross section of an infection peg in the wall. Observe a layer of densely-stained material surrounding the peg and the collar below.
Glutaraldehyde and osmium fixed; uranium and lead stained. 56,000x.

Figure 136 Cross section of a peg through the collar region of a haustorium in an infected cell. The peg hole which is surrounded by a thick darkly-stained material appears empty and is surrounded by inner granular and outer fibrous collar material. Note the host plasma membrane, host cytoplasm and tonoplast.
Glutaraldehyde and osmium fixed; uranium and lead stained. 45,000x.


P
Co
135
T
Co
Pm
P
136

Figure 137 Cross section of a haustorial neck. Observe the numerous worm-like densely-stained material in the vicinity of the neck. The haustorial sheath is invaginated. Glutaraldehyde and osmium fixed; lead stained. 55,000x.

Figure 138 Section of a haustorial neck in the same infected susceptible clover cell (Figure 137). A membrane bounded substance is observed in the neck. 55,000x.

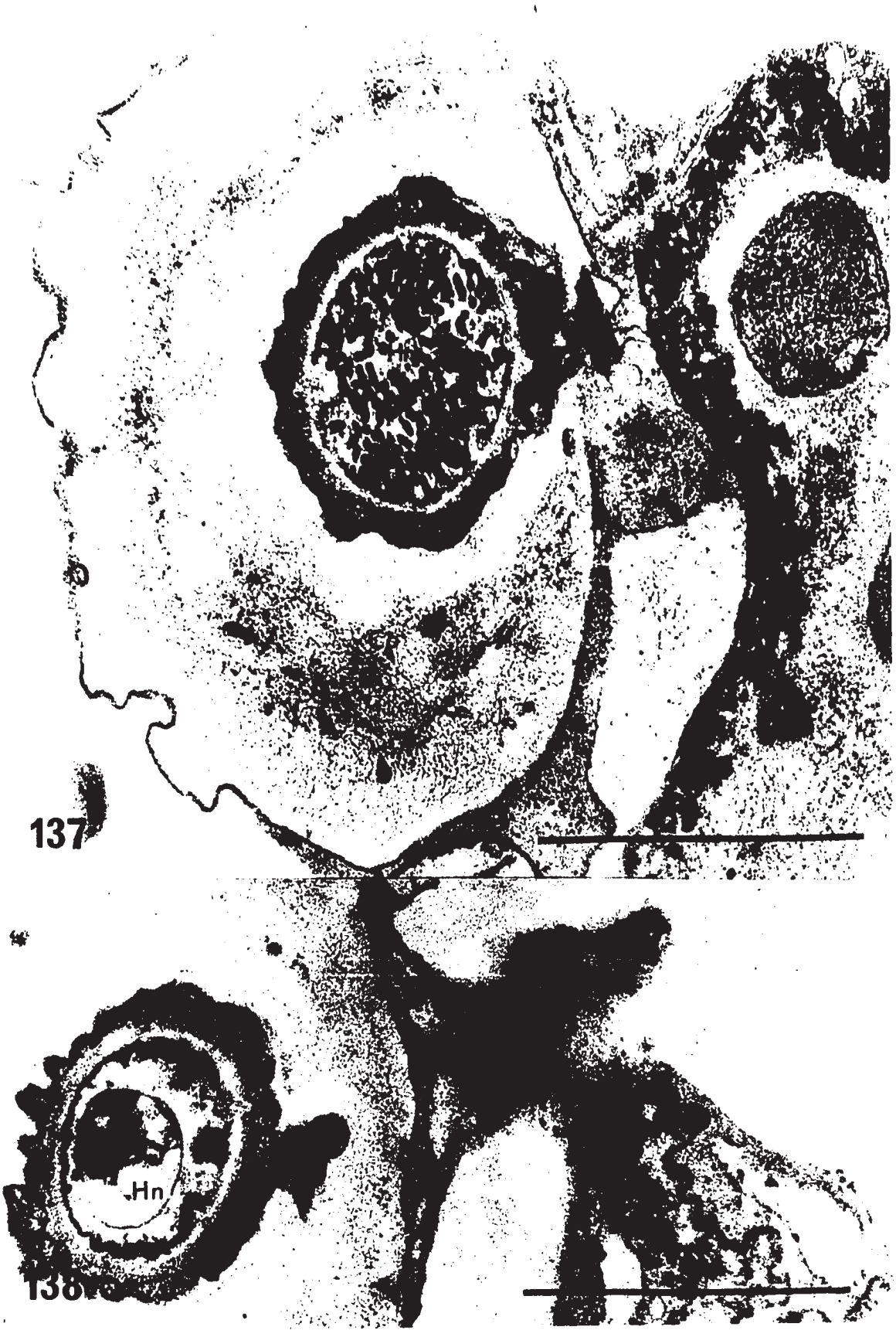

137
Hn
138

Figure 139 Section of an infected area of a susceptible clover cell, showing the bent cuticle, the epidermal wall, peg hole, haustorial sheath and the collar which appears to be divided into outer and inner region.
Glutaraldehyde and osmium fixed; uranium and lead stained. 29,000x.

Figure 140 Section of a portion of an infected resistant clover cell showing a peg in the host wall.
Glutaraldehyde and osmium fixed; uranium and lead stained. 59,000x.

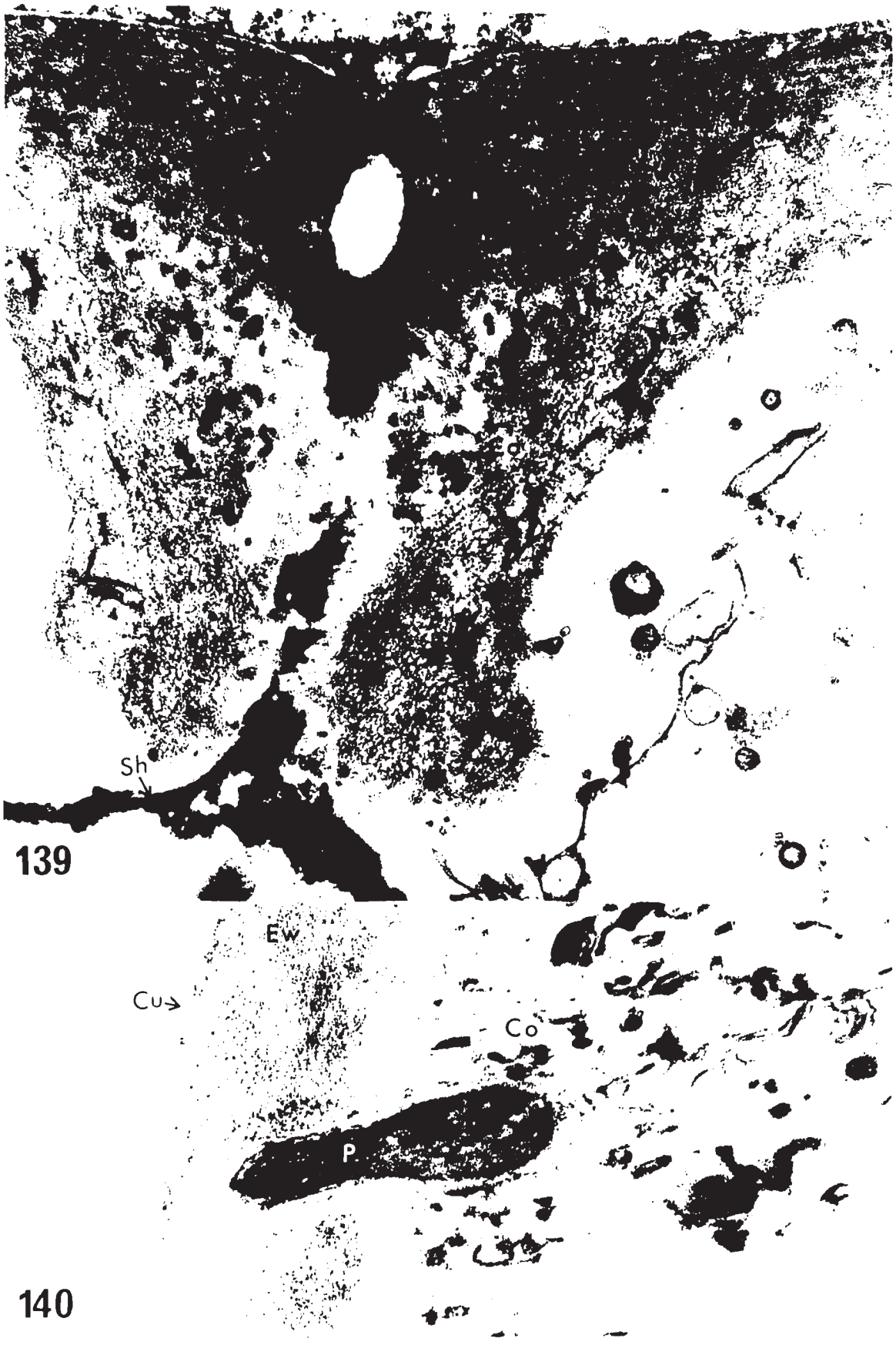
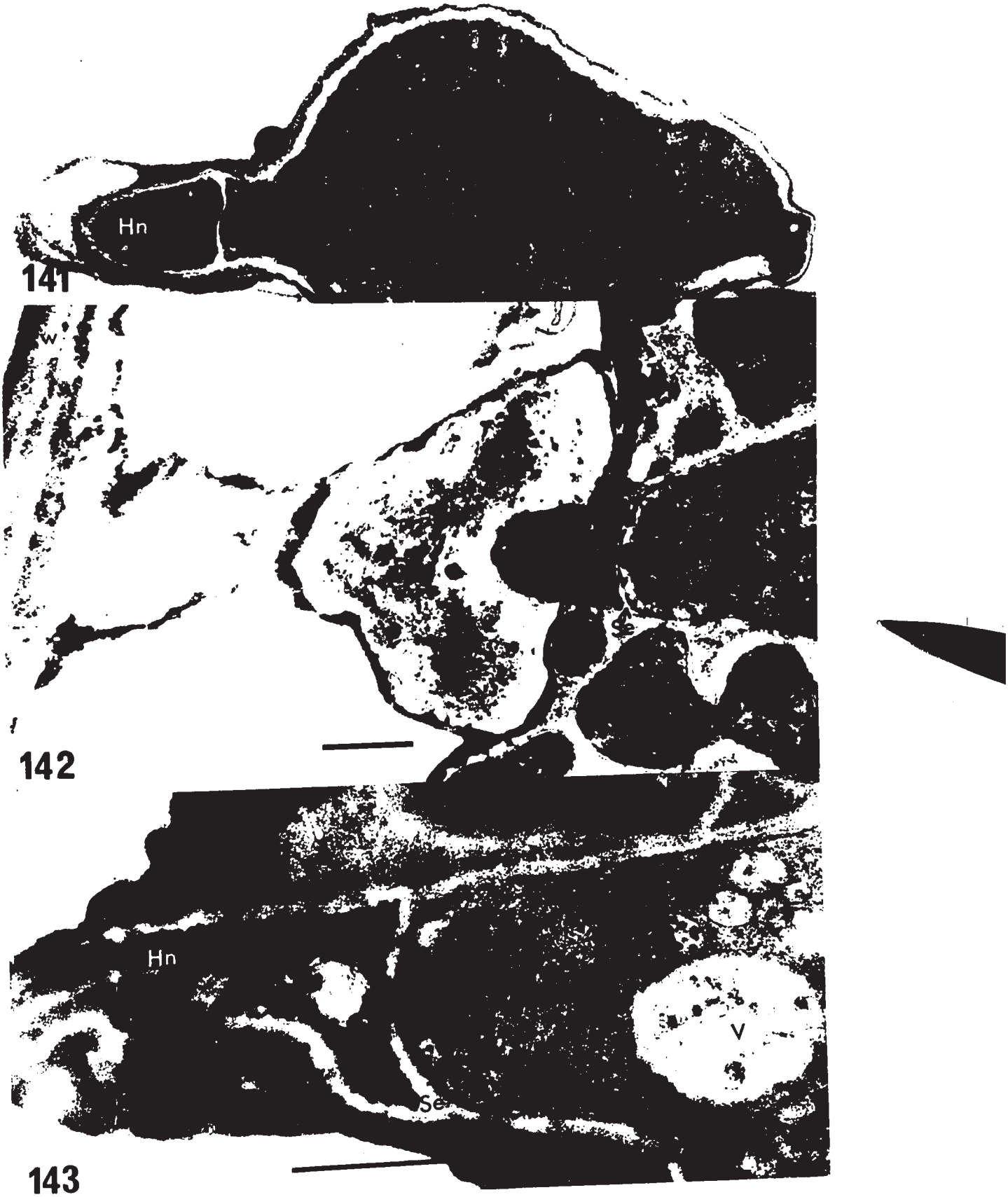

Sh
139
Ew
Cu
Co
P
140

Figure 141 Longitudinal section of a young haustorium showing a septum at the neck and a Woronin body adjacent to the septal pore. A nucleus and numerous mitochondria are present in the haustorium.
Glutaraldehyde and osmium fixed; lead stained. 16,000x.

Figure 142 Section of part of an infected susceptible clover cell, 10 days after inoculation, showing host wall, collar and part of the central body. Note the septum in the neck and 3 Woronin bodies, one of which is in the septal pore.
Glutaraldehyde and osmium fixed; uranium and lead stained. 17,000x.

Figure 143 Section of the neck of a haustorium, 10 days after inoculation, showing the septal pore plugged by a Woronin body.
Glutaraldehyde and osmium fixed; uranium and lead stained. 43,000x.


Hn
141
W
Se
142
Hn
V
Se
143

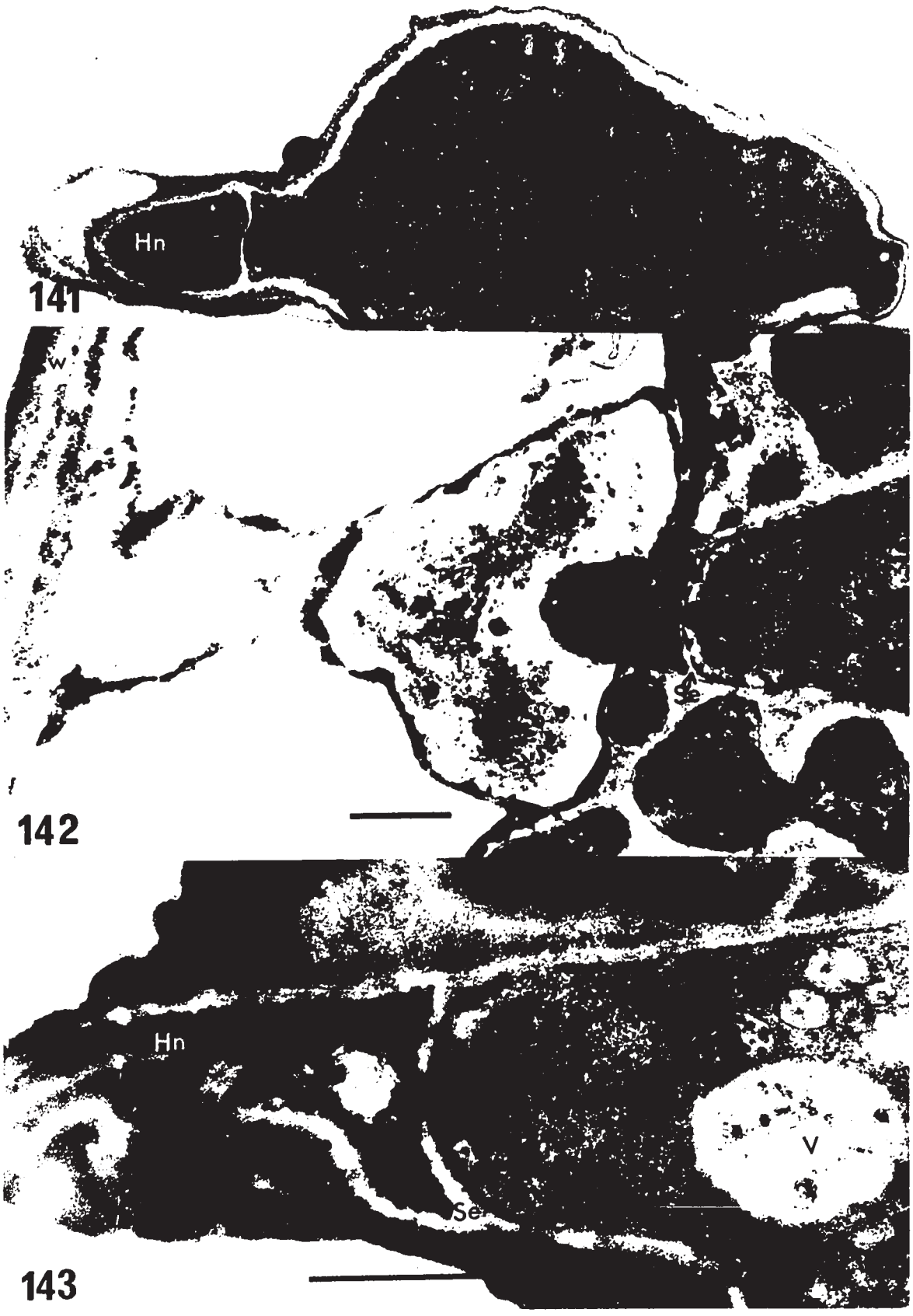

Hn
141
W
S
142
Hn
V
Se
143

Figure 144 Section of part of an infected susceptible clover cell, 5 days after inoculation. A fully developed haustorium with numerous branching appendages or hyphae are in the sheath matrix. Vacuoles are in the branches. Glutaraldehyde and osmium fixed; uranium and lead stained. 4500x.

Figure 145 A highly magnified portion of the haustorium shown in Figure 144. A branch is connected with the central body. Note that there are groups of separated vesicular bodies in the central body (arrows). A myelinoid body, lipid granules, ribosomes and mitochondria are also observed in the cytoplasm. 21,000x.

Figure 146 A highly magnified portion of part of the sheath and haustorium shown in Figure 144. Note the wavy greatly invaginated sheath. 25,000x.

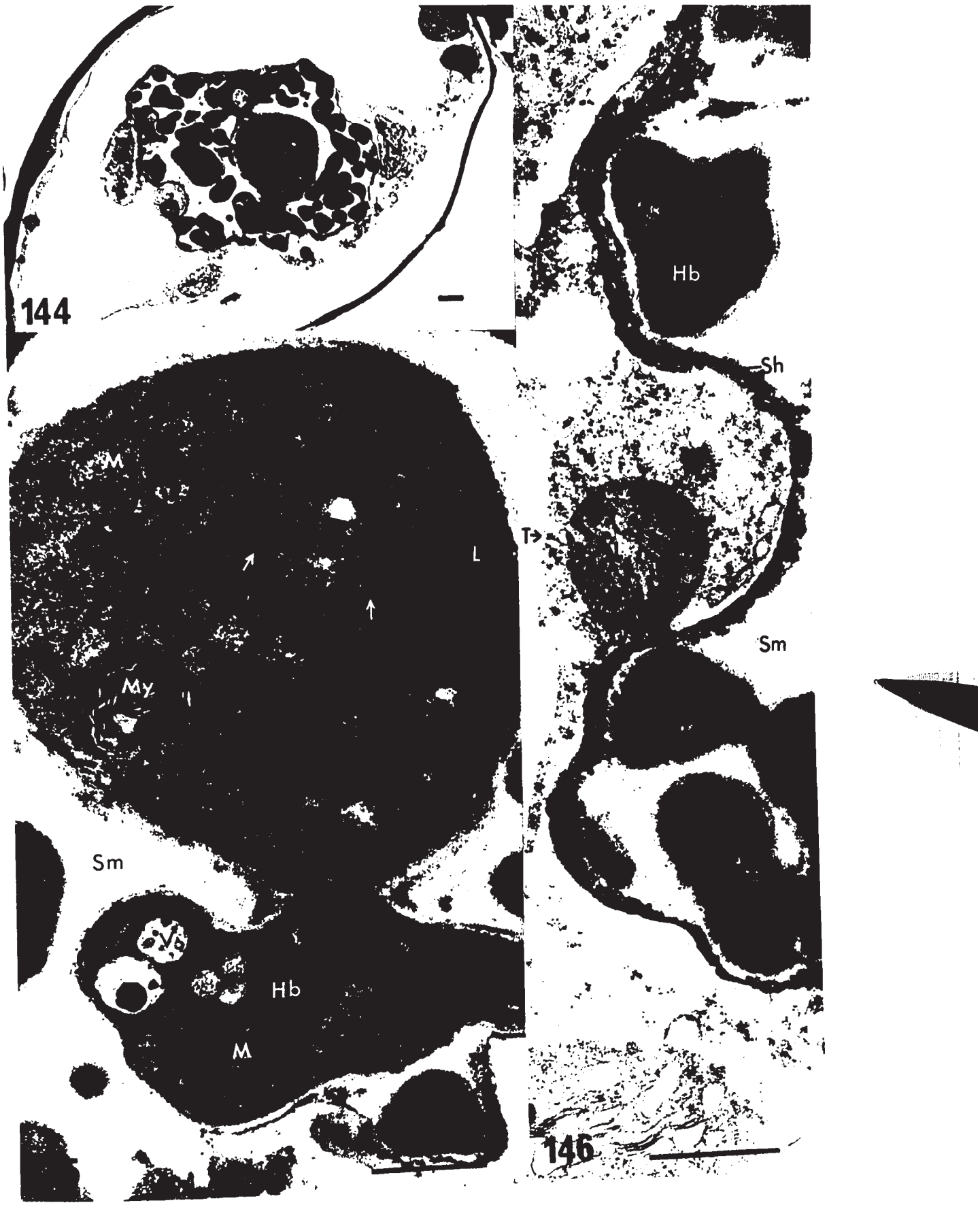

144
M
My
L
Sm
Vb
Hb
M
Hb
Sh
T
Sm
146

Figure 147 Section of an infected susceptible clover cell, 5 days after inoculation and part of mesophyll cells. Note the large host nucleus situated beside the haustorium. Glutaraldehyde and osmium fixed; uranium and lead stained. 7600x.

Figure 148 A highly magnified portion of Figure 147. Observe the densely-stained bodies on the sheath membrane and in the sheath matrix (arrows), and several lipid bodies in the central body. The chloroplast appears normal. 27,000x.

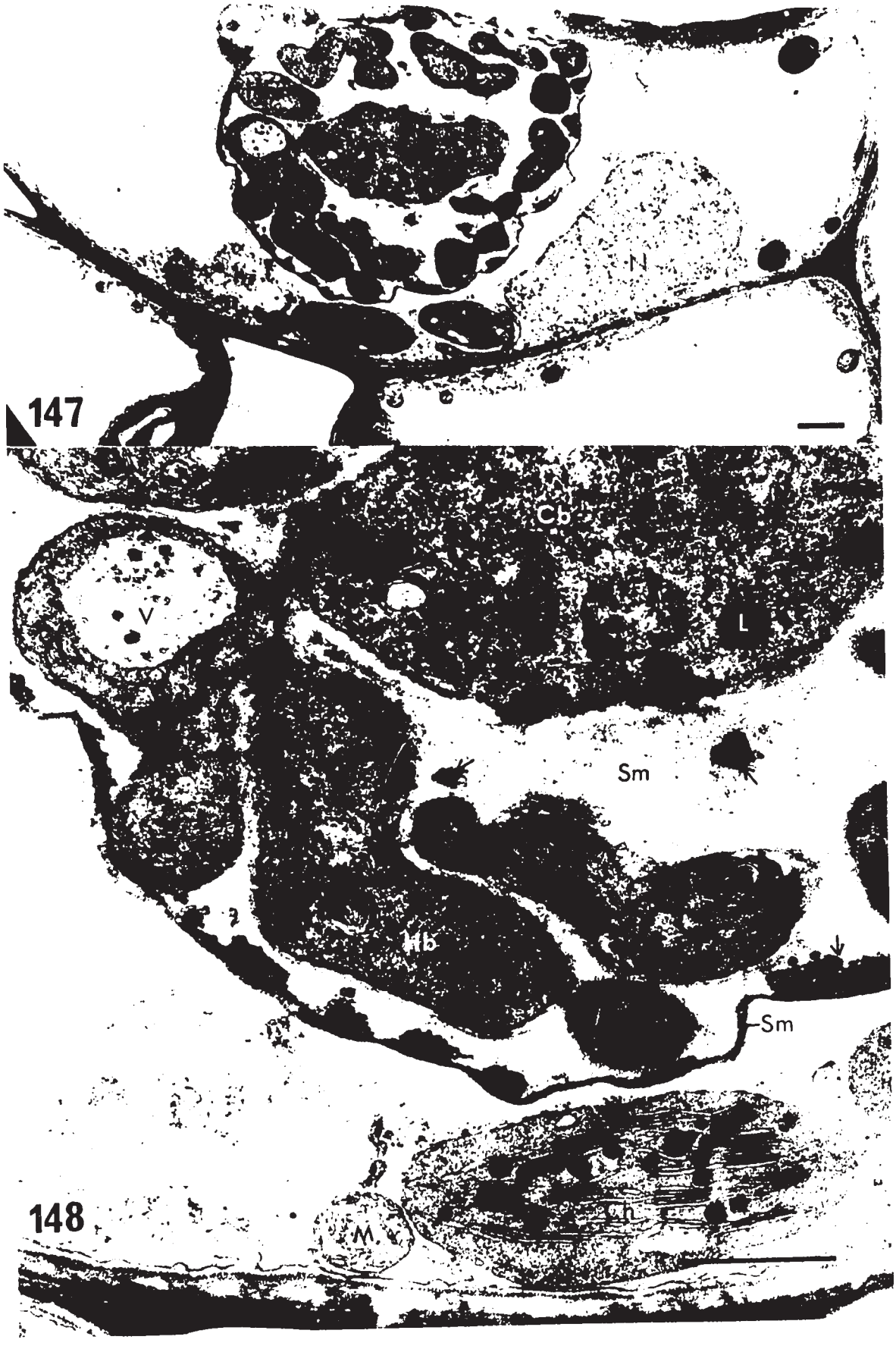

147
N
Cb
V
L
Sm
Hb
Sm
148
M.

Figure 149 Section of part of an infected susceptible clover cell, 5 days after inoculation. A prominent nucleus with nucleolus is in the central body and vacuoles with a variety of inclusions are present. Glutaraldehyde and osmium fixed; uranium and lead stained. 13,000x.

Figure 150 A highly magnified portion of Figure 149 showing an elongated chloroplast, host cytoplasm and the wavy host plasma membrane. 35,000x.

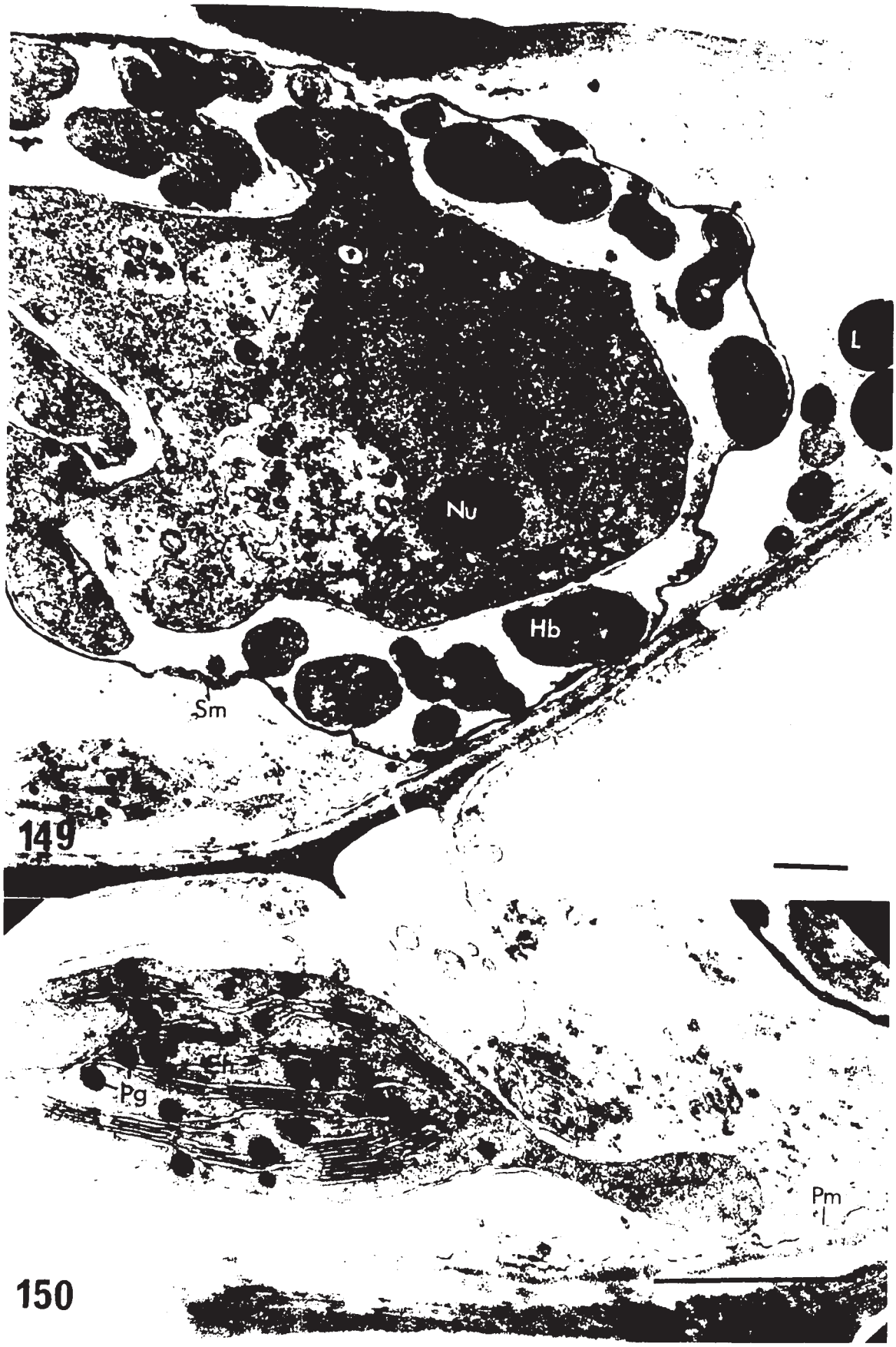
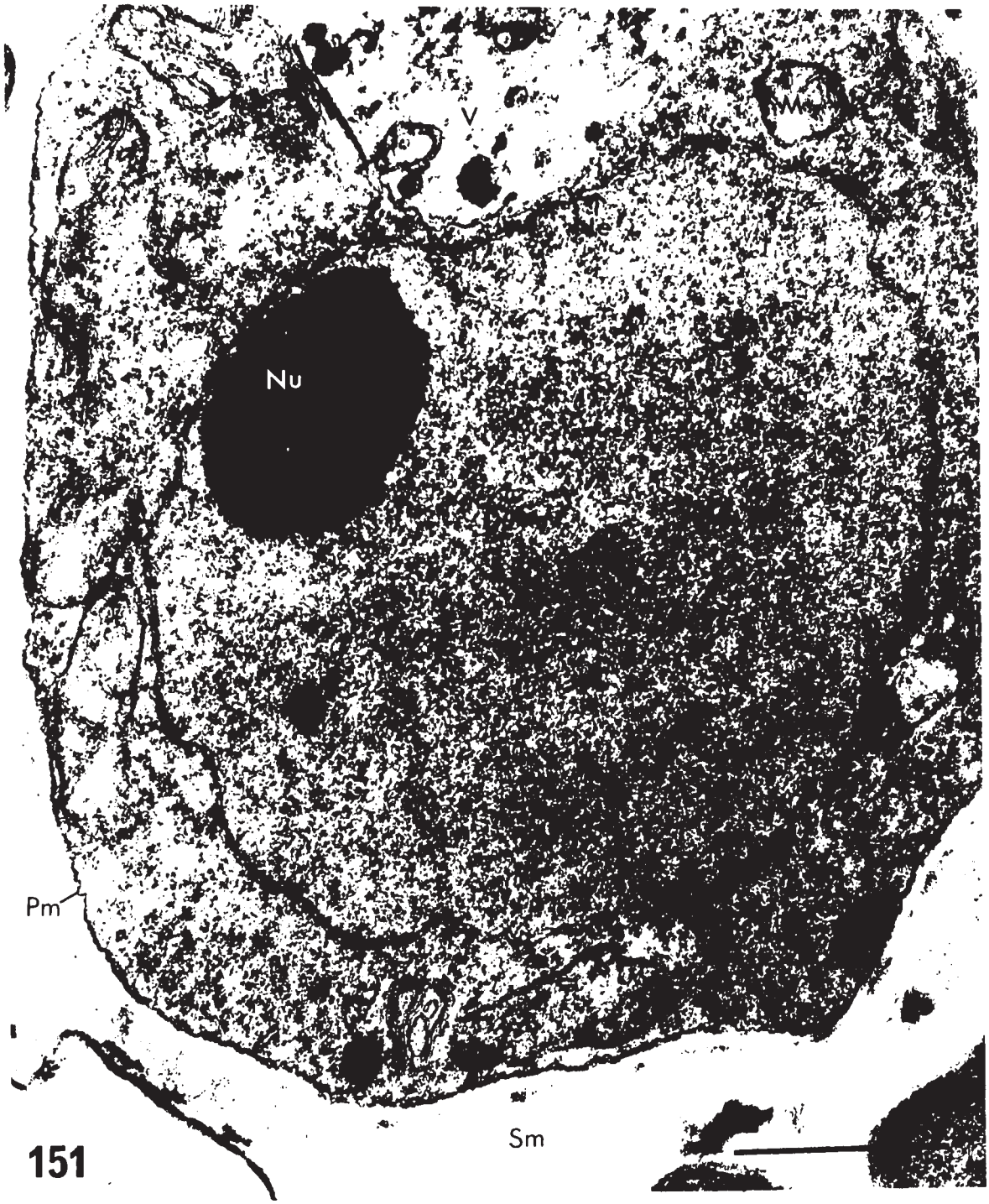

V
L
Nu
Hb
Sm
149
Pg
Pm
150

Figure 151 A highly magnified portion of the haustorium shown in Figure 149. A nucleus and nucleolus with a light area at one side of it are present along with the double-membraned nuclear envelope, mitochondria with tubular cristae, dense ribosomal particles and the plasma membrane of the haustorium.

Glutaraldehyde and osmium fixed; uranium and lead stained. 33,000x.

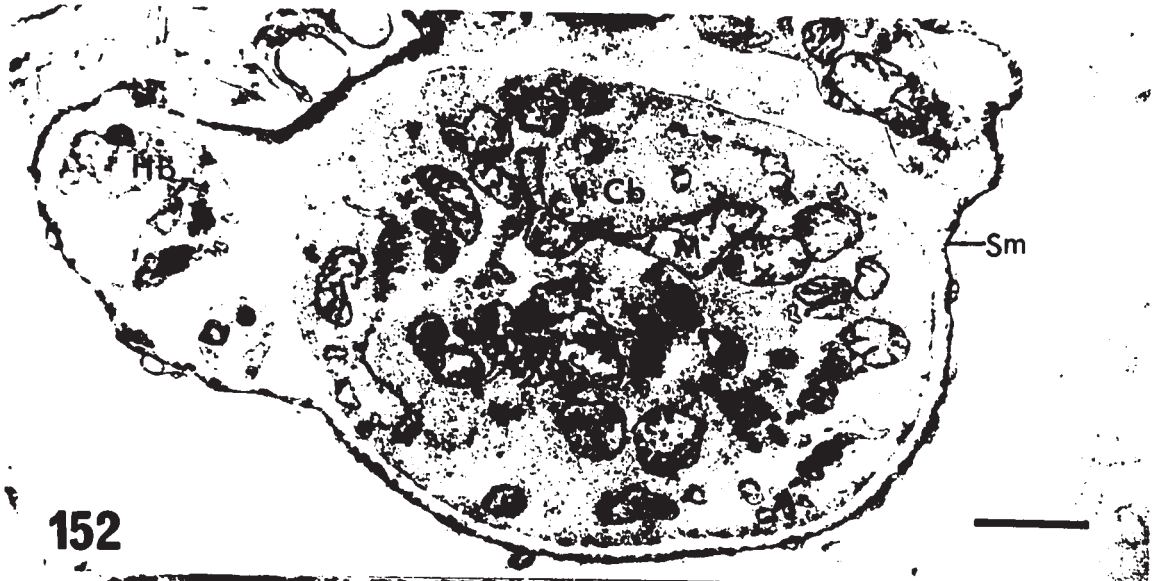


151

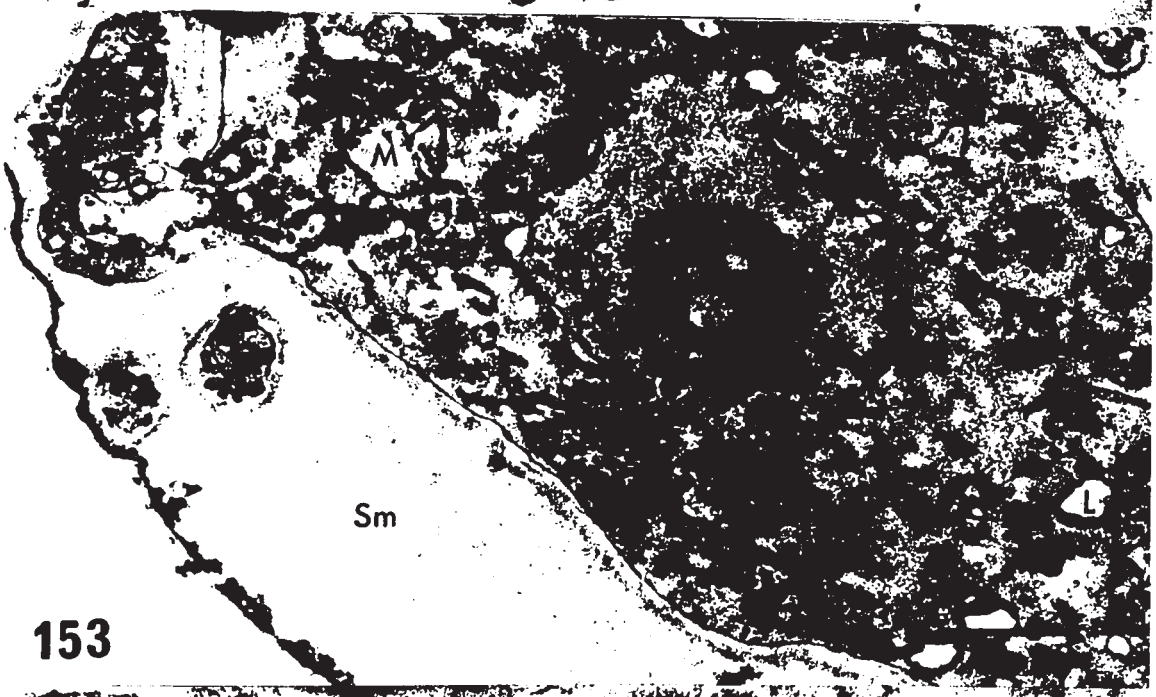
Figure 152 Section of a haustorium in a susceptible clover cell, 5 days after inoculation. Note the wall of the central body is electron transparent, whereas the sheath membrane is more electron dense than other membranes. Observe also the numerous mitochondria in the central body.
Potassium permanganate fixed; unstained.
15,000x.

Figure 153 Section of part of a haustorium with a nucleus and several lipid bodies in the central body.
Potassium permanganate fixed; uranium and lead stained. 18,000x.

Figure 154 A portion of a haustorial branch containing 2 lomasomes.
Potassium permanganate fixed; lead stained.
91,000x.



152



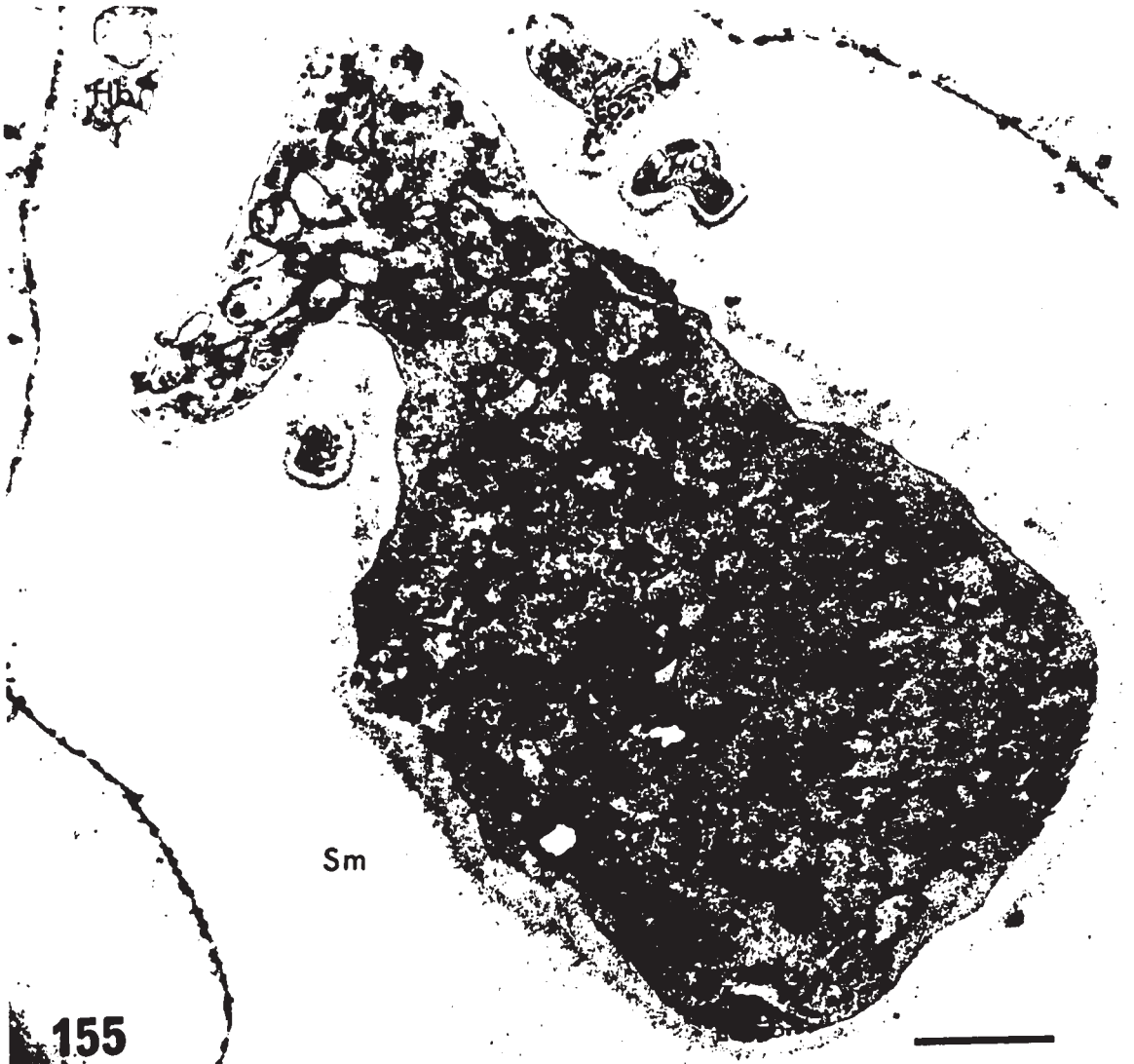
153

154

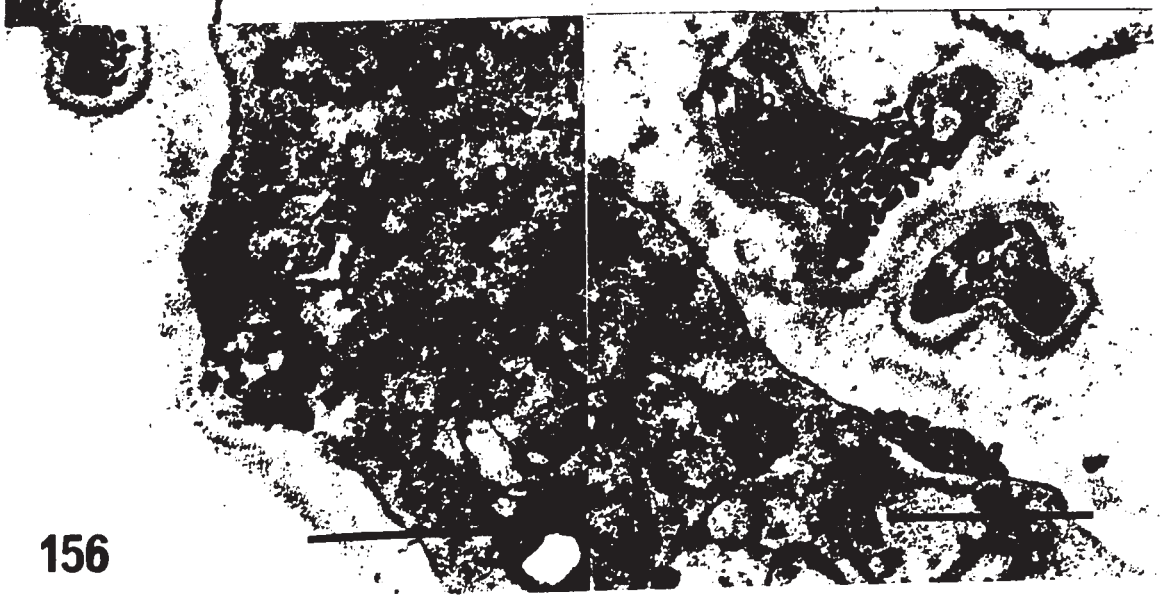
Figure 155 Section of a haustorium showing lomasomes around the central body and in a branch. Note the fibrous netted material in the sheath matrix.
Potassium permanganate fixed; lead stained.
19,000x.

Figure 156 A highly magnified portion of Figure 155 showing a probable point of origin of a haustorial branch. Note the thinning and protrusion of the haustorial wall.
27,000x.

Figure 157 A highly magnified micrograph of Figure 155. Note the vesicular structures in the lomasomes. 27,000x.



155



156

Figure 158 Serial section of a haustorial central body adjacent to the section shown in Figure 155. Observe that the vesicles in the cytoplasm are similar to those in the lomasomes and those in the vacuole. Note also the fibrous netted material throughout the sheath matrix. Potassium permanganate fixed; lead stained. 24,000x.

158

Figure 159 Section of a portion of 2 haustoria in the same susceptible clover epidermal cell, 10 days after inoculation. A thin layer of host cytoplasm is sandwiched between the 2 invaginated haustorial sheaths. Note they do not contact each other.

Glutaraldehyde and osmium fixed; lead stained. 29,000x.

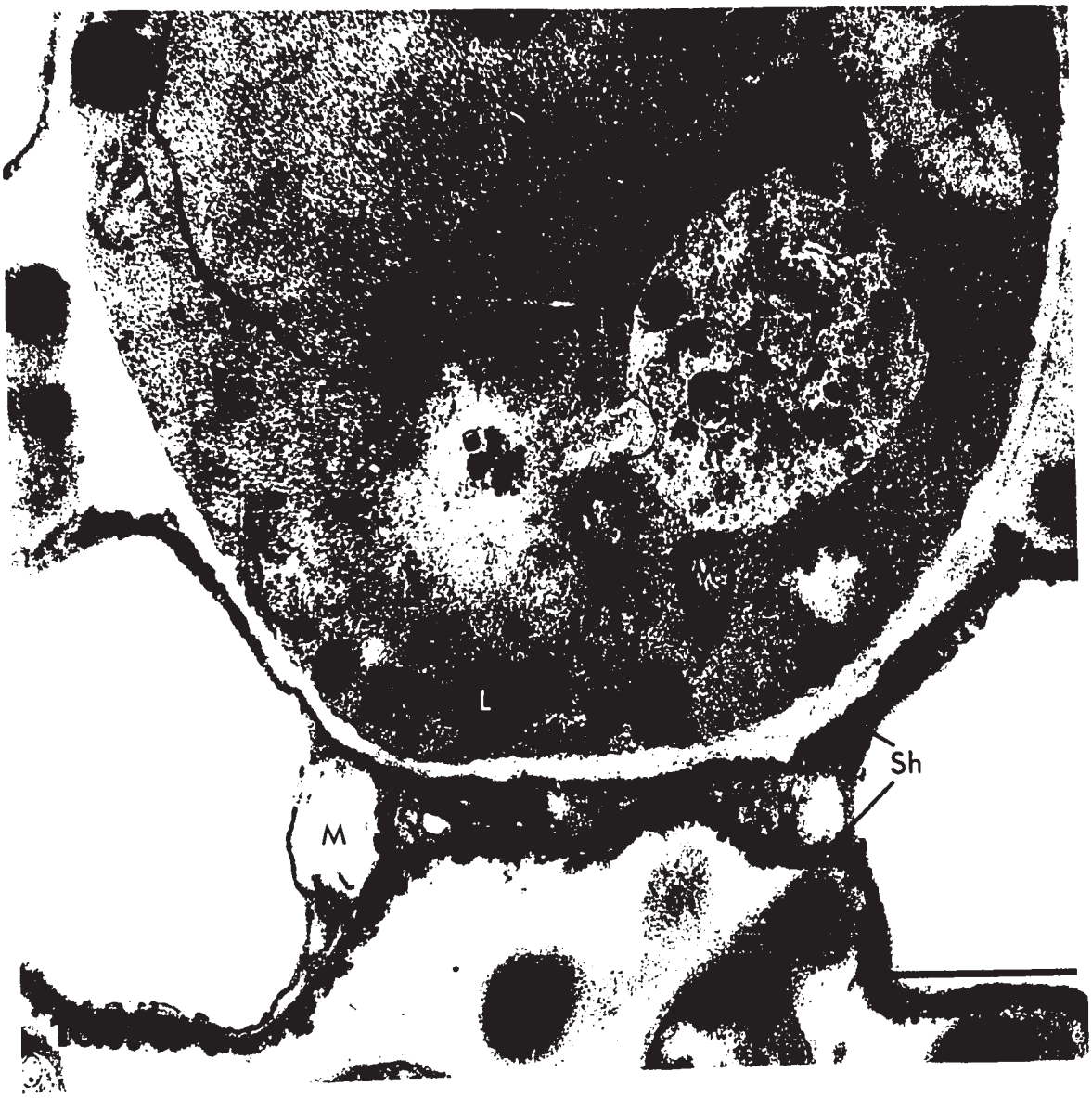

L
Sh
M

Figure 160 Section of part of a haustorium, 5 days after inoculation, showing the highly invaginated sheath membrane. The thickened sheath membrane is about 6-10 times thicker than the host plasma membrane. Glutaraldehyde and osmium fixed; uranium and lead stained. 85,000x.

Figure 161 Portion of a haustorial sheath, 7 days after inoculation showing the highly invaginated and thickened sheath membrane. Note also the fibrous material in the sheath matrix. Glutaraldehyde and osmium fixed; Uranium and lead stained. 112,000x.


Si
T
Pm
Sm
Sm
Si
Hb
Sh
Sh
T
Go
0.1 μ
160
161

Figure 162 Portion of the haustorium from Figure 155 showing the sheath membrane, fibrous netted material, sheath matrix and a lomasome at the edge of the central body.
Potassium permanganate fixed; lead stained.
38,000x.

Figure 163 Section of part of an haustorium showing the surface view of the vesicular lomasome in a haustorial branch.
Potassium permanganate fixed; lead stained.
56,000x.

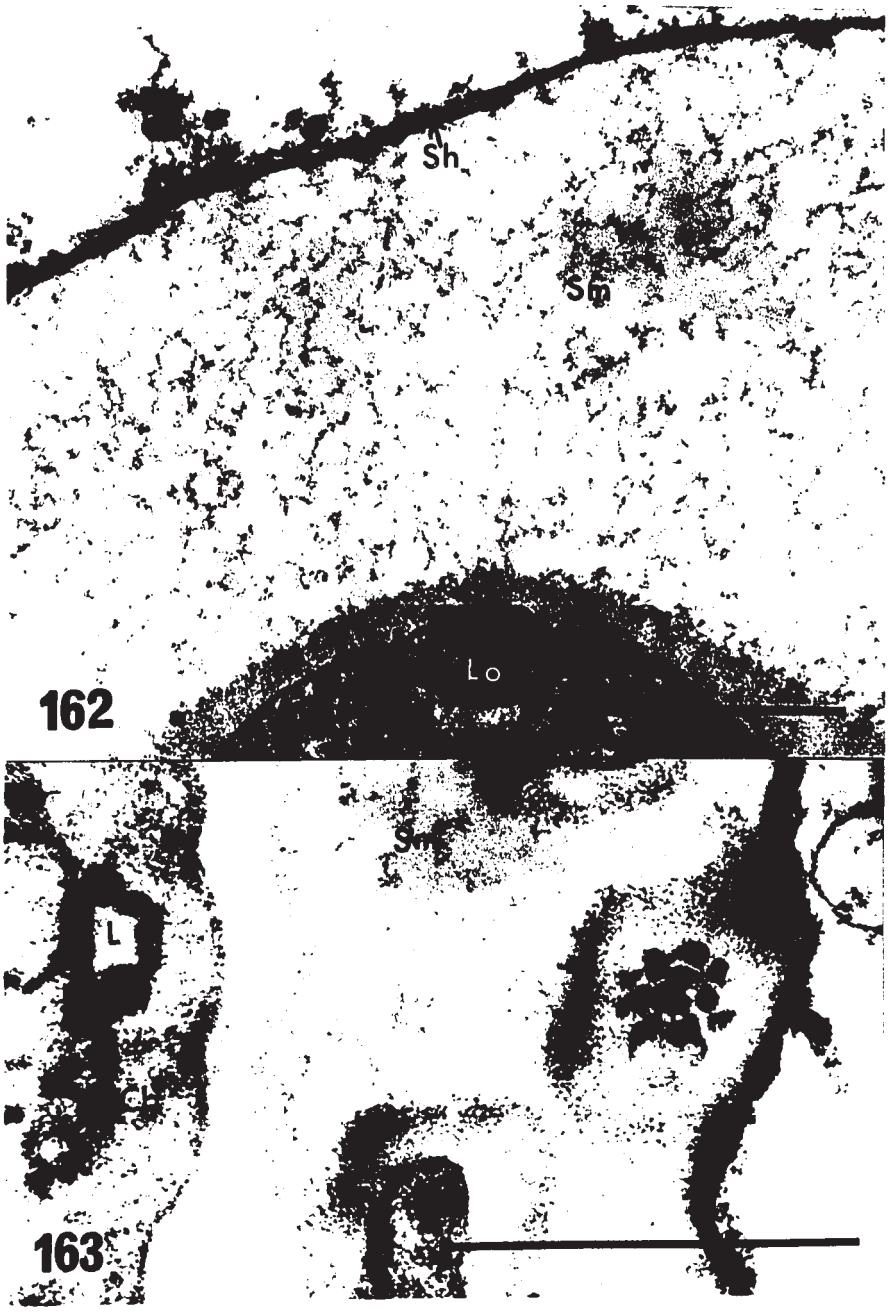

Sh
Sp
Lo
162
Sm
L
163

Figure 164 Section of part of an infected susceptible clover cell, 10 days after inoculaton, showing a haustorium with many vacuoles and lipid bodies as well as a nucleus in the central body. A host nucleus is situated beside the haustorium.
Glutaraldehyde and osmium fixed; uranium and lead stained. 6,000x.

Figure 165 A highly magnified portion of Figure 164 which shows vacuoles and lipid bodies in the central body. A lipid-like body is present in the sheath matrix (arrow).
36,000x.

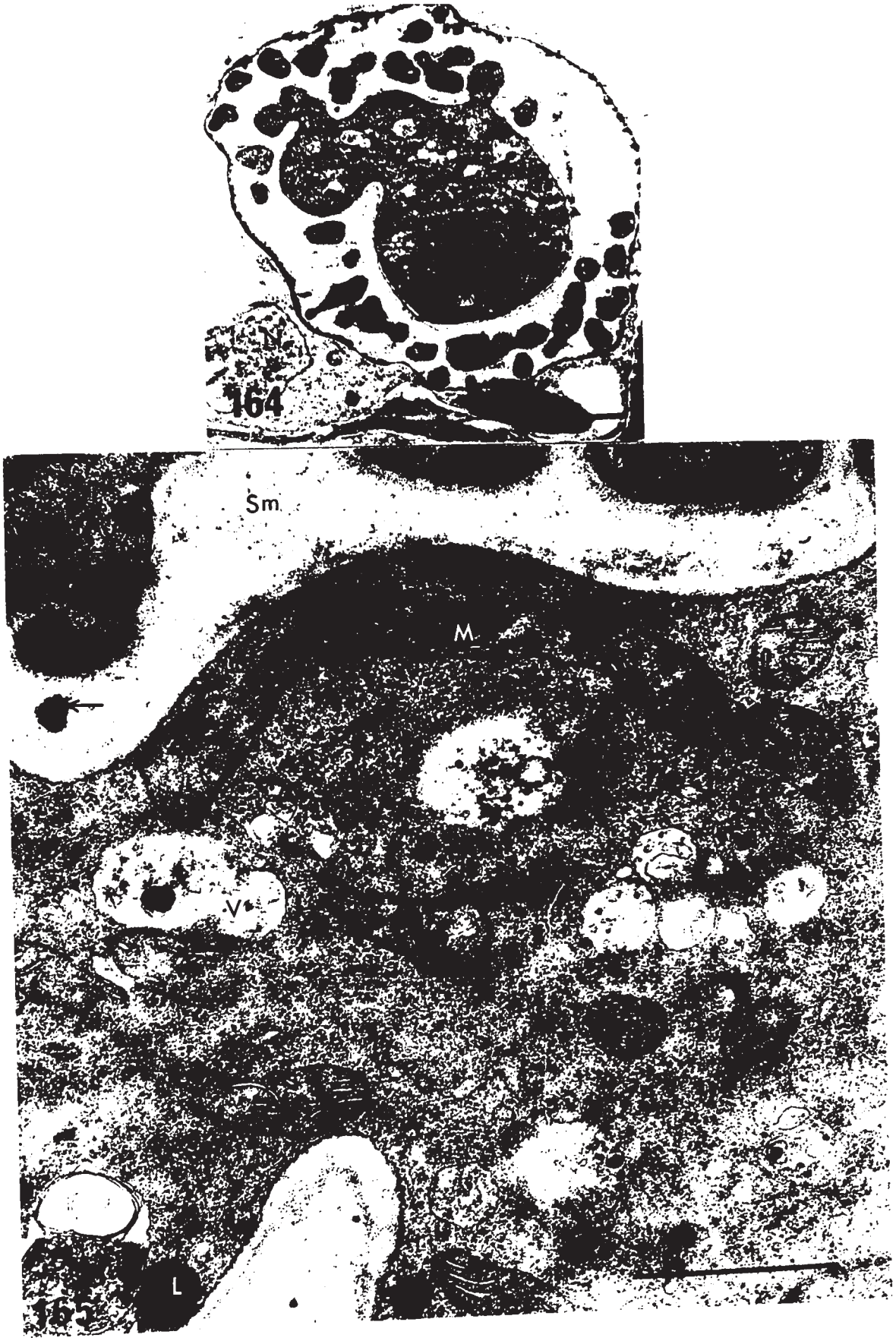

164
Sm
M
V
L
165

Figure 166 Section of a portion of a haustorium in a susceptible host cell, 10 days after inoculation. It shows the appearance of an older haustorium. Observe the lipid bodies, vacuoles with lipids and other unknown densely-stained material. The nucleus has a fuzzy or discontinuous nuclear envelope. Note the 2 large lipid-like bodies in the sheath matrix. Glutaraldehyde and osmium fixed; uranium and lead stained. 25,000x.

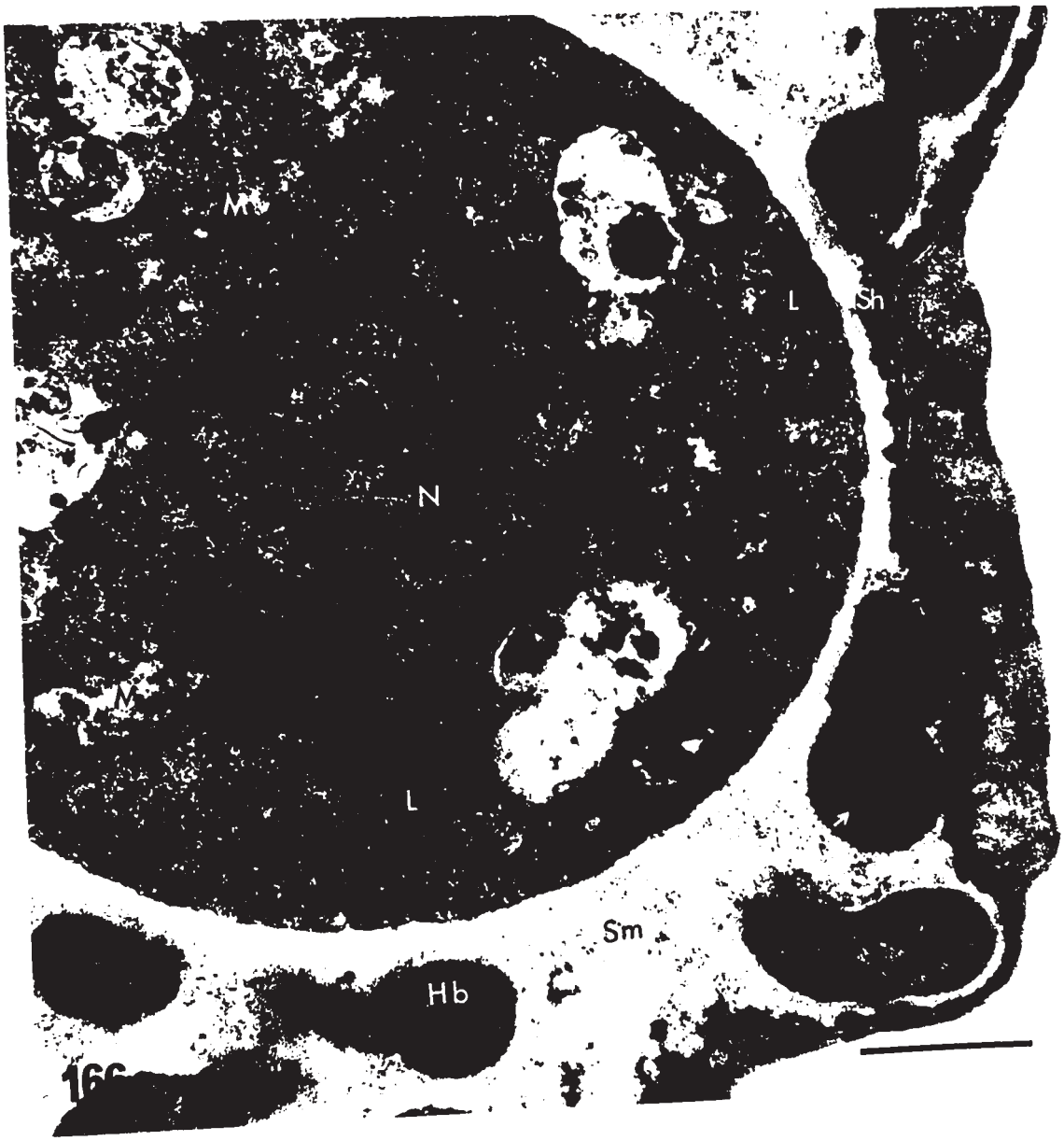
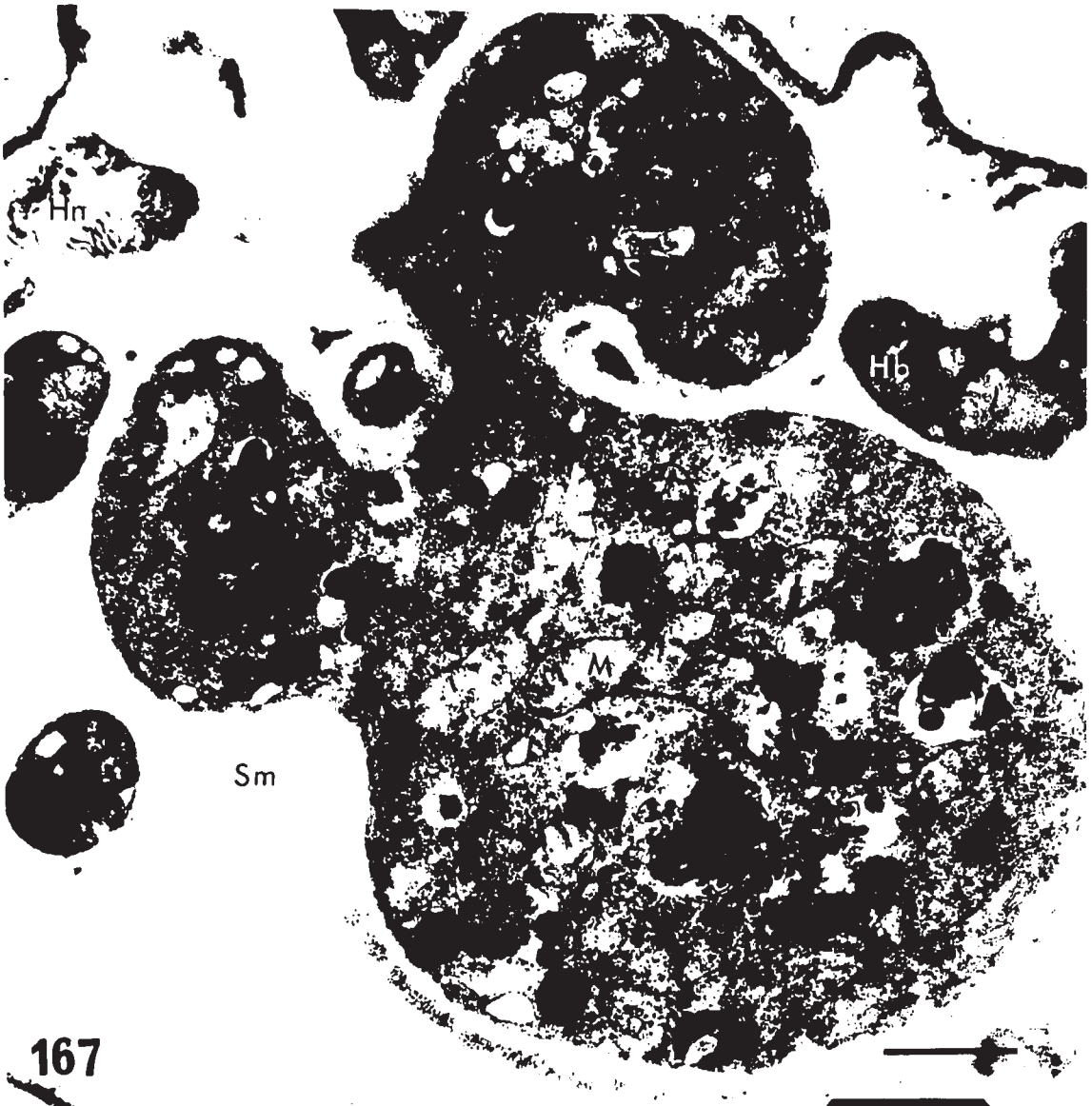

M
L
Sh
N
M
L
Sm
Hb

Figure 167 Section of a haustorium in an infected susceptible clover cell, 2 weeks after inoculation. The old haustorium has numerous lipid bodies and vacuoles with masses of densely-stained material. Glutaraldehyde and osmium fixed; uranium and lead stained. 19,000x.

Figure 168 Section of an infected susceptible epidermal cell, 2 weeks after inoculation. The apparently dead haustorium is surrounded by the disintegrated cytoplasm of the host cell. Note the sheath membrane appears intact.
Glutaraldehyde and osmium fixed; uranium and lead stained. 6,500x.



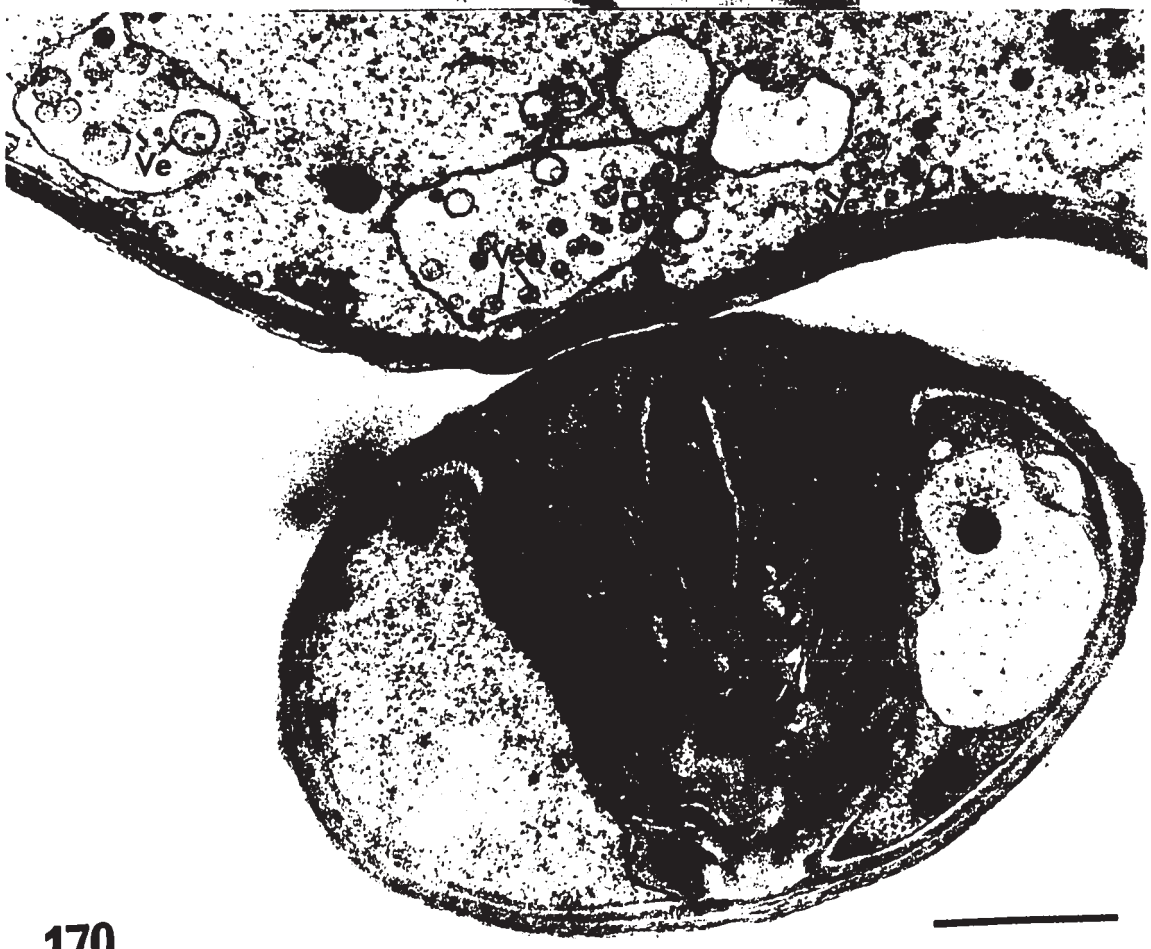
167

168

Figure 169 Two hypha of the same strain which have crossed each other. The penetration peg appears loop-like and surrounded by a layer of material.
FAA fixed; Azure B stained. 3,000x.

Figure 170 Electron micrograph of a longitudinal section through a hypha. Its penetration peg has penetrated into another hypha of the same strain. Observe that vesicular structures near the point of origin of the penetration peg. Note there is a wide electron-dense zone around the penetration peg. Glutaraldehyde and osmium fixed; uranium and lead stained. 25,000x.

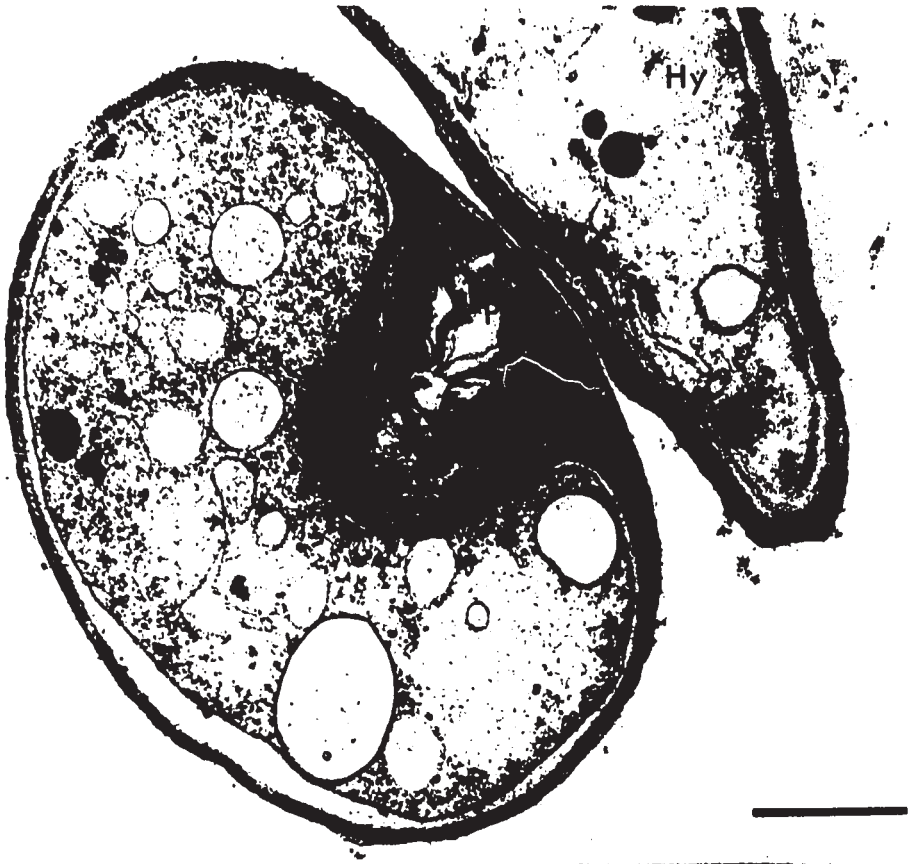
169



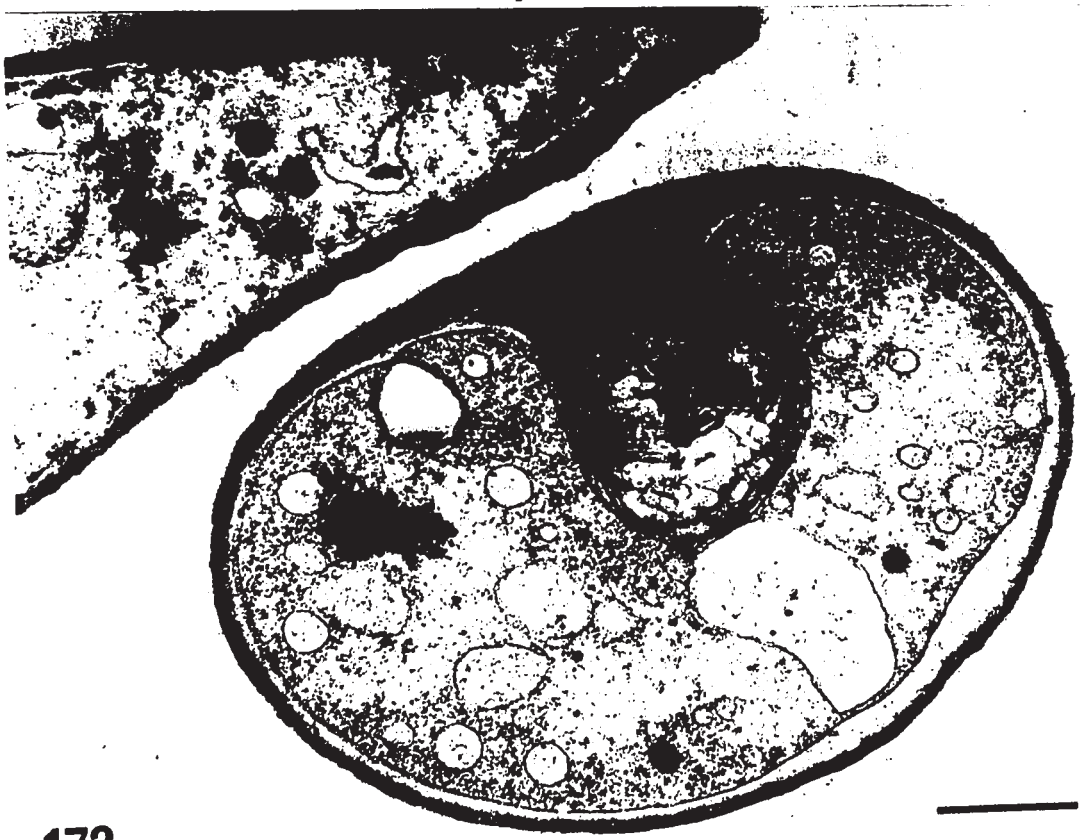
170

Figure 171 Section of a part of 2 hyphae, showing an oblique view of a peg. Observe the new wall surrounding the penetration peg. Both the infected and the invading cell appear to be living.
Glutaraldehyde and osmium fixed; uranium and lead stained. 21,000x.

Figure 172 Serial section adjacent to the one shown in Figure 171. Observe the vesicles in the peg.
19,000x.



171



172

Figure 173 A longitudinal section through a hypha with an infection peg which has penetrated an identical strain of hypha. Observe that the wall appears to have been dissolved at the pores. There is also an electron-dense zone around the infection peg. Glutaraldehyde and osmium fixed; uranium and lead stained. 21,000x.

Figure 174 A cross section through 2 hyphae. Observe that the peg enlarged greatly after penetrating the wall and that the plasma membrane has withdrawn some distance in advance of the penetration peg. Glutaraldehyde and osmium fixed; uranium and lead stained. 10,000x.

Figure 175 A cross section through a penetration peg which has not enlarged after penetrating the fungal wall. Observe that the fungal wall does not appear to be mechanically broken at the point of entrance. Glutaraldehyde and osmium fixed; uranium and lead stained. 61,000x.

173

174

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