1967

Light And Electron Microscope Studies Of Sphaerotheca Macularis

Norman Lancelot Mitchell

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LIGHT AND ELECTRON MICROSCOPE STUDIES
OF SPHAEROTHECA MACULARIS (WALLR. EX FR.) COOKE

by

Norman Lancelot Mitchell
Department of Botany

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada
August, 1967

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ABSTRACT

The ultrastructure of the strawberry mildew *Sphaerotheca macularis* was studied by light and electron microscopy to elucidate some physiological peculiarities common to powdery mildews. Comparative observations were made on two other species of powdery mildews, *Erysiphe polygoni* and *Erysiphe cichoracearum*.

The conidia are shown to be highly vacuolated structures with relatively sparse cytoplasmic contents. The vacuoles contain water, and evidence is given that they probably contain reserve nutrient material also. Conspicuous myelinoid bodies are associated with the vacuoles and it is postulated that they serve a synthetic function in the utilization of the reserve material in the vacuoles.

A mucilaginous layer of wall material surrounds the conidium as well as the hyphae, and it is shown to serve three important functions. It insulates the conidium against loss of water, fastens it to the surface of the host thus preventing it from being washed off by rain, and provides the necessary anchorage required by the hyphae to develop the pressure needed to penetrate the host cuticle.

Obvious cytoplasmic changes accompany germination. The
number and size of mitochondria increase. The endoplasmic reticulum is greatly enriched. Ribonucleoprotein particles become abundant and aggregated granulations resembling glycogen particles accumulate.

The wall of the germ tube is formed from the inner layer of the conidial wall. The germ tubes respond positively to light stimulus and are produced on the illuminated side of the conidia.

Evidence is given that mitochondria multiply by fission as well as being formed from the endoplasmic reticulum.

The septa of hyphae and conidiophores are not typical of those generally seen in Ascomycetes. Instead of tapering toward the middle the wall thickens at the centre and the pore is in the centre of a biconcave disc.

Nuclear division is neither direct nor typically mitotic. Chromosomes are clearly visible and mitotic stages are evident but an extra-nuclear spindle is absent. A conspicuous peripheral granule, associated with the nucleus, functions in the segregation of the chromosomes.

Penetration of the host cuticle is mechanical but both mechanical pressure and enzyme action are believed to be involved in the penetration of the cellulose layer. During penetration the thickness of the host wall is progressively increased in advance of the penetration peg. This could affect resistance to penetration in host plants having a thick cell wall.
Haustoria were observed to be separated from the host cytoplasm only by a single unit membrane which is believed to be the host plasma membrane. No encapsulating layer was present. Loosely scattered, electron-dense, deposits on the inside of the membrane enclosing the haustoria are believed to be waste products of metabolism which serve no specific function.
ACKNOWLEDGEMENT

The author wishes to express his gratitude and sincere appreciation to the following persons whose assistance and encouragement have been of invaluable help in making the accomplishment of this work possible.

To Dr. W. E. McKeen, Professor of Botany, University of Western Ontario, London, Canada, for proposing the problem for research, for his advice and direction and for his continual encouragement.

To Dr. C. J. Hickman, Head of the Department of Botany, University of Western Ontario, London, Canada, for making the Department and its facilities available.

To Dr. C. F. Robinow for his assistance in developing a technique for staining fungal nuclei.

To Dr. D. A. McLarty, Professor of Botany and Dr. A. M. Wellman, Associate Professor of Botany, University of Western Ontario for reading the manuscripts.

The author would also like to acknowledge his indebtedness to the National Research Council of Canada for providing the necessary financial help.

Finally, very special gratitude is extended to all the members of the technical and secretarial staff of the Department of Botany, University of Western Ontario, and to the author's fellow graduate students for their many acts of kindness, their thoughtfulness and their encouragement at all times.
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CHAPTER I

INTRODUCTION

Whether or not the references made by early Bible writers to mildews as agents of crop destruction (1 Kings 8:37; Deut. 28:22; Hagai 2:17) were applicable to any particular group of fungi or to parasitic fungi in general, is open to much speculation. However, it is evident from the literature that the term mildew as applied to members of the Peronosporaceae or downy mildews and the Erysiphaceae or powdery mildews, has been in use for well over two centuries. Salmon, in his monograph of the Erysiphaceae (98) indicated that the powdery mildews were named by Linnaeus at least as early as 1753.

The wide range of agricultural crop plants that are hosts to this ubiquitous group of fungi makes the name powdery mildew a household word to farmers almost everywhere. Their economic importance is readily appreciated when one realizes that, according to Eriksson (41), "The Erysiphaceae, next to the rusts, includes the largest number of forms found in any of the groups of parasitic fungi, many of which cause considerable damage to cultivated crops". As pointed out by Yarwood (117) the powdery mildews as a group attack most cultivated crops.
Speaking of the grape powdery mildew, *Uncinula necator*, Eriksson (41) stated that during the years 1852-55, sometimes as much as nine-tenths of the grape crop in southern Europe was destroyed by this fungus. Consequently, farmers began to regard the growing of grapes as impossible and the labourers migrated in large numbers to America. A recent study done by Large and Doling (65) on the effects of powdery mildew on barley and oats in nine experimental stations in England and Wales (1957-60), showed a mean loss in yield of nine cwt per acre.

The pathological importance of powdery mildews is further emphasized by the attention paid to them by plant pathologists and mycologists. Ten years ago Yarwood (117) observed that since 1900, over 3,000 publications had appeared on the powdery mildews. The last decade has seen an even greater and ever accelerating interest in powdery mildew research.

The importance of mildew as a parasite of strawberry must be assessed from two aspects, i.e., from the importance of strawberry as an economic crop and from the extent to which the fungus is capable of reducing production.

A report quoted by Jhooty (63) from the Canadian Department of Agriculture Bulletin of 1956 stated that strawberry is second only to apple in crop value in Ontario. This report further showed that between the years 1950-1956 Canada alone produced approximately 28,224,000 quarts of strawberries annually, valued at $6,358,000. The value of this crop on a
world wide scale is obvious.

Damage to strawberry crops by powdery mildews, though not usually of epidemic proportions, can, however, be quite extensive. Evidence to this fact was given by Salmon (98) who reported in 1900 that the number of strawberry crops he observed ruined by powdery mildew fungi demanded that serious attention be given to the subject. Peries (87) stated in 1962, that strawberry mildew had been known in the United Kingdom for over one hundred years and that the disease has been observed in all parts of the world in which strawberries are grown. Of the varieties grown in the United Kingdom, he claimed that none was known to be resistant to mildew and two species are extremely susceptible. The importance of this fungus as a subject for research, however, transcends its immediate economic importance. The powdery mildews, being obligate parasites, are among the few remaining fungi which have continued to defy attempts to grow them successfully on artificial media. A clearer understanding of the interrelationships between these fungi and their hosts at the subcellular level may help to solve this problem.

Another characteristic of the powdery mildews that is of considerable importance as a subject of research is their extreme biologic specialization. Within the different species of powdery mildews are many subspecies, varieties and races most of which cannot be distinguished by morphological differences but can most often be distinguished by their ability
to grow on different varieties or species of host plants.

Yarwood, (117), in his review of the powdery mildews (1957) stated "A pure culture of a powdery mildew derived from a single spore usually will attack only species within a single genus, occasionally will attack several genera within a family and rarely will attack several families of host plants".

The extent to which this host specificity exists was demonstrated by Peries (88) working with Sphaerotheca macularis (Wallr. Ex. Fries) Jaczewski. He inoculated the leaves of twenty-five different species of weeds and cultivated plants known to be hosts of a fungus which is morphologically identical to the one on strawberry, with conidia of strawberry mildew. The results showed that strawberry mildew would grow only on one other plant — Potentilla fragariastrum, grown continually under greenhouse conditions. All attempts to transfer mildew of other plants to strawberry were unsuccessful.

Associated with this biologic specialization, and perhaps closely related to the cause of it, are certain differences in the physiology of the various varieties of powdery mildews, conflicting reports of which have lead to some amount of confusion in the literature. Such discrepancies no doubt have contributed largely to the interest now being given to powdery mildew research.

Perhaps the points of greatest disagreement among workers on the powdery mildews are those relating to water content and
osmotic pressure of conidia and their moisture requirements for germination and development. The literature is replete with evidence, but a few examples will suffice.

Yarwood (114), Cherwick (30), Schnathorst (99, 100, 101), Boughey (14) and several others have shown that high moisture conditions have a deleterious effect on powdery mildew fungi. Indeed, Boughey has claimed that throughout the world the incidence of powdery mildews decreases as rainfall increases and Yarwood (115) proposed that powdery mildews could be controlled by spraying with water.

On the other hand, Delp (38) listed a number of workers who found that rain, mist and other damp conditions were favourable to powdery mildew development. Moore (75) found that wet conditions favoured powdery mildew of garden snap beans and Jhooty (63) found that the infection of strawberry by Sphaerotheca macularis reached its highest intensity at 100% relative humidity and decreased accordingly as the humidity decreased. These discrepancies were sufficiently evident in 1957 for Yarwood (117) to remark that the relation of moisture to disease has been one of the most controversial aspects of our knowledge of powdery mildews.

Similar differences exist on the matter of the germination of mildew conidia at different moisture levels (18, 19, 38). It is now generally known that powdery mildew conidia, unlike most other fungal spores, will germinate without an external source of water, and some at very low humidities. Brodie (20)
showed that conidia of *Erysiphe polygoni*, *Erysiphe graminis*, *Uncinula salicis* and *Miscrosphaera alni* germinated at 0% relative humidity. Brodie and Neufeld (19), Clayton (31), Delp (38) and Yarwood (114) found that conidia of various mildews will germinate at humidities approaching zero relative humidity. But in 1947, Grainger (45) showed that conidia of *Erysiphe graminis* causing mildew of corn were not able to germinate on glass slides below 80% relative humidity, and Arya, according to Jhoooy (63), found that conidia of *Erysiphe graminis* on wheat failed to germinate below a relative humidity of 93%. Berwith (12) and Brodie (2) showed that high water stresses inhibited the germination of conidia of *Erysiphe cichoracearum*, *Podosphaera leucotricha*, *Sphaerotheca humuli* and *Sphaerotheca pannosa*. Data given by Jhoooy (63) for *Sphaerotheca macularis* showed 87% germination at 100% relative humidity with a rapid decrease in germination to 7% at 8% relative humidity. The most controversial reports regarding this aspect of powdery mildew biology have centred around conidia of *Erysiphe graminis*, probably because it has been most thoroughly investigated. However, after studying three forms of this species, Manners and Bossain (68) could find no significant difference in their water requirements for germination.

It is clear from the literature that powdery mildew conidia vary widely in their moisture requirements for germination. But the fact that a large number do germinate at relative humidities far lower than that required for most other fungal spores suggests a need for an investigation of their water
In 1942 Brodie and Neufeld (19), describing the structure of conidia of *Erysiphe polygoni*, noted that the protoplast contained numerous globules that appeared like vacuoles. Their attempts to stain these globules with neutral red were unsuccessful, and linking this with the fact that, "the protoplast shrinks but slightly during plasmolysis", these authors stated "It would seem justifiable to conclude that the mature conidium contains very little free water prior to germination".

In 1950, however, Yarwood (ll6) using the fresh and dry weight method to determine the water content of several fungal spores, found that the surface-dry conidia of *Erysiphe polygoni* contain about 70% water, whereas other representative airborne fungus spores contain only about 10%. He later proposed that the explanation of the mildew's capacity to germinate at such low humidities was probably due to the high water content of the conidia and their extremely efficient system of water conservation (l17).

In 1964, Jhooty and McKeen (64) working on conidia of *Sphaerotheca macularis* and *Erysiphe polygoni* obtained a percentage of water content of 53 and 69% respectively. They showed that conidia of *Sphaerotheca macularis* germinated best at 99 to 100% relative humidity on glass slides and would not germinate if the humidity was below 93%. On the contrary, conidia of *Erysiphe polygoni* germinated readily at 3% relative humidity at the same temperature.
Somers and Horsfall (106) found that the water content of *Erysiphe cichoracearum* and *Erysiphe graminis* varies with the humidity conditions prevailing during conidia formation, with a maximum of 66 and 69% respectively of their fresh weight as water. They found, too, that although germination was greatest when turgidity was highest, no consistent correlation between these two factors existed. Clearly, there must be a great deal of physiological variation even within closely related races of powdery mildews.

Reviewing these differences in the response of powdery mildew conidia to different moisture stresses, Schnathorst (101) suggested that the powdery mildews could be divided into three categories on the basis of these responses as follows: those which germinate only at low-moisture stress, those which germinate optimally at low moisture stress with a small percentage of conidia germinating at high-moisture stress, and those which germinate well through a wide range of moisture stress. He admitted the need for further subdivisions in the first two categories.

However, as noted by the same author, most, if not all studies on moisture relations in powdery mildews have shown that free moisture is inhibitory to the germination of their conidia. Peries (87) claimed that free water was lethal to conidia of *Sphaerotheca macularis*.

If, as stated by Yarwood (117), mildew conidia carry their water with them, they would not need moisture from the environment for their germination. However, according to
Gregory (47), fungal spores are characterized by having small water content and a lack of vacuoles, and Brodie and others feel that mildew conidia do not contain true vacuoles and contain very little free water. On the contrary, Corner (34) after studying several species of mildews, stated that mildews carry their water of germination in the highly vacuolate cytoplasm of the conidia. It is clear that if mildew conidia do contain water to the extent of 70% of their weight they must either contain vacuoles or an excessively hydrated cytoplasm. The need for electron microscopic investigation of the structure of these conidia is evident.

In his paper "Observation on the Mechanism of Germination of the Conidia of Various Species of Powdery Mildew at Low Humidity", Brodie (20) stated that the protoplast of the conidium in the Erysiphiaceae probably lacks much free water and that what cell sap there is may be quite concentrated. He proposed that the high osmotic pressure of these conidia might be an aid to the spores in absorbing water from relatively dry atmospheres. He found the osmotic pressure of *Erysiphe polygoni* and *Erysiphe graminis* conidia to be 63 atmospheres and 68 atmospheres respectively. Several other workers have obtained unusually high osmotic pressures on mildew conidia but here, too, there is not much agreement. Thatcher (107) found the osmotic pressure of *Erysiphe polygoni* to be 18 atmospheres as compared with Brodie's 63 atmospheres for the same fungus. And Jhooty and McKeen (64) obtained
values of between 17.8 and 21.5 atmospheres for conidia of *Sphaerotheca macularis*. However, their table of osmotic values showed that between 5 and 10% of the conidia had osmotic pressures above 34 atmospheres.

Somers and Horsfall (106) offered a third explanation for the ability of mildew conidia to germinate at high moisture stress by proposing that water retaining power and not absolute water content is the important factor in germination of powdery mildews at low humidity. They found that the water contents of conidia from the same host will vary considerably and that germination potential was greatest when percentage turgidity was highest, but analysis of 15 different germination tests showed no consistent correlation between these factors. For example, at least 50% of the conidia of *Erysiphe cichoracearum* were nonviable even when all the spores were turgid and hyaline. Indeed, Brodie (20) felt it was unadvisable to theorise until further knowledge of the nature of the protoplast was available. One of the purposes of this study was to provide further evidence as to the nature of the protoplast.

While some aspects of the biology of the mildews have been extensively investigated, even if perplexingly confused, others have been almost totally neglected. This is especially true with regard to the structure and mode of division of the nucleus.

In 1905 Harper (48) published an exhaustive description
of sexual reproduction and the organization of the nucleus in some mildews. Speaking of *Phyllactinia corylea* in particular and of *Erysiphe* and other powdery mildews in general he placed strong emphasis on a peculiar and characteristic attachment of the chromatin of the nucleus to a conspicuous deeply stained body which he called the central body. He claimed that this central body persists throughout all stages of the dividing and resting nucleus as a permanent structure through the whole life history of the mildews and gives the nucleus a characteristic polar rather than a radial structure. He described the nucleolus as being conspicuous and lying usually in the antipolar region of the nucleus which is the region antipodal to the central body.

During nuclear division the central body divides and the daughter centres migrate away from each other on the surface of the nuclear membrane. From these, the spindle fibres develop. Harper claimed to have observed all the stages of classical mitosis during ascospore formation in *Phyllactinia corylea*. He did not describe nuclear division in the vegetative structures of the fungus.

In 1936 Allen (7) published her cytological study of *Erysiphe polygoni* in which she described the structure of the vegetative nucleus. She wrote: "The nucleus always contains abundant chromatin, a large protruding nucleole at one end and usually one or two dark-staining smaller granules located elsewhere on the nuclear membrane". She did not describe nuclear
division and suggested no function for these dark-staining granules.

In 1938, Colson (32), also working on the sexual stages of *Phyllactinia corylea*, described the nucleus as a colourless sphere, empty except for the nucleolus; but at the two-nuclei stage of division of the ascogonium, one nucleus possesses a very large nucleolus, a conspicuous lateral granule and a quantity of granular chromatin which completely fills the nuclear area. The structure she here called the lateral granule, is the same body referred to by Harper as the central body, as indicated by her drawings. She too reported the presence of spindle fibres at each end of the cell. Olive (84) in his review of the structure and behaviour of fungal nuclei, mentioned several other authors who have investigated nuclear division in powdery mildews, all of whom found the process to be typically mitotic, complete with spindle. However, most reported investigations of nuclear division in the powdery mildews have been confined to the sexual stages perhaps because, as Olive stated; "The vegetative nuclei are quite small and difficult to study". Basing his conclusion on the findings of a large number of works on fungal nuclei, most of which were confined to the sexual stages, Olive stated "In most respects the fungus nucleus does not differ in structure or in behaviour during division from the nuclei of higher plants and animals". Speaking of nuclear division in the ascus of *Sphaerotheca* and *Podosphaera* Olive said "A typical meiotic prophase is followed by the appearance of a distinct spindle
with four bivalents....and conspicuous centrosomes are located at the spindle poles". But he added: "However, the report that the chromosomes divide transversely in the second and third divisions leads one to question the accuracy of the observations".

Robinow (95) and Bakerspigel (9) working on different members of the Mucorales confirmed a previous report by Leger (1896) that the nuclei in the vegetative hyphae of the Mucorales divide by constriction. When Robinow (96) later found that the nucleus of *Basidiobolus ranarum* divides by ordinary mitosis with spindles and a metaphore plate, he noted that this was most remarkable because there were indications that vegetative nuclei of fungi divide, as a rule, not by ordinary mitosis, but by a modified form of mitosis which involves neither an obvious spindle nor a metaphase plate. His earlier work on *Lipomyces lipofer* (94) served to confirm this view.

In 1964, Moore (80) provided outstanding evidence in support of this point of view with his electron microscopic studies on *Cordyceps militaris*. He produced a long list of the fungi in which nuclear division has been studied and stated that in none of the reports had the presence of a spindle apparatus been substantiated. He advocated a non-mitotic interpretation of the mode of division in fungal nuclei, except for *Basidiobolus ranarum*, and in place of the term "amitosis" used by Robinow he substituted the term "Karyochorisis", meaning "nuclear sundance". Thus he avoided any reference to mitosis. His electron micrographs of *Cordyceps militaris*
show that the nucleus divides by an invagination of the inner membrane of the nuclear envelope which first separates the nucleus into two or more subunits or karyomes. Later the outer membrane invaginates to separate the units into daughter nuclei.

In 1938 Lindergren and Rumann (66) described nuclear division in *Neurospora crassa*. They reported that they never found spindles except in the ascus and further added that for the asexual tissue of many Ascomycetes it was not possible to find evidence that their nuclei divide by mitosis.

Bakerspigel (10) also studied nuclear division in *Neurospora crassa* and reported it to be another fungus in which the vegetative nuclei do not appear to divide in the manner of classical mitosis. Again, neither spindle nor metaphase plate was observed to be involved in the process. He observed a densely stained granule which he suggested may play a significant role during division. The nucleolus, which he called the central body, elongates and divides by constriction, and he believes this to be significant in the segregation of the chromatin.

A recently reported account of division in vegetative fungus nuclei by Brushaber et al.(22) has added yet another version to the already confused issue. They studied nuclear division in several pathogenic fungi and found that they were all similar. As they summarized it, division occurs perpendicular to the long axis of the hypha. Anaphase movement is unilateral and unsynchronized and a spindle forms only between
separating chromosomes. Thes authors found abundant meta-
phase plates from which the chromosomes migrate singly or in
groups. They did not know the nature of the spindle and ad-
mitted it was possible that some of the material interpreted
as spindle might have been lagging chromosomes.

In the light of these reports it seems very desirable that
the structure and mode of division of somatic nuclei of pow-
dery mildews be examined, considering especially the conspic-
uous central body observed by Harper in the sexual stage, which
could add an even more interesting variation to the methods
of fungal nuclear division.

Another aspect of the biology of powdery mildews to which
considerable attention has been given recently is the relation
of the parasite to the host plant. Powdery mildews are char-
acterized by a superficial hyaline mycelium, which is in con-
tact with the host cytoplasm only by haustoria. With the
exception of Phyllactinia and Leveillula species, they in-
vade only the epidermal cells. Since the further development
of the fungus depends on a continuing supply of food from the
invaded cell, the parasite manages to penetrate into the cell,
establish itself in it, and tap its available food supply
without seriously damaging the host cell wall or its contents.
The problem as to whether the mode of penetration is mechanical,
chemical or both, has been a subject of much debate.

One of the earliest descriptions of the development of
haustoria in the Erysiphaceae was given by Smith in 1900 (105).
In this work, Smith observed that the cell wall around the point of penetration was more or less altered and dissolved. He mentioned a depression on the outer surface of the cell at the point of penetration appearing as if it were dissolved away, and postulated that this dissolution of the cellulose suggested that the Erysiphaceae probably produce an enzyme for this purpose. Some of the workers feel that both the cuticle and the cellulose wall are penetrated after chemical dissolution with an enzyme. Caporali (23), speaking of Sphaerotheca pannosa var. rosae on rose leaves stated, "The haustoria of the parasite penetrate the interior of epidermal cells by lysis of the cuticle and of pectocellulose layers underneath". After using certain staining techniques, he, like Smith, observed a colourless area around the point of penetration and an inward swelling of the cellulose layer of the host cell toward the mesophyll. He claimed that this process of lysis affects all the constituents of the cuticle and of the cell membrane, before penetration.

Woodward (113), working with Podosphaera leucotricha on apple, also agreed that penetration was effected by the action of an enzyme. He suggested that the observed enlargement of the passage through the cuticle and the swelling of the cuticle which accompanies penetration might both be due to enzymatic action. In defence of his claim for enzymatic dissolution of the cuticle and cell wall, he pointed out that some means of maintaining contact between hyphae and cuticle during pene-
tration must be shown to exist before the theory of penetration by mechanical force could be accepted since a relatively great force must be exerted if penetration is purely mechanical. He added that although hyphae of *Podosphaera leucotricha* conformed closely to the leaf surface they were not firmly attached until haustoria had been formed.

Several other authors have reported the dissolution of host cell walls by enzymes produced by fungal parasites but the fungi involved are usually saprophytic or facultative parasites. Woodward (113) referred to De Bary as saying, in 1886, that the cuticle of the stems of broad beans was softened by a toxic substance produced by the invading fungus *Sclerotinia libertiana* and that this toxic substance diffused through the cuticle causing the death of the cell. Ward (108) also claimed that *Botrytis cinerea* produced an enzyme which dissolved host cuticle. On the other hand Blackman and Wellsford (13), also working with *Botrytis cinerea*, could find no evidence for chemical dissolution of the cuticle. They showed instead that the cuticle was punctured by mechanical means after which an enzyme was produced which dissolved the subcuticular layer resulting in the swelling of the cellulose wall. These authors showed that attachment to the host which was necessary for mechanical penetration, was provided by a mucilaginous sheath investing the germ tube.

Several workers support the view that penetration of the cuticle is mechanical. Corner (34), in reference to *Ery*—
*Siphe graminis*, *Sphaerotheca pannosa* and *Podosphaera leuco-tricha*, stated that cuticular penetration was evidently mechanical, neither physical nor chemical alteration being noticeable about the point of penetration. He added that a thick cuticle prevents penetration. Penetration of the cellulose layer, he claimed, is clearly both mechanical and chemical, the swelling in this layer being caused by acytase diffusing from the penetration process. He too observed a depression in the epidermis at the point of penetration caused by the tip of the germ tube being closely pressed to the cell wall, but he could find no obvious means of fixation of the germ tube to the surface. Staining by indian ink did not reveal a mucilage sheath.

Peries (88), working with *Sphaerotheca macularis* on strawberry, also agreed that penetration of the cuticle was mechanical. He stated: "There was no evidence for the presence of a cuticle dissolving enzyme at the point of penetration and it appears that penetration is achieved entirely by mechanical pressure". On the point of host resistance he pointed out that young leaves were more susceptible than old leaves and that resistant varieties have thick leathery leaves. McKeen, Smith and Mitchell (72) also found that penetration of the cuticle of *Helianthus annuus* by *Erysiphe cichoracearum* was mechanical.

Evidence that mildew hyphae do have a means of fixing themselves to cell surfaces, as stated by Woodward (113) to
be necessary for mechanical penetration, was provided by Nour (83) in his studies on *Leveillula taurica* and other powdery mildews. He found that the washing of leaves of *Euphorbia heterophylla* after inoculation with conidia of *Leveillula taurica* (dev.) Arn. had little or no effect on the degree of infection if the washing was done more than three hours after inoculation. He concluded that the results he obtained could only be explained by the assumption that the conidia and germ tubes probably exude a sticky substance which glues them on to the leaf surfaces. He did not demonstrate the presence of this substance but he added that if this explanation was correct, it was additional evidence of the adaptability of powdery mildews to a wide range of environmental conditions.

The structure of haustoria in the Erysiphaceae was probably first described by De Bary who gave an accurate account of them. In a more detailed work on the subject Smith (105) described the penetration of the cell by a slender penetrating tube which, on entering the cell, swells up to form the body of the haustorium. In so doing, it invaginates the host plasma membrane which surrounds the haustorium. Finally a nucleus, which assumes a very elongated form, migrates into the haustorium. Concerning the sheath which surrounds the haustorium, Smith claimed that it does not belong to the protoplasm of the cell but consists of disintegrated cellulose from the distal end of the cellulose ingrowth through which
the haustorium made its way. He showed the haustorium of
*Erysiphe graminis* to be branched at both ends of its long
axis.

Later investigations have confirmed most of Smith's
findings except for his explanation of the sheath around
the haustorium. Concerning this matter, a rather lively
debate is still in progress. Caporali (28) believes the
sheath is a secondary membrane of pectic material which
is secreted by the haustorium. Ehrlich and Ehrlich
(39), who observed a similar sheath around haustoria of
stem rust of wheat, were less positive in their opinion of
its origin. They wrote: "The nature of the material com-
posing the encapsulation is uncertain, but it appears to
originate from the haustorial protoplast, and at least
a portion of it may be fungal cytoplasm".

In their later work on haustoria of *Erysiphe graminis*
(40) these authors confirmed their previous view on the
origin of the haustorial encapsulation but proposed a new
explanation for the origin of the sheath membrane. Their
electron micrograph of the haustorium showed a membrane
separate from the host plasma membrane and surrounding the
haustorium starting from outside the cell wall. They
proposed that this membrane was at least partly of fungal
origin or a special membrane produced by the host in
response to the presence of the pathogen. This view, they
claimed, is supported by the fact that Hirata and Kojima (59) were able to pull the intact sheath with the haustorium from the host cell without destroying the semi-permeable nature of the sheath boundary. Additional supporting evidence for this view may be drawn from the results of Dekhuijzen (36) who successfully isolated haustoria of Sphaerotheca fuliginea from cucumber leaves still completely invested in the haustorial sac.

Hirata and Kojima agree with Smith and Caporali that the membrane bordering the sheath is derived from the protoplasmic membrane of the host but differ with them on the mode of its development. Hirata and Kojima believe the membrane of the sac is transformed into a thicker and firmer membrane by the addition of calcium.

These authors claim that the sac around the haustorium is a protective device for the cell, controlling the diffusion of the constituents of dead haustoria into the host protoplasm thus protecting the host cell from death (59).

In an earlier paper Hirata (58) suggested that two types of haustoria are distinguishable in epiphytic powdery mildews, i.e., types represented by Erysiphe graminis in which the processes from the ends of the ellipsoidal body extend straight out like fingers and those represented by Erysiphe cichoracearum in which the processes are convoluted and cover the body of the haustorium. In this work Hirata
expressed the view that the substance surrounding the vesicle of the haustorium is derived from the host plasm.

Peyton and Bowen (89), working on the host parasite interface of *Peronospora manshurica* on *Glycine max*, saw no evidence of a true host wall around the haustorium. They introduced a new term -- a zone of apposition -- to describe the sheath between the host and parasite plasma membranes. This zone they claim has staining properties different from those of normal host cell wall. They described "secretory bodies" which apparently discharge material through the host plasma membrane into the "zone of apposition". Hence, this zone contains secretory products of the host plasma membrane. They did not find any plasmodesma-like discontinuities in the fungal cell wall like the channel-like areas described by Ehrlich and Ehrlich (40) as connecting the host cytoplasm with the haustoria of *Erysiphe graminis*. Shaw and Manocha (104), similarly, could not find any in cells of wheat leaves infected by *E. graminis*. These authors claim that the haustoria merely invaginated host protoplasm from which they were separated by granular encapsulations which they believe is secreted mainly by the host.

McKeen et al (72) are in agreement with the above view but express certain reservations about the connection
between the membrane around the haustorium and the host cytoplasmic membrane. This connection, they claim, is always obscured by a "zone of confusion" around the penetration peg.

On this point Bracker (16) expressed a similar view when he said, "Examination of many electron micrographs indicated that the sheath membrane is continuous with the host ectoplast, but continuity between the two membranes is frequently obscured because of complex ectoplast invagination involving the collar channel". He added, "The sheath membrane is thicker and more resistant to certain adverse conditions than other cytoplasmic membranes". These varied opinions would indicate that there is much yet to be learned about the haustorial sac and further research is required in this area.

The use of the electron microscope may help to solve the number of questions raised and left unanswered by previous research. But to the present there has been surprisingly little work in this field on the Erysiphaceae. In fact, perhaps the first electron microscopic investigation of the fine structure of any powdery mildew conidia has recently been released from this laboratory and is presently in press (Canadian Journal of Botany). Reference has already been made to published works in electron microscopy of the
haustoria of certain mildews (40, 72, 16) and, to this author's knowledge, the only other publication on electron microscopy of a powdery mildew was a note on the structure of the conidial wall, again from this laboratory (73).

In 1964, Hawker (56) reviewed the then available findings on the fine structure of fungi as revealed by electron microscopy, and she noted that mycologists were slow to make use of this new technique, adding that with few exceptions electron microscopy of the components of the fungal cell began with the present decade. With the development of more refined techniques suitable for dealing with fungal material the pace has quickened somewhat and much information of considerable interest is now available on fungal ultra-structure. Much of the work done in this field, however, has been done on the yeasts, largely because of their commercial importance and the ease with which they are obtained and grown.

The numerous reports on the fine structure of *Saccharomyces cerevisiae* agree in general that the yeast cell is surrounded by a wall of at least two layers, the outer one being dense to electrons, the inner wall thicker and less dense. Underlying the cell wall is the cytoplasmic membrane described as being a thin sinuate structure in resting yeast cells (2) but smooth and closely adherent to the cell wall in budding cells (51).
The cytoplasm is granular and contains sparse endoplasmic reticulum. Mitochondria are variously reported as being few or abundant, their number apparently varying with the physical condition of the cell (56). Yeast mitochondria are not essentially different from those of higher organisms but the cristae are generally fewer (50). Indeed Moore et al (79) point out that "there is apparently nothing structurally characteristic of fungal mitochondria".

Also characteristic of the yeast cell is a central vacuole now shown by electron microscopy to be a separate organelle, distinct from the nucleus. The nucleus is surrounded by a double perforated membrane and contains one to three more deeply stained areas. The presence of any structures identifiable as chromosomes is disputed (51).

Highly electron dense storage granules have also been reported by various workers. In addition to these generally observed organelles, Linnane et al (67) reported the presence in Tilletia utilis, of certain myelin-like membrane systems which these authors propose are concerned with the morphogenesis of mitochondria. They claim that these membranated bodies are found in cells grown under strictly anaerobic conditions and that such cells do not contain mitochondria. On aeration of resting cell suspensions "the reticulate membranes appear to line up in a parallel array and fuse and infold to form primitive mitochondria containing a few cristae". The same is claimed to be true for Saccharomyces cerevisiae.
Similar myelinoid membranous bodies were described by Jarvie (61) as seen in *Penicillium levitum*. Reports on the fine structure of filamentous fungi are less numerous but the available reports show a close similarity to that of the yeast cell. For the lower fungi, studies on *Rhizopus sexualis* by Hawker and Abbott (54), *Botrytis cinerea* by Buckley et al (23) and Hawker and Hendy (52), *Pythium debaryanum Hesse* by Hawker (53) and Hawker and Abbott (55), *Allomyces macrogyrus* Em by Blondel and Turian (14) and *Blastocladiella emersoni* by Cantino et al (27), will suffice to give a general impression.

For the motile spore of *Blastocladiella emersoni*, Cantino et al (27) reported a single, large, posterior, eccentrically disposed mitochondrion possessing a classical structure with numerous cristae arising from the inner membrane. The cytoplasm is somewhat granular but devoid of any obvious endoplasmic reticulum. A nuclear cap, packed with ribosomes and distinct from the nucleolus, overlies the nucleus and is separated from it by a double membrane. The nuclear membrane is perforated as in the yeast nucleus. Other cell contents include electron dense lipid-like bodies which are enclosed in a double membrane. A flagellum is described, having the usual nine-plus-two structure.

Similar motile cells of *Allomyces macrogyrus* show numerous small mitochondria, two types of which were recognized by Blondell and Turian (14), i.e. those of the motile cells
having numerous, characteristic, deeply penetrating cristae and those of the hyphae which are elongate and have few cristae which do not penetrate deep into the matrix of the mitochondria. The endoplasm contains few tubules some of which aggregate to form structures resembling dictyosomes. The nucleus, like that of Blastocladiella emersonii, has a nuclear cap of granular structure and probably contains ribosomes. Numerous "R" particles or ribosomes are scattered throughout the cytoplasm. Lipid bodies and other storage bodies are also apparent.

Mature sporangiospores of R. nigricans and R. sexualis are surrounded by a thick, reticulate, single-layered electron-dense wall unlike the two-layered wall of yeast cells. The endoplasmic reticulum is sparse. Mitochondria are globose in immature spores but large and contorted in dormant spores, and tend as in yeast, to be more in the periphery of the cell.

At the beginning of germination, mitochondria increase in number and it is suggested by Hawker (54) that the increase might be brought about by the division of the large contorted ones.

Formation of a germ tube is preceded by the laying down of an inner cell wall resembling that of the vegetative hyphae. The outer wall stretches and finally breaks and the germ tube emerges enveloped in the newly formed elastic wall.

Conidia of Botrytis cinerea show a double-layered wall like that of yeast cells. The endoplasmic reticulum occurs
as short double strands near the periphery of the cytoplasm and appear to originate from the plasmalemma. These membranes are longer and more centrally located in germinated conidia. Mitochondria are numerous and of varied shapes. Lipid-containing storage bodies are described as well as other highly electron dense storage bodies surrounded by a single membrane and containing coarsely granular material. In the case of the "storage granules" reported by Cantino (27) for Blastocladiaella, the surrounding membranes are double. In addition to these storage bodies, myelinoid membranes were reported by Buckley et al (23), some lined with dense material, others completely solid. These authors suggested that these loops and circular structures in the vacuoles of germinants probably represent stages in the mobilization of cellular reserves.

Reports by Hawker (53) and Hawker and Abbott (55) agree that the fine structure of Pythium debaryanum differs considerably from that of yeast or the young hyphae of other fungi. They claim that the young hyphae show more numerous and more regularly distributed cisternae than in any other fungi they had examined. The endoplasmic reticulum occurs in layers and typical golgi bodies resembling those of some algae are present.

The mitochondria differ from those of yeast and species of Rhizopus in having shallower undulating cristae of irregular thicknesses and depth of penetration. Tubules are sometimes
found in the centre of the mitochondria. Lomasomes, structures first described by Moore and McAlear (77), were found to be present. The nuclear membrane is irregular and has more numerous pores and larger discontinuities than those of other fungi they studied. The cell wall is not electron dense and shows no structure in section. On the basis of these differences these authors suggest a certain affinity between this fungus and certain algae and other green plants. Of the higher fungi studied, Hawker (56) cites the organization of the vegetative hyphae of Neurospora crassa as described by Shatkin and Tatum (102) as being typical of the group, individuals differing only in detail. Cytoplasmic structure is similar to that of the yeast cell.

According to Shatkin and Tatum (102) the outer surface of the cell wall of *N. crassa* is a dense fibriform meshwork which is continually sloughed off. Their micrographs also show a thick inner electron transparent wall.

Cytoplasmic continuity is maintained throughout the hyphae by incomplete septation. The septal pores are shown to be simple openings in the wall toward which the septum often tapers. In some cases, the septum maintains a uniform thickness. Such septal pores agree with those described by Moore and McAlear (78) for other Ascomycetes and Deuteromycetes (78). The pore is closed by a septal-pore-plug claimed to be composed of two bodies separated by two light layers and a central dense one. The plasmalemma coats the cell wall
and is highly convoluted. It consists of two dense layers with a less dense region between. Invaginations in the plasma membrane are suggested to be associated with absorption of nutrients into the cytoplasm by a process resembling pinocytosis. The endoplasmic reticulum divides the cytoplasm into an extravesicular area of dense and homogenous material and a membrane-enclosed less dense area. Dense particles resembling ribonucleoprotein particles (ribosomes) are dispersed throughout the cytoplasm.

Mitochondria are highly convoluted, their cristae usually lying perpendicular to the long axis of the mitochondria, but occasionally parallel to it as in yeast. The matrix contains dense granules 30-40 millimicrons in diameter. Branching forms of mitochondria suggest the development of new ones from pre-existing ones.

Several nuclei were observed in each cell. The nuclear membrane is a double structure containing several pores 40-70 millimicrons wide and connections between the nuclear membrane and the endoplasmic reticulum were reported. The nuclear matrix was reported to contain dense and light regions as well as fine coiled fibrils. An area of dense particles surrounding an island of low density in the nucleus was suggested as probably corresponding to the nucleolus. Other cell contents observed include lipid or polysaccharide storage granules and dense hexagonal crystals scattered in the cytoplasm.
Other structures described for other members of the septate fungi are ingrowths of the cell wall which Moore and McAlear (77) called lomasomes. These were found to be present in several species of Phycomycetes, Ascomycetes and Basidiomycetes. They were described as sponge-like structures continuous with the wall with their interior limits defined by the plasma membrane. It is of interest that these authors have never found lomasomes in conidia, ascospores, basidiospores or any of the cells from which these arise. According to them, lomasomes represent the only structure found to be peculiar only to fungal cells.

In another paper (76) these authors reported the occurrence of golgi dictyosomes in cells of the Ascomycete Neobulgaria pura. The same authors were again responsible for pointing out an important difference in pore structure in the major taxonomic groups of fungi by their description of the dolipore, which is found only in the septae of higher Basidiomycetes. (78). The complex structure of this pore, as revealed by the electron microscope, makes it an important factor in fungal taxonomy.

Reference was previously made to several works dealing with electron microscopic investigations of haustoria and the host-parasite interface. Electron microscopic studies on dividing nuclei of yeast by Conti and Naylor (33), Hashimoto et al (50) and of Cordyceps militaris by Moore (80) give additional evidence in support of the non-mitotic interpretation of nuclear division occurring in these fungi.
Although this summary must necessarily exclude a large number of valuable contributions to the literature on the fine structure of fungi, it is believed that the examples referred to here adequately represent the important findings in this branch of research on fungal microstructure.

Electron microscopy has proven to be an invaluable tool in the elucidation of several controversial problems that necessarily arise from the investigation of the structure and functions of fungal organelles, many of which are below the limits of resolution by the light microscope. Despite the ever increasing extent to which this tool is being employed, many important problems remain unanswered or unstudied. As was mentioned previously, electron microscopy has not been applied in the numerous attempts that have been made to solve the equally numerous riddles presented by the powdery mildews. This work is one step in that direction. An attempt was made to explain the structure of the unusual conidia found in the powdery mildews and to investigate their alleged capacity to maintain a high water content and/or an unusually high osmotic pressure. An effort was also made to throw some light on the mode of division of the nucleus which, according to the few available reports, is atypical of most fungal nuclei in the possession of a conspicuous lateral granule which persists throughout all stages of the nucleus, and finally, to find additional evidence for a correct interpretation of the relationship between the fungus and its host.
Although the main portion of this work is based on electron microscopy, it was felt that a correct understanding of the structures observed in the electron microscope could only be achieved by a previous study of the macrostructure of the organism by light microscopy. The extremely thin sections of material that must be used in the electron microscope could lead to serious misinterpretation of form and structure if the structure of the whole organism is not taken into consideration. For this reason, careful light microscopic observations were made before electron microscopy was employed.
CHAPTER II

EXPERIMENTAL METHODS

1. Growing the fungi

The fungus which is the subject of this investigation is \textit{Sphaerotheca macularis} (Wallr. Ex. Fries) Cooke, which grows parasitically on strawberry. For comparison, two other powdery mildews, \textit{Erysiphe polygoni} and \textit{Erysiphe cichoracearum} DC, were examined. The strawberry mildew was grown on two species of strawberry, \textit{Fragaria ovalis} Wilson and \textit{Fragaria chiloensis} L. Dusch. Most of the mildew was obtained from \textit{Fragaria ovalis} which was found to be much more susceptible than \textit{F. chiloensis}, the leaves of which were only infected when they were young and growing vigorously.

The plants were grown continuously under greenhouse conditions with temperatures ranging from a minimum of 65°F to a maximum of 75°F. During the summer the maximum temperature fluctuated with the external atmospheric temperature but the minimum did not fall below 65°F.
Young plants were rooted in 4-inch pots and were later
transferred to 6-inch pots or boxes. They were watered daily
and fertilized once per week with a commercial (Plant Prod)
28:14:14 N.P.K. fertilizer mixture during the summer, and a
similar 20:20:20 mixture in winter. Plant Prod is supplied
by Plant Products, Streetsville, Ontario. Owing to the fre-
quently infestation of the greenhouse plants by mites, the plants
were sprayed regularly with a miticide known commercially as
Plantfume 103 and obtainable from Plant Products Corporation,
Blue Point, L.I., New York. This was very effective in kill-
ing the mildews as well, and usually 3-4 weeks were required
after each spraying before fresh infestations occurred, usually
on freshly produced leaves.

Erysiphe polygoni was obtained from leaves of red clover
(Trifolium pratense Linneaus) and E. cichoracearum from the
sunflower plant, Helianthus annuus L. These plants were also
grown in the greenhouse under conditions similar to those pro-
vided for the strawberry plants.

ii. Collecting conidia

Conidia were collected in different ways according to
the treatment to which they were to be subjected. Those that
were to be stained without previous fixation were collected
by simply pressing the infected leaf to the surface of a dry
glass slide. The same method was used for those conidia which
were to be incubated for germination purposes.

When conidia were to be fixed before staining, the surface
of the slide was first coated with a thin film of egg albumin
in distilled water to form a slightly viscous paste. The film was allowed to dry until it became tacky and then the leaf was pressed onto the slide and allowed to remain until the slide was dry. When the leaf is removed the spores are left behind sufficiently secured to the slide to withstand the various fixing and washing processes to be carried out without being washed off. The same method was used for collecting the mycelium from the leaf. However, this method could not be used for conidia of *S. macularis* which were to be incubated as it was found that the egg albumin was inhibitory to the germination of these conidia.

When conidia were needed in chains, another method of collection was used. A dilute sugar solution was put on glass slides to make a thin film and this was allowed to dry until it became very sticky. The leaf was pressed on to the slide very gently to avoid breaking the chains of conidia. After removing the leaf, the slide was covered with a cover glass and drops of water were added to one edge of the cover glass to dissolve and wash away the sugar from the conidia. This leaves a much cleaner preparation than when egg albumin is used and allows for better staining and photographing of the specimen. The cellotape method, as described by Butler and Mann (25), was also employed but it proved to be less desirable than the above method as the tape tended to wrinkle on wetting. Long chains of conidia could not be properly
focused for photography. Another problem encountered in staining the cellophane preparations, was that the tape became very deeply stained thus seriously interfering with the illumination of the specimen. However, the cellophane proved to be very useful in the method used for staining nuclei of growing hyphae. The whole process of fixation and staining was carried out with the fungus still attached to the infected leaf. The leaf was then dried with blotting paper and the surface was pressed against a piece of cellophane which removed the superficial growth of mycelium, stained and ready for microscopic examination. In this way the tape remained clean and it could be mounted without serious wrinkling.

iii. Germinating conidia

Conidia to be incubated were collected on perfectly dry glass slides or cover glasses by simply touching the slides with the infected leaves. These slides were then placed in a Petri dish the bottom of which was covered with a layer or two of glass beads. Enough water was put in the Petri dish to keep the chamber humid but care was taken that the water did not cover the glass beads so that the slides were not wetted, as free water was found to inhibit germination of the conidia almost totally. A filter paper was put in the lid of the Petri dish and was moistened just enough to make it adhere to the top. The dish was then covered and placed
in an incubator at 18°C.

The length of time allowed for incubation varied with the different mildews. For *E. polygoni* an incubation period of three to five hours was adequate for the production of germ tubes two to four times the length of the conidia. With *S. macularis* and *E. cichoracearum* significant germination was not evident before eight to nine hours of incubation.

Also, *S. macularis* conidia were sometimes incubated on strawberry leaves in a humidity chamber as described by Jhooty (63). The percentage germination was often greatly increased in this way. With *E. polygoni*, however, the conidia were placed on dry slides at all times as this method consistently gave very high percentage germination.

iv. **Light microscopy**

a. **Staining and examining haustoria**

For counting and examining haustoria, epidermal strips were removed from the infected host and mounted in water on glass slides. The epidermis of strawberry leaves proved very difficult to remove, but the stolons could be stripped easily and were more often used. Strips of infected epidermis were stained with very dilute solutions of methylene blue or crystal violet prepared by dissolving 0.1 gram of the dye in 100 ml of water. These solutions were further diluted by adding four to five drops of the stain from an eye dropper to ten ml. of distilled water. Such dilute dye solutions cause a minimum of shrinkage, stain the host tissue only very
slightly but stain the fungus quite well after a few minutes. Thus the haustoria stand out clearly in the much more lightly stained host cells. Preparations were examined under a Zeiss (Jena) compound microscope.

b. Testing cell wall permeability

Conidia were collected in chains as described above and various dyes such as methylene blue, cotton blue, fast green and crystal violet were used to determine whether or not the conidial wall was equally permeable at the sides and at the ends of the cells. The stains were prepared as described above.

The conidia were mounted in a drop of water and covered with a cover glass. A drop of dilute stain was introduced at one end of the cover glass so that the dye diffused slowly inwards and surrounded the conidia. This prevented the dye from covering the top of the conidia and obscuring the observation of its penetration into the cytoplasm. The same procedure was used in attempts to stain the vacuoles, but for this purpose neutral red was used.

c. Fixing and nuclear staining

Various methods were used in an attempt to stain the nucleus to reveal as much as possible of its structure and to determine its method of division. To study undividing nuclei fresh ungerminated conidia were used, as well as growing hyphae obtained from leaf surfaces. For dividing nuclei, conidia were incubated to the stage where germ tubes were
about twice the length of the conidia and about to form their first cross walls.

Of the many fixing agents tried, two were found to give satisfactory results and were used for most preparations. Helly's fixative, as modified by Robinow (95), consisted of 5 gm. mercuric chloride and 3 gm. potassium dichromate in 100 ml. distilled water. To each 10 ml. portion of this mixture used, 0.6 ml. of 40% formaldehyde was added just before use. To prevent shrinking of the nucleoplasm and plasmolysis of the conidia which sometimes resulted from the use of the above fixative, the fluid was sometimes further modified by diluting it to half strength so that the final solution consisted of 5 ml. Helly's fixative 5 ml. water and 0.6 ml. formaldehyde. Cells were fixed for ten minutes in small Columbia jars, and were then washed in several changes of 70% alcohol. Preparations that were to be stained the following day were stored in 70% alcohol in a refrigerator at 10°C. Some preparations were treated with acetone for twenty minutes as suggested by Hartman (49) and Aist and Wilson (3). The other fixing agent employed was a 3:1 mixture of absolute alcohol and glacial acetic acid (Carnoy's solution). This fixative was most often used for preserving chromosomes in dividing nuclei.

For staining nuclei, particularly to demonstrate chromosome configurations, the HCl/Giemsa technique was used. The correct degree of hydrolysis was determined by trial and
error and was found to vary with the type of fixative used. For preparations fixed in Helly's fluid good results were obtained by hydrolyzing for nine to ten minutes in 1 N HCl at 60°C, whereas for acetic acid/alcohol-fixed preparations, four to five minutes were sufficient.

Following hydrolysis the material was washed in running tap water and rinsed in a Giemsa buffer prepared by dissolving one Giemsa buffer tablet in 100 ml. of distilled water giving a pH of 6.8. The staining fluid was prepared by adding eight to ten drops of saturated Giemsa stain to 10 ml of Giemsa buffer in a Columbia staining jar. Preparations were differentiated by emersing them for two or three seconds in a Petri dish of water containing a small drop of acetic acid. They were then rinsed in buffer and mounted in a drop of very dilute buffered Giemsa solution. The mount was sealed with wax to prevent it from drying out. Sealing was done by heating the point of a blunt pair of forceps, pressing it against a piece of wax and pulling the melted wax along the edge of the cover slip. Such wet mounts could be stored for several days in a humid chamber kept in a refrigerator.

To demonstrate the presence of a spindle and to determine the role of the nucleolus during division, the iron hematoxylin method was tried as used by Robinow (94,96). Germinated spores were fixed either in Helly fluid as described above or in osmium tetroxide vapour for four minutes in a small Petri dish with glass beads. They were then washed in 70%
alcohol and mordanted for 1-2 hours in 2% iron alum and stained for 1 hour with 5% hematoxylin in absolute alcohol which was diluted to 0.5% in distilled water. Stained preparations were differentiated in half strength iron alum mordant, washed in tap water, mounted in water and sealed with wax.

Several attempts were made to obtain instructive preparations using the Feulgen technique. Various methods of fixation were tried such as Helly's fixative, acetic acid-alcohol, buffered gluteraldehyde, formaldehyde and osmic acid vapour. Treatment with HCl was also varied through a series from 3-10 minutes in 1N HCl at 60°C. Different lengths of time were allowed for staining, ranging from 2-12 hours. Different brands of basic fuchsin were also used. Fixation with Helly's fluid was carried out as previously described. The fixed preparations were washed in 70% alcohol to remove all the fixative. Preparations were then hydrolyzed in 1N HCl for 9-10 minutes and stained in Schiff's reagent for 4 hours. Stained preparations were briefly differentiated in SO₂ water consisting of 90 ml tap water, 5 ml 1N HCl and 5 ml 10% potassium metabisulfite. They were then washed for 20 minutes in running tap water, mounted in water and examined.

d. Staining for proteins

Total nucleoproteins

In order to demonstrate the presence of proteins within the nucleus, cover slips bearing germinated conidia were emersed in Carnoy's fixative for 10 minutes, washed in 70% ethanol
and stained with alcoholic mercuric bromophenol blue (B P B). The staining solution as outlined by Mazia et al. (71) consisted of 10 g of mercuric chloride and 100 mg of bromophenol blue in 100 ml of 95% ethanol. After 15 minutes of staining, preparations were washed for 20 minutes in 0.5% acetic acid and then in water for 15 minutes, mounted in water and examined. This procedure is specific for proteins.

To detect any difference in the staining reaction of the nucleus when the nucleic acids were removed, cells were treated with hot 15% trichloroacetic acid for 15 minutes in a boiling water bath, washed thoroughly in 70% alcohol, then in water, stained with the B P B stain and compared with untreated preparations.

**Histones**

It was thought necessary to employ a technique that was specific for histones in order to demonstrate the presence of basic proteins in the nucleus. The method used here was developed by Alfert and Gestwind (6) and is claimed by these authors to be very specific for histones. The technique was followed as outlined by Jenson (62) who made some modifications in the fixing procedure as used by the original authors. Cells were fixed in a formaldehyde-aceto-alcohol (F A A) fixative consisting of 90 ml 50% ethanol, 5 ml glacial acetic acid and 5 ml 40% formaldehyde. The DNA was then extracted by heating for 15 minutes in 15% trichloroacetic acid in a boiling water bath. They were then washed in three changes of
70% ethyl alcohol and stained for 30 minutes in a 0.1%
aqueous solution of Fast green FCF which was adjusted to pH
8.1 with a trace of NaOH, using a pH meter. The stained
material was washed for 5 minutes in distilled water after
which it was mounted in water and sealed with wax.

As a further attempt to determine what structures were
being demonstrated by the Giemsa stain, conidia and growing
hyphae were treated with a 0.1% solution of DNA-ase enzyme
for 4 hours at 25°C. Some were treated before fixation and
others after fixation with acetic acid-alcohol. These were
then stained, some with Giemsa and others by the Feulgen
reagent. Preparations were examined and compared with stained
untreated material.

Other stains used successfully for demonstrating inter-
phase nuclei include azure B and cyrstal violet. Prepara-
tions which were previously fixed with Helly's fixative were
stained for 15 minutes in a solution of azure B consisting
of 10 drops of the stain to 10 ml of water. Unfixed material
was stained with a more dilute solution of crystal violet
consisting of 4-5 drops of 0.1% crystal violet to 10 ml of
water.

The microscope used was a Zeiss (Jena) compound micro-
scope equipped with an apochromatic oil emersion objective
lens X90, N.A. 1.25 and compensating ocular 15X and Zeiss
achromatic oil emersion condenser, N.A. 1.2. A green filter
was used and photographs were taken with Kodak Panatomic X
plate films and developed in Kodak D76 developer.
V. Electron microscopy

In order to prevent the fungal material from being lost in the various processes involved in preparing specimens for electron microscopy, the material was first embedded in blocks of 2% Agar-Agar. Spores were collected on dry 22 mm. square cover glass on which they were incubated to the desired stage when germinated conidia were needed. The cover glass was put in a small Petri dish and liquid 2% agar which was cooled to the point of setting, was poured around the cover glass so that the agar flowed over the cover glass from all sides into the center. The spores float along with the advancing edge of the agar and accumulate in a small area on the surface of the agar. The agar was allowed to set and a thin covering layer was poured on top to prevent them from being washed off in the fixing fluid. Blocks 2-3 mm. square were cut from this spore-bearing block of agar and placed in the fixative. As a result of many attempts to work out a satisfactory fixing procedure the following modification of the glutaraldehyde-osmium tetroxide procedure as outlined by Pease (86) was adopted. Blocks were first fixed in a phosphate-buffered 5% solution of glutaraldehyde at pH 6.4 for 2 hours at 10°C. They were then washed in buffer to remove excess glutaraldehyde which is believed by Sabatini and others as reported by Pease (86) to retard the fixing action of Osmic acid. Good results have been obtained by post fixing in osmium overnight but preparations so treated tend to be very dense to electrons and produce over contrasted micrographs.
Fixation with potassium permanganate was also tried but was later discontinued as, apart from the superior results obtained with the glutaraldehyde-osmium tetroxide method, agar blocks that were fixed in permanganate became very black during the dehydration process and made it difficult to see the material in the blocks while they were being trimmed for sectioning.

Fixed preparations were dehydrated in a series of 50% to absolute ethyl alcohol and then treated with two changes of propylene oxide, each for 15 minutes in order to facilitate penetration of the embedding medium. The blocks were then shaken for several hours in an Epoxy resin plastic medium consisting of a 5:5:4:0.3 mixture of Epon 812, Dodecenyl succinic anhydride (DDSA), Methyl nadic anhydride (NMA) and the catalyst Benzyl dimethylamine (BDMA). To this mixture, 1/3 the volume of propylene oxide was added, to ensure complete penetration of the plastic into the fungal cells. The blocks were then resuspended in freshly prepared plastic from which the propylene oxide was omitted, incubated at 36°C for 3 hours and then transferred to gelatin capsules—one block to a capsule—filled with the same plastic. The capsules were cured overnight in an oven at 70°C and were ready for trimming and sectioning by next morning.

Before the blocks were trimmed they were examined under the light microscope to locate the position of the material in the capsule. One could then select a particular spore or
group of spores and, using a binocular microscope, trim down to it without much difficulty. With permanganate or prolonged osmium fixation this is more difficult as the blocks become black and opaque. Blocks were sectioned with a Porter-Blum microtome using glass knives prepared with a glass cutter and taped pliers as described by Porter (90). Sections were cut 0.06 to 0.15 microns thick, were floated off on water and collected on 200-mesh copper grids. Techniques in ultramicrotomy were followed as outlined by Pease (85,86) and Porter and Siegel (87).

In most instances osmium fixed material was sufficiently electron dense to obtain high contrast without further staining but, in some cases, especially when germinated conidia were used, it was necessary to stain the sections before microscopy.

Sections were stained on the grids in a 2% uranyl acetate solution for 15-20 minutes. Drops of the staining solution were pipetted on to the surface of a piece of sheet wax which was previously heated to get rid of all free dust, and the grids were floated sections-side down on the drops. After 15-20 minutes they were washed by dripping distilled water on them from a small pipette and dried on filter paper. Sections were examined in a Philips 200 E.M. microscope fitted with a 33 mm camera and using 60 K.V.
CHAPTER III

EXPERIMENTAL RESULTS

The conidium

1. Cell wall structure and permeability

As seen under the light microscope, conidia of Sphaerotheca macularis are egg-shaped structures measuring 20 to 30 microns in length and 10 to 15 microns in diameter. In dry mounts the conidia appear dark and opaque and some conidia seem to have a dark cap at either end. Mounted in water they are seen to be completely hyaline, highly reticulate internally and show no evidence of a cap at the ends.

The conidial wall is smooth and thin being about 1 to 1.5 microns thick and is readily seen to consist of two layers. The outer layer is darker than the inner one and takes up dyes such as methylene blue or fast green very quickly. The inner layer usually remains unstained even after long submergence in the dye (Fig. 1).

The outer wall varies somewhat in thickness but is always thicker than the inner one which is of constant
thickness all around the conidium. Quite often immature terminal conidia have been observed partly broken off from the conidiophore and attached to one side by the outer wall. The broken end of the outer wall can be seen as a ring around the end of the conidium projecting beyond the end wall of the conidium which here consists of inner wall material only, derived from the septal wall between two adjoining conidia.

Conidia are borne in chains on a conidiophore, each conidium being cut off by a single-layered septum derived from the inner lateral wall (Figs. 2, 3, 6 and 10).

Light and electron microscopy show that abscision of the terminal conidium occurs by a gradual splitting of the septum giving rise to the end walls of the two adjacent conidia (Fig. 2 and 3). The splitting begins at the outside and advances toward the centre, so that just before the terminal conidium falls off it is held to the subterminal conidium by a small delicate connection (Figs. 3 and 4).

Before the abscission process begins the young conidia are almost isodiametric. Toward the end of the chain the conidia show a bulge in the middle and as abscission progresses the previously flat ends of the conidia begin to curve away from the point of previous attachment as the conidia round up to their mature shape.

Since the end wall is derived from the septum of the conidiophore which does not have an outer layer (Figs. 6 and
and 23), the outer spore coat grows over the end wall progressively as it becomes exposed by the splitting of the septal wall.

Figure 3 shows that the outer coating of the wall does not completely surround the conidium before division of the septal wall is completed and the conidium is ready to fall off. If the conidium is broken off mechanically before the abscision process is completed the end of the wall remains incompletely coated, leaving a ring at the end (Fig. 5). This ring is never observed at both ends of the same spore as the wall at the terminal end is always complete.

In *E. cichoracearum* the mature conidia remain connected in long chains of up to twenty, held together by a small "mucilagenous" link at the ends of each spore. The intact chain falls off and if left undisturbed, intercalary as well as terminal conidia will germinate while still attached. If a drop of water is added to the slide the conidia immediately break apart and the end walls appear completely smooth as the papillate connection -- evidently of an elastic of fluid nature as indicated by (Fig. 9) -- draws back over the end of the conidium. Electron micrographs show that the septal wall is considerably thicker than the inner lateral wall (Figs. 6 and 22) so that splitting of the septum produces an end wall which, in *S. macularis*, is of equal thickness with the lateral wall. However, in *E. polygoni* the end
wall is much thinner than that of the sides (Fig. 7) but the mucilagenous coat is considerably thicker at the ends and is in general much thicker than that of *S. macularis*. Both layers of the spore wall are almost transparent to electrons but the outer layer is conspicuously more so, and if sections are not properly stained with osmic acid or heavy metals this layer may not be observed at all. Even after staining with uranyl acetate it is not always possible to see the outer wall and it is evident that much of it is removed by the alcohol and propylene oxide used in preparing the material. From Figure 9 it is apparent that the outer wall is capable of great elasticity and is readily sloughed off and lost. Its thickness varies considerably in different parts of the fungus and even in different parts of the same spore (Figs. 8 and 14). It has a structureless appearance in the electron microscope and it is capable of forming an adhesive attachment to surfaces with which it comes in contact (Fig. 37). When treated with Sudan Black B or Sudan III the inner wall remained unstained but the outer wall became deeply stained indicating the presence of some fatty material. Much of the stain could just have accumulated by surface adhesion, but the fact that structures inside the conidia were also stained deeply by these reagents is evidence that some penetration did occur and the difference in staining reaction of the two layers of wall material indicated a difference in chemical nature.
When conidia were treated with ether in water they became much more permeable to methylene blue and other dyes. This gave additional evidence that the outer wall probably contains some fatty material. Treatment with cellulase had no effect on cell wall permeability.

While testing the permeability of the conidial wall to different dye solutions, it was observed that a large proportion of the conidia remained unstained for a considerable time and that, when the dye finally began to penetrate, it first entered at one end and gradually penetrated throughout the cell from that point.

The author suspected that the relative impermeability of the side walls was due to the presence of the "mucilagenous" outer layer of the wall and that the greater permeability of the ends of the conidia was due to incomplete covering of the inner wall by this layer. Since the outer wall is never present in the septa between the conidia on the conidiophore, dyes should move along the length of the conidial chain from one cell to another across the septa more readily than they would penetrate the side walls. To test this, chains of conidia were collected and stained as described earlier, using dilute solutions of methylene blue, neutral red and fast green. The conidia were stained without previous fixation so as not to interfere with the natural texture of the cell wall. It was observed that in each case the dye penetrated the tip of the terminal conidia.
very slowly and then moved slowly down the chain from cell to cell. Similarly, the dye entered the broken end of the conidiophore at the other end and moved slightly more rapidly up the chain, so that the cells in the middle were the last to be stained (Fig. 10). This process often took more than an hour to complete in chains of eight to ten cells. For example, the rate of penetration of three chains of conidia was timed as recorded in Table I.

In chain No. 1 the dye entered the broken basal cell of the conidiophore and continued up to the sub-terminal cell in a total of 17 minutes, while the terminal cell took 12 minutes to be penetrated. In the second chain the terminal cell was partly separated from the sub-terminal cell, and the sub-terminal cell was damaged at its end. The dye entered the broken sub-terminal cell after 10 minutes and into the basal cell after five minutes and continued along the length of the chain from both directions. Nine cells were penetrated after 14 minutes. The 10th and terminal cell which was evidently completely surrounded by its outer wall was not penetrated until 35 minutes after the dye was applied. In the third chain the basal cell was connected to the parent hypha and cut off from it by a septum. The dye penetrated very slowly from the terminal cell backwards going through the 8 cells in 68 minutes. The experiment was repeated several times with similar results and then the chains were first mounted in water and a drop of ether added
Table I. The rate of penetration of methylene blue solution into chains of conidia of *S. macularis*.

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<td>34</td>
<td>24</td>
<td>14</td>
<td>4</td>
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</table>

* Basal cell
to the edge of the cover glass and left for 4 to 5 minutes. The dye was then applied and the penetration was observed under the microscope. In chains so treated the dye still entered the end wall more readily but it also penetrated the side walls within a few minutes and almost the whole chain was stained simultaneously. Similar results were obtained by treating the chains with 2 per cent sulphuric acid.

In general, single spores were more quickly penetrated by the dye and terminal conidia of some chains took considerably longer to be penetrated than others. Tests were made to determine how young conidiophores would be penetrated before any conidia had been broken off.

Epidermal strips were removed from infected strawberry leaves and mounted in water so that the chains of conidia and young conidiophores projected off the edges of the strip. When these were stained, it was observed that some chains as usual stained fairly quickly, but the newly formed chains remained unstained long after the majority of mature chains were stained throughout. It was clear that the outer mucilaginous wall was forming a barrier to the entry of dyes into the conidia and that the breaking off of the terminal conidia, especially if they are removed prematurely, was leaving an unprotected point of entry for external fluids. Figure 8 shows a section of a conidium with such
a gap in the outer wall. As an additional test of this hypothesis, a comparison was made between the rate of dye uptake in conidia that had fallen off the chains naturally after maturity and those that had been broken off before maturity. Glass slides were placed immediately underneath the surface of heavily infected strawberry leaves and left overnight in the greenhouse to collect conidia that would fall off naturally. Many such conidia were collected the following morning, most of them in long chains. Additional mounts of conidia were prepared by removing infected leaves, shaking them vigorously to remove conidia that were ready to fall off, and then touching the leaf to a glass slide to collect more immature conidia. These two sets of conidia were stained with methylene blue in the usual way. The results did not show a spectacular difference but did indicate that the conidia which had fallen off naturally absorbed the stain more slowly than those that had been broken off prematurely.

These results all seem to confirm the hypothesis that greater permeability of the end wall of some conidia is due to incomplete sealing off of the point of abscission by the outer 'mucilaginous' coat surrounding the wall.

ii. The vacuoles

The conidial protoplast gives the appearance of a complex reticulum of spherical vacuolated globular bodies separated by slender granular strands of protoplasm. Appearing to be
floating in the globules are small refractive bodies which are constantly moving randomly about. Close observation reveals that these moving bodies do not really float inside the vacuolate bodies but above them, since they are in a different focal plain and can be observed to cross over the cytoplasmic strands separating one 'vacuole' from another.

The empty appearance of the vacuolate structures and the large proportion of the conidia occupied by them prompted the question as to what their nature and contents might be.

In the light of the capacity of powdery mildew conidia to germinate at unusually high moisture stresses, it was considered of great significance to determine whether or not these conidia might not have large water-containing vacuoles.

During the experiments in which chains of conidia were stained, it was observed that when dyes entered the ends of the conidia they did not enter the "vacuoles" but that the cytoplasm became coloured around them. It was observed also that, as long as the "vacuoles" were intact, penetration of the dye throughout the conidium proceeded extremely slowly whereas those conidia which were shrivelled or not turgid with "vacuoles" absorbed the dye immediately. Furthermore, penetration was much faster when strong concentrations of dye were used than with dilute solutions. However, when more concentrated dye solutions were used the "vacuoles" disappeared and the cytoplasm became amorphous and shrivelled.
Attempts to stain the "vacuoles" with dilute neutral red were always unsuccessful so long as the cell content was in its normal reticulate form, but stronger solutions ruptured the "vacuoles" and the whole conidium was stained red. This suggested that the dye was not penetrating the intact "vacuolar membranes".

The author attempted to test this by using a pH indicator which would colour the "vacuole" if it penetrated. The dye bromcresol purple was used. This dye reacts to give a yellow colour at pH 5.2 or less and a purple colour at pH 6.8 or over. When conidia were treated with this solution, the bands of cytoplasm around the wall and between the "vacuoles" became yellow after some minutes but the colour of the "vacuoles" remained unchanged. A drop of NaOH solution was added to give a higher pH and the colour of the cytoplasm changed to purple but again the "vacuoles" remained unchanged. This was further evidence that the molecules of the dye were not entering the "vacuoles" since, whatever the pH of the "vacuolar" fluid, it should give a colour reaction with the indicator. Phenolphthalein solution was also used with similar results. The next step was to try and rupture some of the "vacuoles" so that the barrier between the "vacuolar" cavity and the cytoplasm was removed, and to stain again with neutral red. It was previously observed that these "vacuoles" would swell and coalesce into larger ones whenever the conidia were left in water for an hour or more.
Fresh turgid conidia were mounted in a drop of water and covered with a cover glass and a drop of ether was added to the water under the cover glass and left for a few seconds. More water was then added to wash out the ether so that the membranes were only slightly ruptured. When observed under the microscope, some of the "vacuoles" were seen to coalesce into one or two large central ones with a few smaller ones remaining intact. These conidia were then stained with dilute neutral red. After a few minutes it was observed that the large central "vacuoles" that were formed from the breaking up of smaller ones, were stained red with neutral red whereas the adjacent small undisturbed "vacuoles" remained unstained. Conidia in which all the "vacuoles" were intact had no stain in any of their "vacuoles". It was, therefore, evident that these globular bodies do contain water but that the membrane surrounding them is not permeable to dyes such as neutral red as are other cell vacuoles. Figure 11 shows a conidium with its vacuole stained with neutral red and another conidium with the vacuole unstained but with smaller bodies deeply stained.

Electron microscopy also confirms the presence of numerous vacuoles in the conidia. Figures 8, 13, 14, 16 and 17 show sections of whole conidia of *S. macularis* with several vacuoles separated by strands of cytoplasm. Sections of *E. polygoni* show a similar structure as seen
in Figures 7 and 18. In some cases the vacuoles appear to be completely empty (Fig. 8) while others have randomly dispersed granular material (Figs. 14 and 18). It may also be observed from an examination of Figures 14, 22, 23 and 34 that the vacuolar membrane stains differently from the membranes of the endoplasmic reticulum, and does not have the typical unit membrane structure of other cell membranes. The vacuolar membrane has never been seen to be continuous with the endoplasmic reticulum.

iii. Osmotic pressure and water relations

The realization that conidia of the powdery mildews examined are highly vacuolated structures naturally lead to a reconsideration of the question of osmotic pressure in these conidia. As shown in the introduction, this has been a subject of some controversy, some authors having obtained osmotic pressures ranging as high as sixty to ninety atmospheres for some powdery mildews. This author felt that it was difficult to reconcile the idea of a cell having more than fifty per cent of its contents as water while at the same time maintaining osmotic pressures several times higher than is average for plant cells. Tests to determine the osmotic pressure of the conidia were carried out, using known concentrations of both sucrose and sodium chloride solutions.
Molar concentrations of both solutions were prepared and from these a graded series of each was made up, ranging from 0.1 to 1.0 molar concentration. Slides of conidia, freshly collected from different strawberry leaves, were labelled to correspond with the different concentrations of plasmolyte, and each slide was flooded with its corresponding solution and covered with a cover glass. Conidia were also mounted in 2M solutions and in pure water. The slides were then placed in a tray, the bottom of which was covered with paper towels saturated with water, and the tray was covered with aluminium foil to keep the chamber humid and avoid any significant evaporation of the solution.

The slides were left for two hours and then examined under the microscope. To determine the percentage plasmolysis in each case, all the conidia in ten different fields of the 20X power of the microscope were counted using one of four categories: normal, shrunken, plasmolyzed and fully turgid. Not less than one hundred conidia were counted for each solution.

Conidia were considered normal if they showed no obvious change in form, their vacuoles intact and the cell walls unwrinkled. Under the category shrunken, were placed those conidia in which the cytoplasm showed signs of shrinkage, having contracted vacuoles and cell walls and the vacuolar membranes broken or folded. In such cells the plasma membrane remained adpressed to the cell wall. Plasmolyzed
cells were those in which the cytoplasmic membrane was withdrawn from the cell wall and fully turgid (swollen) cells those which in water or very dilute solutions appeared more turgid than normal cells, their vacuoles coalescing into one or two large ones or the cell walls ruptured by swelling. These four categories were used because of the previously observed behaviour of the conidia in water and in dye solutions. Conidia were seen to become more turgid in water and their vacuoles swell and burst forming fewer and larger vacuoles.

In more concentrated solutions the vacuoles shrunk and lost their spherical shape, the reticulate structure of the conidia disappeared and the cell wall sometimes became somewhat wrinkled. Yet the plasma membrane was only rarely observed to be withdrawn from the cell wall except after several hours of treatment. It was, therefore, assumed that the changes taking place in the cell, when exposed to various concentrations of diffusable substances, indicated a change in the water relationship of the cell and that incipient plasmolysis should be assumed to have occurred when such shrinking as previously described are observed.

The results of the experiment are tabulated in Tables II and III.

It is shown by the results of these tests that the percentage of fully plasmolyzed cells at any concentration
Table II: Osmotic behaviour of *S. macularis* conidia in sodium chloride solutions of graded concentrations over two hours.

<table>
<thead>
<tr>
<th>Molar concentration NaCl.</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
<th>1.0</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic value in atmospheres</td>
<td>0.0</td>
<td>3.8</td>
<td>7.6</td>
<td>11.4</td>
<td>15.2</td>
<td>19.0</td>
<td>22.8</td>
<td>26.6</td>
<td>30.4</td>
<td>34.2</td>
<td>38.0</td>
<td>72.0</td>
</tr>
<tr>
<td>% Normal</td>
<td>80</td>
<td>67</td>
<td>70</td>
<td>76</td>
<td>65</td>
<td>40</td>
<td>5</td>
<td>7</td>
<td>15</td>
<td>5</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>% Fully turgid</td>
<td>20</td>
<td>23</td>
<td>18</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>% Shrunken</td>
<td>0</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>29</td>
<td>41</td>
<td>84</td>
<td>86</td>
<td>62</td>
<td>77</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>% Plasmolyzed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>19</td>
<td>11</td>
<td>7</td>
<td>23</td>
<td>18</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Note - Not less than 100 conidia were included in each sample.
Table III: Osmotic behaviour of *S. macularis* conidia in sucrose solutions of graded concentrations over two hours.

<table>
<thead>
<tr>
<th>Molar sucrose solution</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
<th>1.0</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic value in atmospheres</td>
<td>0.0</td>
<td>2.6</td>
<td>5.3</td>
<td>8.1</td>
<td>11.1</td>
<td>14.3</td>
<td>17.8</td>
<td>21.5</td>
<td>25.5</td>
<td>29.7</td>
<td>34.6</td>
<td></td>
</tr>
<tr>
<td>% Normal</td>
<td>80</td>
<td>98</td>
<td>88</td>
<td>92</td>
<td>51</td>
<td>31</td>
<td>58</td>
<td>72</td>
<td>69</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Fully turgid</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Shrunken</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>48</td>
<td>62</td>
<td>30</td>
<td>23</td>
<td>16</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Plasmolyzed</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>12</td>
<td>5</td>
<td>15</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note - not less than 100 conidia were examined per sample.
of either sucrose or sodium chloride was very small, and in the case of sucrose solution more than seventy per cent of the conidia were normal after two hours in a solution of 34 atmosphere pressure. However, a comparison of the two tables reveals that shrinking of the cytoplasm was strongly evident in NaCl solution from 15 atmosphere pressure increasing rapidly at higher concentrations, and that similar shrinking occurred at 14.3 to 17.3 atmosphere pressure in sucrose but, strangely, decreased at higher concentrations. Such shrinkage clearly indicates that the solutions were hypertonic to the cell sap and evidently must be strongly so before any withdrawal of the plasma membrane can be observed.

Even in strongly hypertonic solutions the plasma membrane may remain in place because of the simultaneous shrinking of the rather thin cell wall, the cytoplasm and vacuoles.

When the conidia are mounted in pure ether they are immediately plasmolyzed, the whole cell shrinks and the cytoplasm rounds up in the centre of the cell. If water is then added to these cells, the cell wall soon becomes flush and smooth again as the space between the wall and the withdrawn plasma membrane becomes filled with water. However, the cytoplasm, killed by the ether, remains shrunken and rounded up in the cell.

Using then, the first evidence of shrinkage of the cytoplasm as the indication that the point of incipient plasmolysis had been reached, the data from both tables would
give an osmotic pressure for the conidia of *S. macularis* in the region of 14 to 19 atmospheres. Some conidia do have lower osmotic pressures, however, a condition that seems to vary with the age and physiological state of the conidia.

iv. Water content in relation to vacuolar area

Assuming that almost all of the free water in the cell was contained in the vacuoles, it was considered possible to obtain a fair estimate of the proportion of this water to cell cytoplasm by estimating the relative proportions of the cell occupied by these fractions. First an attempt was made to plasmolyze the cells completely and measure the amount of empty space in proportion to the total cell area. To do this, the conidia were mounted in 25% potassium hydroxide in which they were readily and fully plasmolyzed. The plasmolyzed cells were photographed as shown in Figure 12 and the pictures were projected on a gridded screen on which the measurements were made. The results are given in Table IV.

The indications were that more than 50% of the conidia consists of vacuoles containing water which can be withdrawn during plasmolysis. As a means of comparison electron microscopy was employed. Serial sections were cut completely through a group of conidia and the sections collected on
<table>
<thead>
<tr>
<th>Cell number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Avg. of 10 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent-age empty space to total conidial area</td>
<td>57</td>
<td>55</td>
<td>52</td>
<td>59.5</td>
<td>53</td>
<td>55</td>
<td>48</td>
<td>48</td>
<td>55</td>
<td>43</td>
<td>52.55</td>
</tr>
</tbody>
</table>

* Determined by measurements on plasmolyzed conidia
grids.

All the sections that were found in the electron microscope (167 in all) were examined and photographed. The negatives, all clearly showing the vacuoles as distinct from the cytoplasm, were projected from a 16 mm. projector on a gridded screen and the total area of the spore and the area of the vacuole were calculated. Since it was not always possible to tell which section belonged to which spore, all the figures were totalled and the average of vacuolar area to total spore area was calculated. It was found that 53.5% of the total area was vacuole. This figure was surprisingly close to that obtained by the plasmolytic method and although these results can only be taken as an estimate, it is clear that at least 50% of the volume of these conidia is occupied by vacuoles which, from their positive staining reaction with neutral red, maybe expected to contain water. Evidence will be presented later that there are other substances present in the vacuoles.

v. Ultrastructure

a. The cell wall

Electron microscopy reveals that the conidia of \textit{S. macularis} are surrounded by a double layered wall consisting of a thin inner relatively electron-transparent layer 0.15 to 0.2 microns thick and an outer almost completely electron-transparent layer of variable thickness sometimes
several microns thick and at other times almost completely absent (Figs. 13, 14 and 46).

Absence of this layer may be only apparent owing to its extreme transparency to electrons and longer exposure of the negatives usually reveals its presence in micrographs. Adequate staining of the sections usually ensures a good resolution of this layer which generally shows an electron dense outer edge after prolonged osmium fixation or uranyl acetate staining. The outer layer is often much thicker at the ends of the conidia but in some cases it appears to be absent or more diffuse.

A layer of similar texture is found around the hyphal wall and is several times thicker on the lower side of the hypha when it touches the surface of the epidermis of the host (Fig. 37). This material, apparently mucilagenous, attaches the mycelium to the host cell wall so that attempts to remove the mycelium often results in bits of host cuticle coming off with it. Possible functions of the mucilage coat as a means of attachment will be discussed later.

b. The plasma membrane

Lying close against the inside of the wall is the plasma membrane which is clearly seen in plasmolyzed cells (Fig. 47). The strongly osmiophilic nature of this membrane makes it readily resolvable as a black line skirting the inner wall
(Figs. 24 and 25). In *S. macularis* the plasma membrane is relatively smooth but in conidia of *E. polygoni* this membrane is highly convoluted. The typical unit membrane structure has never been clearly defined in the plasma membrane although this structure may be easily distinguished in the endoplasmic reticulum of the same cell.

c. The vacuoles and associated mycelloid bodies

The mature conidium contains surprisingly little cytoplasm. Almost all of the middle portion of the cell is occupied by a complex of vacuoles of various sizes and numbers. The extent of vacuolation shown in any one section depends on how close the section was to the cell wall. Figures 15 - 17 represent sections close to the wall and the centre of the cell respectively. The cytoplasm occupies a narrow strip around the cell next to the cell wall ranging from 0.5 to 1.0 micron thick or about one twentieth the width of the cell. From this narrow coating of cytoplasm, thin cytoplasmic strands project out to form the delicate ramifying boundaries between the vacuoles. There may be as many as a dozen separate vacuoles in a section taken from the centre of the conidium (Figs. 16 and 18) but closer to the wall the vacuoles are fewer until they are completely absent within the cytoplasmic sheath. The strands between the vacuoles may contain little else but the two vacuolar membranes which may run side by side, and during the dehydration and impregnation processes of specimen preparation, these thin membranes may be ruptured connecting
two or more adjoining vacuoles. Fragments of these membranes are apparently pushed into the vacuoles by the penetrating fluids. This may account for the presence of particles resembling fragments of cytoplasm in some vacuoles.

Sometimes the vacuoles appear completely empty (Fig. 8) but not infrequently are quite granular as in sections of \textit{E. polygoni} which, more often than not are densely granular (Figs. 7 and 18). In addition, membrane-bound bodies are sometimes seen inside the vacuoles, but serial sections show that these bodies are not isolated in the vacuoles but are ingrowths from the cytoplasm projecting into the vacuole, as is shown in Figures 19 and 21 to 23. Sections cut at an oblique angle to the plain of the projections show them as being isolated from the cytoplasm (Fig. 31).

A conspicuous and very interesting feature of these projections is that they usually enclose a myelinoid body consisting of a few to a large number of membranes, arranged in concentric circles. The innermost circle usually encloses a cavity with contents of low electron density (Fig. 23). The enclosed cavity may itself enclose another structure consisting of a system of tubes surrounded by dense material (Fig. 25). Highly magnified micrographs show that the membranes of these myelinoid bodies are continuous with the vacuolar membrane which forms a loop into the vacuole and turns back upon itself several times thus forming a highly convoluted and complex structure (Figs. 21 to 23). Neither these membranes nor the vacuolar membranes show the double-membrane
structure of the endoplasmic reticulum or mitochondrial membranes.

d. The nucleus

Usually on one side of the cell and lying close to the wall is the single nucleus. It occupies a position in the cell where the cytoplasm is of its greatest width and in very highly vacuolated cells it is almost isolated in the middle of the cell except for radiating strands of cytoplasm by which it is connected to the rest of the cell (Figs. 14, 16 and 17).

The nucleus is surrounded by a double layered nuclear envelope which may be fairly smooth in outline or highly convoluted (Figs. 26 and 27). Alternating light and dark bands were sometimes seen along the membrane but no nuclear pore was observed. The nuclear membrane has never been seen to be continuous with the endoplasmic reticulum. The shape of the nucleus varies with its physiological state but is usually spherical in the interphase state in mature resting conidia, and measures three to four microns in diameter. Sitting eccentrically on top of the nucleus is a conspicuous nucleolus which is of high electron density and stands out in strong contrast to the lighter grey nuclear matrix. Inside the dense nucleolonema may be seen, depending on the plane of section, one to three electron-transparent areas, distinct from the nucleolar ground substance (Figs. 28 and 29). These
may be considered to be the pars amorpha. In cells prepared for light microscopy, three corresponding darkly stained granules are observed, stained similar to the chromosomes and standing out very distinctly in the unstained nucleolus of hydrolyzed cells (Fig. 65 c and h). In nuclei undergoing division the electron-dense nucleolonema assumes a reticulate appearance with dense coiled strands embedded in a diffused matrix (Fig. 30). Inside the nucleus and lying close to the nucleolus may be seen one or two satellite bodies depending on how the section was cut (Figs. 28, 31 and 70). Away from the nucleolus on the periphery of the nucleus is an electron-dense area which is continuous with the nuclear envelope (Figs. 6, 27 and 31). This dense area corresponds with a dark-staining granule seen in light microscope preparations stained with azure B, Giemsa and iron hematoxylin and less conspicuously with Feulgen.

Additional electron-dense bodies are usually found occupying various positions in the nucleoplasm of both interphase and dividing nuclei. These range in size from one large body about 0.3 microns in diameter which is present in nearly all sections of the nucleus to several small ones of about 0.1 to 0.2 microns in diameter. Often these bodies occur at random (Figs. 26 and 32) or arranged in somewhat linear fashion in the nucleus (Fig. 33). Their presence in nondividing nuclei indicates that they are not identical with the mitotic chromosomes seen in the light microscope,
but may be associated structures.

e. The endoplasmic reticulum

Mature resting conidia contain sparse endoplasmic reticulum of the agranular type. Figure 34 shows the membranes of the endoplasmic reticulum to be of typical unit-membrane structure and smooth. Although the cytoplasm usually contains abundant ribonuclear protein particles they are always scattered in the ground substance of the cytoplasm and are never attached to the membranes of the endoplasmic reticulum. In mature conidia the membranes tend to be short and randomly arranged but in younger conidia which are still attached to the conidiophore, the endoplasmic reticulum tends to run parallel to the cell wall in long continuous wavy lines of two to four layers (Figs. 23 and 31). The reticulum is usually most abundant around the region of the nucleus and along the septum between adjoining conidia. Often the membranes of the endoplasmic reticulum form loops which swell into a vesicle producing internal cristae like typical mitochondria. These may be indications that mitochondria can be formed from the endoplasmic reticulum as is strongly suggested in Figure 37.

In newly formed conidia as well as in germ tubes and growing hyphae, the cytoplasm often appears to be divided up into a peripheral area containing all the mitochondria, the nucleus and lipid bodies and a central area containing structureless cytoplasm and several small vesicles which
appear to become larger in older cells and give rise to the vacuoles (Figs. 35 and 36). Separating these two areas are concentric rings of membranes of the endoplasmic reticulum. Figure 37 is a typical illustration of this as it generally occurs in growing hyphae. The ground substance of the cytoplasm has a light grey amorphous appearance with darker granular material scattered throughout. There may also be isolated vesicles which do not seem to be attached to the intercommunicating tubules of the endoplasmic reticulum.

f. Mitochondria

Mature ungerminated conidia contain relatively few mitochondria. They are usually rounded to ovate in outline and quite small, measuring about 0.5 microns across and 1-1.5 microns in length. The cristae are very few, usually not more than 4 to 5 being present in sectional view (Figs. 37 and 38). Cristae are tubular and usually of equal diameter throughout their lengths except at their ends which are usually expanded. They are generally oriented at right angles to the length of the mitochondria but are sometimes seen to run parallel to their lengths. The cristae do not run the entire width of the mitochondrion but extend about a half to two thirds the way across, so that an uninterrupted cavity is left in the middle. Two long cristae from opposite sides of the mitochondrial membrane may overlap in the middle but they have not been seen to touch each other. Inside the cavity of the mitochondria small electron dense granules are sometimes observed.
g. **Lipid bodies**

Dispersed in the peripheral layer of cytoplasm are numerous, spherical, extremely electron-dense bodies ranging in size from 0.5 to 2 microns in diameter (Figs. 27 and 28). These inclusions are strongly osmiophyllic and resemble similar bodies shown by other electron microscopists (42) to be lipid bodies or liposomes. They do not appear to have a limiting membrane although similar bodies shown in Figure 34 do appear to be surrounded by a membrane. In the light microscope, bodies of the same shape and dimensions are seen undergoing rapid brownian movement and appear to be inside the vacuoles. When dyes are applied these bodies stain independently of the vacuoles. They stain strongly with Sudan III and IV and Sudan black B indicating that they are fat containing bodies. However, they stain equally readily with neutral red, methylene blue and other basic dyes.

Sections prepared for electron microscopy were treated with lipase to see if these bodies would be digested away but the results were not convincing. There is a tendency for these "lipid" bodies to accumulate around the periphery of the nucleus, especially nuclei of incubated conidia of *E. polygoni* (Fig. 28). In *S. macularis* there are usually two or three large ones close to the nucleus on the side opposite to the nucleolus. In median sections where the vacuoles occupy the centre of the cell the "lipid" bodies are found close to the wall. They are rarely ever found in the strips of cytoplasm between the vacuoles and never within the
vacuole.

h. Unidentified bodies

Three structures of uncertain identity have frequently been observed in the conidia. The first, shown in Figures 24 and 25, is a structure somewhat larger than a mitochondrion, spherical in outline and of considerable electron density. It is surrounded by an equally dense membrane and embedded in its amorphous ground substance are several small tubes with electron-transparent cavities. Most of the tubes are oriented along the long axis of the whole structure but some run transversely and appear to be continuous with the inner membrane like the cristae mitochondriæ. Figure 25 shows that this body is surrounded by another membrane continuous with the vacuolar membrane and appears similar to the myelinoid bodies previously described.

Another structure quite frequently observed in conidia of *S. macularis* but never in *E. polygoni* or *E. cichoracearum* is shown in Figures 40 to 43. It is a long, apparently cylindrical rod from 3.5 to 5 microns long, by 0.25 to 0.3 microns wide. Serial sections indicate that this rod is a hollow tube divided up into a few electron-transparent chambers separated by dense septa of varying thicknesses. The ends of the rod are usually wider in diameter and more dense to electrons than the middle portions. They appear singly or occasionally in parallel pairs indicating that they may be self duplicating. These structures appear to be limited to the conidia having never been observed in the mycelium.
The third structure is a membrane-bound body, spherical in outline and slightly smaller than the mitochondria. The surrounding membrane seems to be a single structure. The content of the cavity is of considerable electron density and of an amorphous nature. These bodies are found in young cells and occur in groups, usually in the cavities of developing vacuoles (Figs. 25 and 36). As the vacuoles enlarge they gradually disappear. They may contain enzymes which digest away parts of the cytoplasm giving rise to the vacuoles.

In germinating conidia, long grey strands are sometimes observed extending almost the length of the cell (Fig. 44). At first it was believed that they were spindle fibres but repeated occurrence of these fibre-like strands show that they are never associated with the nucleus and often occur in cells in which the nuclei show no sign of division. The possibility that these structures may be microtubules has been considered but their large size makes this doubtful. Germinating conidia proved exceedingly difficult to fix satisfactorily and the possibility of artifacts occurring in these sections must always be considered.

1. **Changes in structure after treatment with water**

Frequent references are made to the fact that free water has a deleterious effect on mildew conidia. Corner (34) observed that in water the vacuolate structure of the cytoplasm gradually collapsed into a granular homogenous state, and he pointed out the need for an examination of the structure of the cytoplasm. Figure 46 shows a section of a conidium in
which all the vacuoles have disappeared after prolonged contact with water. The cytoplasm appears to lack any organized structure, consisting only of randomly scattered fragments of dense material dispersed in a less dense amorphous ground substance. Figure 47 shows a section of a cell in which the vacuoles have been broken down, apparently by plasmolysis of the cell. The plasma membrane can be seen slightly pulled away from the cell wall. Inside the cell is a tangled system of membranes to which are attached small dense granules comparable in size and appearance to ribonucleoprotein particles. There are a few vesicles with contents. In the light microscope, conidia that are exposed to water for a long time appear granular with no vacuoles. If the slightest amount of a wetting agent, such as sodium lauryl sulphate, is added to the water, the conidia assume this empty structureless appearance in a very few minutes. Once this change has occurred in the conidia they will no longer germinate.

vi. Germinated conidia

a. Germ tube formation and their response to light

Conidia of *S. macularis* will germinate on dry slides within 8-10 hours of incubation at 18 to 20°C in an atmosphere of 90-100% relative humidity. *E. polygoni* conidia germinate within 3-4 hours under the same conditions but will germinate very readily at much lower humidities. Conidia of *S. macularis* will remain viable for several
weeks or even months if stored at near freezing temperatures. Conidia were kept in the refrigerator at 5°C for 30 days and then incubated at 20°C. Over 80% of them germinated within 7 hours, whereas fresh conidia normally take 9-10 hours. Conidia also germinated at 15°C after this period of cold storage. Germination of both types of conidia seem to be stimulated by light and the germ tubes evince a very strong response toward light. Figure 48 shows germinated conidia of *E. polygoni* which were incubated in a Petri dish placed on a window ledge of the laboratory. All the germ tubes can be seen to be oriented in the same direction, i.e. toward the light. As the photograph shows, the germ tubes were not only similarly oriented but were all produced on the illuminated side of the conidia. To determine whether or not the germ tubes themselves were responding to the light or if germ tube emergence was just locally stimulated by light, conidia were germinated in the dark and then exposed to unilateral light to see if the randomly oriented germ tubes would turn in the direction of the light stimulus. Another set was incubated on glass slides with an arrow marked on the slide pointing to the light source. After the conidia had germinated the slide was turned 180° so that the germ tubes pointed away from the light. After 1 hour the slides were examined and it was found that in both cases the germ tubes were bent backwards to face the light (Fig. 49). The bending, though more pronounced at the tip, occurred throughout the length of the germ tube. Conidia incubated in the dark pro-
duced germ tubes randomly on all sides.

The number of germ tubes varies from one to three. The wall of the germ tube is formed from the inner wall of the conidium which pushes out through the outer mucilage wall as a little beak on the side of the spore generally about a third of the way from one end. Germ tubes of *S. macularis* have never been observed to form at the ends of the conidia. There appears to be considerable stretching of this inner wall as the contents of the conidia begin to flow into the protrusion. The wall at the neck of the germ tube is very thin, much thinner than the rest of the wall around the conidium. There is increased production of mucilage around the conidial wall at germination, and the mucilage coat is especially thick at the thin area of the germ tube wall next to the body of the conidium (Fig. 50) as well as at the tip of the germ tube (Fig. 51). As the cytoplasm continues to flow into the tube, its diameter increases toward the middle but remains narrow at the neck and tapers toward the tip. In *E. cichoracearum* there is no marked constriction at the neck of the germ tube and the tip swells up into a bulb (fig. 53). A similar effect is seen in *E. polygoni* but the swelling at the tip is not as marked as it is in *E. cichoracearum*. In *S. macularis* the nucleus migrates out into the germ tube where it divides and one nucleus migrates back into the conidium where it may divide again to produce one or two more germ tubes. In *E. cichoracearum* the nucleus tends to divide inside the
conidium, one migrating out into the germ tube and one re-
main ing behind. Apparently, it may also divide inside the
germ tube, with one daughter nucleus migrating back into
the conidium. Even after the mycelium is established on
the host the original conidium can still be seen with its
germ tubes branching off from it.

b. Changes in the cytoplasm at germination

Obvious differences can be observed between sections
of germinated and resting conidia. Most evident is a
marked increase in the granular appearance of the cytoplasm
of germinated conidia (Figs 44, 45, 51, and 52). Two types
of granules are distinguishable. One type consists of small
electron dense granules randomly dispersed in the cytoplasm,
and resembling ribosomes in size and appearance. Particles
which may be aggregates of these smaller ones are sometimes
seen in groups and arranged in linear fashion as shown in
Figures 44 and 45 (arrows).

The other type of particles is less dense to electrons
and forms aggregated rosettes of 500 - 600 milli microns in
diameter (Fig. 52). These aggregated particles appear very
similar to the particles of glycogen found in liver and other
animal cells. Similar particles have occasionally been seen in
sections of the hyphae but not in sections of unincubated conidia.

To test if these were glycogen particles, the iodine
test was applied. Incubated conidia were treated with 95%
alcohol, and mounted in Gram's iodine on glass slides and
examined under the microscope. Most of the conidia were
deeply stained with iodine and showed the yellow-brown iodine colour. Similarly treated ungerminated conidia did not show any difference in their staining reaction, so that this test gave inconclusive results. It is worthy of note that these glycogen-like aggregates show a decrease in concentration toward the tip of the germ tube, where the ribosome particles are especially abundant.

Another noticeable change in the germinated conidia is the marked enrichment of the endoplasmic reticulum. This is particularly true in the region around the nucleus and in the germ tube where the membranes run together in groups forming parallel cisternae (Figs. 51, 53, and 56).

Mitochondria too, are far more numerous in germinating conidia, particularly near the entrance to the germ tube and in the germ tube itself (Figs. 5 and 53). The mitochondria in these cells are much larger than those of resting conidia, are long and tenuous (chondriocon type) with more numerous cristae. A special point of interest is the frequent occurrence of dumbbell-shaped mitochondria with finely attenuated membrane connections which strongly suggest that they are undergoing fission (Figs. 54 to 56). Some appear as if the two halves have just separated, these being similar in form and having their adjacent ends squared as if recently cut off (Fig. 57).

As the germ tube continues to grow, more of the cytoplasm of the conidium flows into it, the vacuoles in the conidium break down and coalesce into one large vacuole filling most
of the cell except for a narrow margin of cytoplasm around the cell wall and the nucleus. The cytoplasm of the germ tube becomes very dense and crowded with mitochondria and membrane loops. A few lipid bodies also migrate into the germ tube but most of them remain behind in the marginal cytoplasm of the conidium. The extreme tip of the germ tube has fewer mitochondria but is dense with granular cytoplasm and RNP particles (Fig. 51). In preparations of germinated conidia stained for light microscopy the germ tube stains strongly with fast green FCF used in the test for total protein.

After the division of the nucleus a septum is laid down close to the conidium cutting off a cell consisting of the conidium and a part of the germ tube (Fig. 58). The existence of a pore in this septum is uncertain as one has not been observed in any of the serial sections made of several germinated conidia. Evidently, little or no material passes from the conidium to the germ tube after the septum is formed. It is often quite difficult to obtain proper fixation of the second cell after the laying down of the first septum as the thick mucilage of the tube prevents the entry of the fixative which seems to enter the conidium first and flows down the germ tube. Figure 60 shows a conidium with the germ tube deeply stained with osmic acid up to the first septum and the second cell very poorly fixed and stained. It is essential that the flow of material from the conidium into the germ tube be stopped early since other germ tubes are usually formed.
later and must be supplied with material from the conidium.

c. Septation and pore structure

Although the mycelium of Ascomycetous fungi is septate, cytoplasmic continuity is maintained throughout the colony by pores in the septa.

Electron microscopy reveals that the pore in the mildew hyphae and conidiophore is not as simple as is generally shown for other Ascomycetes. Sections taken away from the centre of the septum show the wall to be of equal diameter throughout (Fig. 62). Nearer to the centre of the cell the wall becomes considerably thicker in the region of the pore. Just next to the pore the septum becomes dumbbell shaped and the wall is almost three times as thick as at the sides (Fig. 63).

A section taken from exact centre shows the septum swelling slightly towards the centre and tapering suddenly to the pore (Fig. 64). Considering these serial sections together one finds that the septal pore is at the centre of a biconcavity in the greatly thickened mid region of the septum (Figs. 54 to 56).

The septum is formed by an annular ring of wall material growing in from the sides gradually closing in toward the centre. As the ring grows inwards it thickens just behind the advancing edge, thus showing the two wedge shaped ingrowths of Figure 61. The completely formed pore is very small, being not more than 0.2 microns in diameter.
Lipid bodies and other cytoplasmic structures have been observed as if in the process of passing through the pore (Figs. 24, 31, and 64), but the nucleus has never been observed to do this.

Before the terminal conidium matures the pore is plugged, apparently by material secreted through the plasma membrane that bridges the gap across the pore at this stage (Fig. 64).

d. Comparison of conidial and hyphal structure

No outstanding differences were observed between the cytoplasm of the conidia and that of the hyphae. The cell wall was similar in both cases except that there was more mucilage around the hyphae, especially on the side of the cell touching the host epidermis.

Vacuolation was more extensive in old hyphae but was much less in young growing tips. The most striking difference between the cytoplasm of the hyphae and that of the conidia was in the amount of endoplasmic reticulum present. The endoplasmic reticulum was much more extensive in growing hyphae and consistently showed a parallel type of orientation as compared with the random arrangement of the limited cisternae of ungerminated conidia. Mitochondria were also more numerous in growing hyphae whereas lipid bodies were far more numerous in the conidia.
Lomasomes were occasionally found in sections of the hyphae (Fig. 37) but not in the conidia, although ingrowths of the cell wall, resembling lomasomes, have been found in the conidiophore. Also, the nuclei of hyphal cells were smaller than those of the conidia.
PHOTOMICROGRAPHS

Note. All micrographs described as sections are electron micrographs. All electron microscopic preparations were fixed with buffered glutaraldehyde and post fixed with osmic acid. All magnifications are to the nearest 100.
Figure 1. Light micrograph of whole conidia of *S. macularis* slightly stained with methylene blue to show the outer wall. Note the unstained inner wall and the large number of vacuoles. X 2,700

Figure 2. Section of two adjoining conidia of *S. macularis* showing the splitting of the septum at the beginning of conidial abscission. X 18,200

Figure 3. Light micrograph of two conidia of *S. macularis* showing the final stage of conidial abscission. X 3,375
Figure 4. Section of conidia of *S. macularis* showing the final stage of abscision. \( \times 18,200 \).  

Figure 5. Section of the end of a conidium of *S. macularis* showing the ring of outer wall left by the breaking off of an immature conidium. The "fibrous" material may be a newly formed coating over the exposed end. \( \times 22,00 \)
Figure 6. Longitudinal section of two of a chain of conidia. The septum is about twice the thickness of the side wall, and has a disc on both sides at the centre in the region of the pore. Note the nucleus with the peripheral body.
Figure 7. Longitudinal section of conidium of *E. polygoni* showing the end wall (arrow) which is much thinner than the side walls. Note the large granular vacuoles. X 19,800

Figure 8. Longitudinal sections of conidium of *S. macularis* showing the point of abscission with an incomplete outer wall (arrow). Note the large empty-looking vacuoles. X 15,200
Figure 9. Section of conidium of \textit{S. macularis}
showing the stretched and sloughing outer
wall. \textit{X} 26,500

Figure 10. Light micrograph of two chains of
conidia of \textit{S. macularis} stained with
methylene blue. The terminal conidium
on the left remained unstained. The
dye penetrated the chain through suc-
cessive conidia starting from the
broken end of the conidiophore. The
terminal cell of the chain on the right
was damaged and was quickly penetrated.
\textit{X} 2,000
Figure 11. Light micrograph of conidium of *E. polygoni* showing one large central vacuole stained with neutral red solution after treatment with ether. Note the deeply stained bodies inside the lower cell. X 2,700

Figure 12. Light micrograph of conidia of *S. macularis* plasmodized with KOH solution.
Figure 13. Cross section of *S. macularis* condium showing a large vacuole formed evidently, from the breaking down of smaller ones. Note the thin cytoplasmic strands separating the vacuoles and the electron dense particles inside the vacuoles.

X 14,100
Figure 14. Longitudinal section of *S. macularis* conidium. The section is slightly off median and shows dense cytoplasm surrounding the nucleus and two vacuoles with granular contents. A single membrane of the endoplasmic reticulum (E.R.) runs between the two adjacent vacuolar membranes. The outer wall is of varying thickness around the cell. Note the lipid bodies near the nucleus. X 23,800
Figure 15. Section of *S. macularis* conidium taken from the region close to the wall. Note the dense cytoplasm, sparse and irregular endoplasmic reticulum (E.R.) and the absence of vacuoles. X 31,200

Figure 16. Near median section of conidium of *E. polygoni* showing many small vacuoles, scant peripheral cytoplasm and the nucleus. X 4,500
Figure 17. Median transverse section of conidium of *S. macularis*. The cell is mostly vacuole with only a thin layer of cytoplasm along the wall and around the nucleus. Note the electron dense vacuolar membrane, and the outer layer of the wall. X 11,100
Figure 18. Longitudinal near-median section of conidium of *E. polygoni*. Note the relatively dense granulation of the vacuoles, the lipid bodies along the cell wall and the small spines on the wall. Note also that the end wall is thinner than the side wall. X 11,100
Figure 19. Section of old part of germ tube of *S. macularis* showing a myelinoid body projecting from the cytoplasm into a vacuole. The body consists of a large number of concentrically arranged membranes partly enclosing a cavity of low electron density. X 35,500

Figure 20. Section of myelinoid body in germinated conidium of *E. polygoni*. Note that the membranes form a loop and turn back upon themselves. X 55,600

Figure 21. Section of myelinoid body in vacuole of *S. macularis* conidium. The membranes are continuous with the vacuolar membrane and the material contained between the membranes is similar to and continuous with the cytoplasm adjacent to the vacuole. X 37,800
Figure 22. *S. macularis* conidium showing myelinoid body in the vacuole. The outermost membrane is continuous with that of the vacuole. The double-stranded unit membrane structure of the endoplasmic reticulum (E.R.) and mitochondria (lower left) is absent in the membranes of the myelinoid body and also in the vacuolar membrane, which is incompletely formed. Note the expanded mid-region of the septum and the outline of the septal pore. X 40,400
Figure 23. Section of two adjoining conidia of *S. macularis* showing two myelinoid bodies inside the vacuole. Note the parallel arrangement of the endoplasmic reticulum (E.R.) along the cell wall and the dumbbell-shaped structure of the septum in the region of the pore. X 18,500
Figure 24. Section of conidium of *S. macularis* showing an unidentified membrane-bound body with numerous tubes embedded in a ground substance of high electron density. Compare the size and electron density with that of the mitochondria below. X 71,000

Figure 25. Section of *S. macularis* conidium showing a body similar to that of Figure 23 but enclosed by a second system of membranes resembling those of the myelinoid bodies. X 50,500
Figure 26. Section of *S. macularis* conidium showing the nucleus with highly convoluted envelope, a nucleolus and scattered electron-dense bodies which may be sections of chromosomes. The nuclear envelope is imperforate. Note the many mitochondria. X 22,000
Figure 27. Section of conidium of *E. polygoni* showing the nucleus and lipid bodies. Inside the nucleus are electron dense bodies embedded in a less dense matrix. On the top right side of the nucleus (arrow) is an area having the appearance of the dark-staining area of the peripheral granule observed in the light microscope. X 18,000

Figure 28. Section of conidium of *E. polygoni* showing nucleus and associated lipid bodies. Inside the nucleolus is an area of low electron density forming a pattern similar to the dark-staining bodies seen in the nucleolus in light microscopy. X 18,000
Figure 29. Section of nucleus of germinated conidium of *S. macularis*. The nuclear membrane is absent. Inside the nucleolus is an area of low electron density. In the nuclear matrix are dense "chromatinic" bodies, one of which is apparently paired. X 26,000

Figure 30. Section of germinated conidium of *S. macularis* showing the nucleus with a highly reticulate nucleolus. X 37,900
Figure 31. Section of two of a chain of conidia of *S. macularis* showing the nucleus with the peripheral body (arrow). The peripheral body is shown as an electron dense area of the nucleus opposite to the nucleolus and continuous with the nuclear membrane (see also Figure 6). Note the membranes of the endoplasmic reticulum (E.R.) parallel to the wall and the myelinoid body inside the vacuole. X 31,200
Figure 32. Section of conidium of *S. macularis*
showing the nucleus with randomly arranged
"chromatinic" bodies numbering about
twice as many as is usually found in the
nucleus. X 11,100

Figure 33. Section of germinated conidium of *E.
polygoni* showing the nucleus with
"chromatinic" bodies arranged in linear
fashion. The greatly elongated nucleus
was about to migrate out into the germ
tube. X 11,100
Figure 34. Section of a portion of the cytoplasm of *S. macularis* conidium showing the unit membrane structure of the endoplasmic reticulum (E.R.). The membranes are of the agranular type and the ribosomes are free in the cytoplasm. Note the branching of the membranes at centre.

X 58,800
Figure 35. Cross section of newly formed germ tube of *S. macularis* conidium showing an early stage of vacuole formation. The vacuole begins as a small vesicle of low electron density usually enclosing some membrane-bound bodies. X 35,000
Figure 36. Section of germ tube of *S. macularis* conidium showing the first formed vacuole enclosing membrane-bound bodies of varying electron densities. The membrane surrounding the enclosed vesicles is different from the membranes of the endoplasmic reticulum (E.R.) and mitochondria. X 35,00
Figure 37. Cross section of young hyphae of *S. macularis* on strawberry leaf. The endoplasmic reticulum (E.R.) forms parallel cisternae separating the cell into an outer region containing mitochondria and other structures, and an inner portion with small vesicles from which the vacuoles are formed. Mitochondria appear to be formed from loops in the membranes of the endoplasmic reticulum (E.R.) which swell up and produce cristae. The fungus is attached to the host cuticle (arrow) by a thick layer of "mucillage" which is continuous around the cell wall. Note the lomasome at left. X 42,600
Figure 38. Section of mitochondria in growing hyphae of *S. macularis*. X 54,300

Figure 39. Section of mitochondria formed from membrane loop in young hyphae of *S. macularis*. Note the single crista in the small loop. X 65,000
Figures 40 to 43. Sections of conidia of *S. macularis* showing unidentified rods found in different positions in the cell. The rods are divided up into areas of high and low electron density by septa-like partitions. They are sometimes long and slender as shown in Figure 42 (arrow).

Figure 40, X 31,800.
Figure 41, X 11,100.
Figure 42, X 18,800.
Figure 43, X 14,100.
Figure 44. Section of cytoplasm of germinated conidium of *S. macularis* showing long slender unidentified strands. Note the granular nature of the cytoplasm and the tendency of the particles to line up. X 26,500.

Figure 45. Section of cytoplasm of germinated conidium of *E. polygoni* showing a large number of electron dense particles some of which form parallel linear groups (arrows). X 35,000
Figure 46. Section of *S. macularis* conidium showing the effect of water on the conidium. The vacuolated nature of the cell is changed and the cytoplasm breaks up into globular particles. X 5,500

Figure 47. Section of a plasmolyzed cell of *S. macularis*. Note the withdrawn plasma membrane and the ruptured cell contents. X 11,800
Figure 48. Light micrograph of conidia of *E. polygoni* germinated in the presence of unilateral light. The germ tubes all point to the direction of the light sources, and have all been produced on the illuminated side of the conidia. X 2,700

Figure 49. Light micrograph of conidia of *E. polygoni* which were germinated in unilateral light and turned $180^\circ$ away from the light after germination. Note the bending of the germ tubes. X 2,700
Figure 50. Section of germinated conidium of *S. macularis* showing the formation of the germ tube.

The wall of the germ tube is continuous with the inner wall of the conidium.

Note the "mucilaginous" outer wall.

X 7,800
Figure 51. Section of the tip of the germ tube of *S. macularis*. Note the dense granular cytoplasm and the increase in the amount of endoplasmic reticulum (E.R.) from the tip backwards. The structure of the wall at the tip is ill defined. X 58,600
Figure 52. Section of germinated conidium of *S. macularis* showing aggregated particles believed to be glycogen granules. Smaller scattered particles may be ribosomes. The arrow shows what appears to be a mitochondrion formed from the membranes of the endoplasmic reticulum (E.R.). X 35,400
Figure 53. Longitudinal section of germinated conidium of *E. cichoracearum*. The cytoplasm is dense in the germ tube and around the nucleus. The tip of the germ tube is expanded into a bulb. The vacuoles are filled with granular material. X 14,250
Figures 54 and 55. Cross sections of germ tube of *S. macularis* showing mitochondria in formations which indicate that they multiply by fission (arrows). The body in the centre is the nucleus.

Figure 54, X 32,800

Figure 55, X 49,700
Figures 56 and 57. Cross sections of germ tube of *S. macularis* showing mitochondria in forms which denote that they multiply by fission. Note the two squared facing ends at arrow in Figure 57. Note also the concentric rings of membranes in Figure 56.

Figure 56, X 32,800
Figure 57, X 56,800
Figure 58. Light micrograph of germinated conidium of *E. polygoni* showing a nucleus in the germ tube and one in the conidium. Note the septum. X 4,100

Figures 59 and 60. Sections of conidia and germ tubes of *E. polygoni* showing the thickened outer wall covering at the point of germ tube formation (arrow). The cell shown in Figure 60 is poorly fixed and heavily stained with osmic acid up to the first septum, beyond which the fixative did not penetrate.

Figure 59, X 22,000
Figure 60, X 15,100
Figure 61. Section of two adjoining cells on a chain of *S. macularis* conidia showing a developing septum. The advancing end of the septal wall is thickened. X 14,100

Figure 62. Section of two adjoining cells of a chain of *S. macularis* conidia showing a complete septum. The section was cut close to the cell wall and shows a septum of equal width throughout. X 14,700
Figure 63. A near-median section of two adjoining cells of a chain of conidia of *S. macularis* showing the dumbbell-shaped structure of the septum. X 22,000
Figure 64. A median section of two adjoining cells of a chain of conidia of *S. macularis* showing the pore in the septum. The pore was apparently in the process of being plugged when fixed. Note the membrane-bound lipid-like body which was in the process of passing through the pore. A similar body is shown at top left. X 72,000
CHAPTER IV

NUCLEAR DIVISION

Evidence from the literature indicates that most if not all previous investigations of nuclear division in the Erysiphaceae have had to do with the sexual stages.

An attempt will be made here to describe nuclear division in the asexual cells of *S. macularis* and to point out any differences observed in the process as it occurs in *E. polygoni* and *E. cichoracearum*.

1. Structure of the nucleus as revealed by various dyes

Unfixed vegetative interphase nuclei in mildew cells stain readily in dilute solutions of most basic dyes. These dyes must, however, be applied in very dilute solutions of the whole cytoplasm stains deeply and shrinks up so that the nucleus cannot be seen with any degree of clarity. Crystal violet stains the nucleolus very strongly and is quite useful for bringing out the general outline of the nucleus (Fig. 65a).
Preparations fixed with Helly's or Carnoy's fixative and stained with azure B or iron hematoxylin give clear demonstrations of interphase nuclei. With azure B the nucleolus stains a deep blue, the nucleoplasm is light grey and the granule on the periphery of the nucleus near the end opposite to the nucleolus stains a dark blue (Fig. 65b). In hematoxylin-stained preparations the nucleolus and the peripheral granule stain equally strongly but the rest of the nucleus is lightly stained (Fig. 65c). Sometimes the nucleus shrinks during the staining process and the nuclear envelope can then be seen. Also when such shrinkage occurs, a clear ring is seen separating the nucleolus from the body of the nucleus (Fig. 65f).

For demonstrating chromosomes in dividing nuclei the most useful preparations resulted from the HCl-Giemsa technique. Helly's fixative gives satisfactory results when followed with HCl-Giemsa but acetic acid-alcohol causes less shrinking and preserves the chromatin material quite well. The peripheral body shows up very strongly with the Giemsa stain (Fig. 65 d & e).

Unhydrolyzed cells stained with Giemsa give a picture essentially the same as with hematoxylin or azure B except for a clear area in the cytoplasm surrounding the nucleus (Fig. 65 q). This clear ring has also been observed in hematoxylin stained preparations. In hydrolyzed Giemsa
preparations the nucleolus is unstained except for three small darkly stained granules which are connected by three thin, similarly stained strands, forming a triangle (Fig. 65 h). Sometimes only two of the granules can be seen, at other times only one (Fig. 65e). Figure 65i shows the peripheral granule with what appears to be a strand of chromatin material connected to it.

In incubated conidia in which the nuclei are in various stages of division, the bodies in the nucleolus often appear to be separated from the rest of the nucleus and the three granules can be seen connected to one or more threads projecting out from the chromatinic area of the nucleus (Fig. 66 a and c). Electron microscopy shows three clear areas in the nucleolus arranged in the same triangular pattern as shown in the light microscope but the connecting strands, seen in the light microscope, have not been revealed in electron micrographs. Also, in the light microscope the granules stain darkly instead of being clear areas.

ii. Stages of nuclear division

It has been very difficult to follow the process of nuclear division in this fungus because of the many and varied forms seen in stained preparations. Attempts were made to observe the process by phase microscopy but the reticulate nature of the conidia obscured the nucleus. The inhibitory effect of liquid media (even 25% gelatin), on
germination and nuclear division added to the difficulty.

Hence, one cannot describe what happens with certainty, but from the observation of a large number of conidia in various stages of division the behaviour of the nucleus seems to be as described below.

During the early stages of division the peripheral granule appears to be retracted from the surface of the nucleus and becomes elongated (Fig. 66a). The granule then divides and the two halves separate to opposite sides of the nucleus but still away from the nucleolus so that the nucleus often assumes a triangular shape with the two granules at the extremities of the base and the clear nucleolus at the apex (Fig. 66 b - d).

The nucleus in the germ tube gives an entirely different appearance. In the limited diameter of the germ tube the nucleus does not retain its spherical shape but becomes elongated sometimes five or six times as long as it is wide (Fig. 66f), at other times much shorter and thicker. At all times the darkly stained granule is at the advancing end of the nucleus and the nucleolus at the trailing end (Fig. 66e). The nucleus orients itself in this position before it migrates out of the conidium into the germ tube and each daughter nucleus produced in the germ tube is oriented in the same way. The advancing end which contains the granule is often more tapered than the nucleolar end,
particularly when the nucleus is migrating out of the conidia into the germ tube (Fig. 66e) or along the length of the germ tube.

Hydrolyzed Giemsa preparations reveal that the nucleus, even in its interphase state, has a highly reticulate structure. Nuclei even in young conidia, still attached to the conidiophore and which do not divide, show a meshwork of beaded threads surrounding the nucleus in a more or less regular pattern like knitted crochet (Fig. 66a).

These beads on the threads are most likely the structures appearing as electron dense bodies in the metabolic phase of the nucleus in electron micrographs (Fig. 26).

In what may be called the prophase stage of nuclear division the meshwork of the nucleus becomes less organized, the threads becoming extended into a tangled mass of beaded strands which later shorten and thicken with the nodules becoming more swollen (Fig. 66 h - k). Later distinct strands can be seen with several of these nodules or chromomereres along their lengths.

It has not been possible to count these chromatin threads accurately but the number is never fewer than six and more often appears to be eight.

A point of much significance here is that the chromatin threads do not appear to be separated from one another but are attached at one end either to a single large granule or in two groups each group to a separate granule (Fig. 67 a and b).
Most often one group is larger than the other, the larger group having a long coiled thread with three nodules (chromomeres) and can be seen to be coincident with the nodulated body observed in the nucleolus.

Apparently these dark-staining granules are not really a part of the nucleolus but are extensions of the reticulum of the nucleus which project into the nucleolus and become embedded in it. Thus, these granules stain deeply as do the chromosomes in hydrolized preparations whereas the nucleolus is unstained after hydrolysis.

In E. cichoracearum the two groups of chromatin material come together on opposite sides of the nucleolar granules which seem to be free in the middle (Fig. 67 c and d). As the two groups move away from each other they seem to separate from the Giems-positive body inside the nucleolus, since one can often see two groups of chromatin material of equal size and a third much smaller group between them. Later only two groups are seen. The behaviour of the chromosome-like bodies in the nucleolus is always obscure and its nature remains unresolved.

A true metaphase plate, as it occurs in classical mitosis has never been observed. Moreover, separate chromosomes cannot be distinguished, since the beaded threads of the "prophase" stage become closely packed into a very contracted state. Occasionally individual threads can be observed as, for example, in Figure 67e where they appear
to be oriented end up and lying in a circle around a central strand. In this photograph 9 Giemsa-positive bodies can be counted which could be interpreted as 8 chromosomes associated with a central granule.

In this highly contracted state two groups can often be seen lying parallel to each other with a space between the groups (Fig. 67 f and g).

This arrangement has been observed in the conidia but is most commonly seen in the germ tube, where, because of limited space, the groups are long and rodlike and arranged parallel to the long axis of the cell. The chromosome groups have not been observed to line up across the length of the germ tube. However, in E. cichoracearum and E. polygoni this is the usual pattern in the conidia. In electron micrographs of incubated conidia, electron-dense bodies, which are believed to be associated with the chromosomes, are sometimes seen to be arranged in a linear fashion (Fig. 33). Because of the thin sections that must be used, however, the true orientation of the whole structure is difficult to envision.

Figure 70 shows what appears to be newly duplicated chromosomes or pairs of chromatids in a dividing nucleus.

Possibly because of the limited space in the germ tube, the groups are always more compact than in the conidia except at the swollen tips where branching or appressorial formation is about to take place. Here the chromosome
groups tend to spread out more and appear more as they usually do in the conidia (Fig. 67j). In this more dispersed state the two groups of chromosomes may be seen to be each attached at one end to a deeply stained granule with the unattached ends spreading out loosely (Figs. 67a, b and h). The two granules may be on the same side of the chromosome mass or on opposite sides of it, and in the latter position it is usual to see a clear area between the two groups. The two granules are the products of the division of the deeply stained peripheral granule and the unstained area is the nucleolar area. Nuclei in which the two groups of chromatin material have moved away from each other usually show a few clearly defined strands bridging the gap between them. With further separation it can be seen that the strands are paired and arranged alternately in an interlocked fashion like the fingers of two clasped hands (Fig. 68 a to c). With still further separation the free ends of the cross strands trail behind being pulled by the heterochromatic attachment to the peripheral granule. The chromosome masses may move apart across the length of the cell as shown in Figure 68 a to c or parallel to its length as is more often in cells of *E. polygoni* as shown in Fig. 68e. In narrow germ tubes the two groups tend to slide apart, one forward and the other backwards as shown in Figure 68d.

The two groups migrate some distance apart and as they do so they round up (Fig. 68f). A nucleolus is reformed and the daughter nuclei assume the regular shape (Figs. 68g).
At what stage of division the nuclear membrane disappears is not clear but there are good evidences that it does not persist throughout the division of the nucleus. Electron micrographs of incubated conidia show the nucleus in some cases to be without a membrane or with a partly disintegrated membrane (Figs. 29 and 70). Stained light microscope preparations also show the chromatin material spreading and in a diffuse manner indicating the absence of a limiting membrane (Fig. 66 h to k).

The behaviour of the nucleolus during division is obscure. Its presence could not be detected in hydrolyzed Giemsa preparations from which the most satisfactory results were obtained, but unhydrolyzed material showed it to be present in nuclei which were at least up to the late prophase stage.

However, convincing evidence of its presence in nuclei in more advanced stages of division was not obtained. It was always surprising how very few nuclei in the final stages of division could be found in any preparation. Early stages were usually abundant but rarely more than three in the late stages could be found on any one slide of several hundred conidia. It is clear that the final processes of division are exceedingly rapid, lasting no longer than a matter of seconds perhaps. Because of this peculiarity it was extremely difficult to get sections of dividing nuclei for electron microscopy. Since only a few cells can be
sectioned and examined at any one time, the chances of finding a dividing nucleus are very small indeed. Iron hematoxylin was used in an attempt to determine the role of the nucleolus in nuclear division. Again the nucleolus could be seen as a darkly stained body in most cells but there was some indication that it did not persist throughout the process, since some nuclei showed it only as a pale area in the nucleus.

Electron microscopy has not been helpful in determining the role of the nucleolus since sections of nuclei in advanced division stages were not observed. Figure 71 shows a structure which may be a nucleolus somewhat separated from the rest of the nucleus by fine cytoplasmic strands radiating from it on all sides.

At no time in the whole process of division has an extranuclear spindle been observed, and, although some slightly differently stained material has sometimes been seen between the two separating groups of chromosomes (Fig. 68e), the presence of an intra-nuclear spindle has not been convincingly demonstrated.

That the interlocking connecting strands were not spindle fibres was evident from the fact that they were Giemsa positive and were always few in number with apparently equal numbers on either side. They appeared to interlock in alternate order and to be free at one end. They were,
therefore, more likely to be strands of chromosomes. Stains specific for histones and for proteins in general showed that there is a high percentage of heterochromatic material in the nucleus. After the DNA was extracted by boiling in 15% trichloroacetic acid the nuclei stained very clearly in fast green F.C.F. and showed no noticable difference in form.

The test was carried out on germinating conidia and nuclei showing stages of division were seen. The general pattern of arrangement of the nuclear reticulum was similar to that of untreated nuclei.

On the other hand, preparations stained with Feulgen's reagent gave very poor definition of the chromatin material. Chromatin threads could sometimes be seen but it was always very pale and could not be successfully photographed.

Also, when preparations were stained by the HCl-Giemsa method, both dividing and non-dividing nuclei showed a reticulate structure. For example, young conidia that are still attached to the conidiophore show distinct strands in their nuclei, but these nuclei are known not to divide until these conidia germinate. Electron micrographs of such cells also show dense bodies in their nuclei corresponding to those found in dividing nuclei. Results obtained by treating cells with DNA-ase and RNA-ase were inconclusive. Conidia were first fixed with gluteraldehyde as previously
outlined followed by treatment with a 0.1% solution of RNA-ase for three hours at 20°C. They were then washed and post fixed in osmic acid for three hours. The same was done for DNA-ase treated material.

In each case the structures normally found in the nucleus were still present. In the case of RNA-ase treatment the nucleolus was much less dense to electrons but still much more dense than the rest of the nucleus. Hence, the clear areas usually found in the nucleolus were still outstanding. The peripheral granule was not found in sections of nuclei so treated. This was not unusual since that body was only found in relatively few sections of untreated nuclei. Serial sections of nuclei show that the peripheral granule is not deeply embedded in the nucleus and is evidently quite thin. It appears in only very few sections, and most often is not found at all.

Figures 6 and 31 show sections of this body. It does not appear to be a separate structure but a darkly stained electron-dense area of the nucleus continuous with the nuclear membrane. No difference could be detected in its structure from the rest of the nucleus, except for its much greater electron density.

No chromatin strands have been seen attached to this granule in electron micrographs. However, light microscopy provides unmistakable evidence that the chromosome bearing structures of the nucleus are attached to it, at least during
outlined followed by treatment with a 0.1% solution of RNA-ase for three hours at 20°C. They were then washed and post fixed in osmic acid for three hours. The same was done for DNA-ase treated material.

In each case the structures normally found in the nucleus were still present. In the case of RNA-ase treatment the nucleolus was much less dense to electrons but still much more dense than the rest of the nucleus. Hence, the clear areas usually found in the nucleolus were still outstanding. The peripheral granule was not found in sections of nuclei so treated. This was not unusual since that body was only found in relatively few sections of untreated nuclei. Serial sections of nuclei show that the peripheral granule is not deeply embedded in the nucleus and is evidently quite thin. It appears in only very few sections, and most often is not found at all.

Figures 6 and 31 show sections of this body. It does not appear to be a separate structure but a darkly stained electron-dense area of the nucleus continuous with the nuclear membrane. No difference could be detected in its structure from the rest of the nucleus, except for its much greater electron density.

No chromatin strands have been seen attached to this granule in electron micrographs. However, light microscopy provides unmistakable evidence that the chromosome bearing structures of the nucleus are attached to it, at least during
division.

The sequence of events as they appear to follow in nuclear division is shown in Figure 69.
Figure 65. a to i. Light micrographs of powdery mildew conidia stained in different ways to show the nuclei.

(a) Unfixed conidium of *S. macularis* stained with crystal violet. The nucleolus is deeply stained.

(b) Conidium of *S. macularis* fixed with Helly's fluid and azure B. Note the deeply stained nucleolus and peripheral body.

(c) Conidium of *E. cichoracearum* fixed with Helly's fluid and stained with iron hematoxylin.

(d) and (e) Conidia of *S. macularis* fixed with Helly's fluid, treated with HCl and stained with Giemsa. The dense body shown by the arrow is the peripheral body. Note the darkly stained nucleolar body in (e).

(f) Conidium of *S. macularis* fixed with acetic acid-alcohol and stained with iron hematoxylin. Apparently the nucleus had shrunken slightly thus revealing the nuclear membrane and an unstained ring between the nucleolus and the rest of the nucleus.

(g) Germ tube of *S. macularis* stained with Giemsa without previous hydrolysis. Note the unstained ring around the nucleus.

(h) Nucleus of acetic acid-alcohol fixed and hydrolized conidium of *S. macularis* stained with Giemsa. Note the deeply stained granules in the nucleolus and the strand connecting them.

(i) Dividing nucleus of *E. polygoni* fixed with Helly's fluid, hydrolyzed and stained with Giemsa. Note the strand of chromatin material extending out from the peripheral body (arrow). Magnification excepting c and h, X 3,000 c and h, X 4,000
Figure 66. Conidia and germ tubes of powdery mildews fixed with Helly's fluid, hydrolyzed with NHCl and stained with Giemsa to show dividing nuclei.

(a) Nucleus of *S. macularis* showing the peripheral granule slightly elongated and strands extending from the body of the nucleus into the nucleolus.

(b - d) Nuclei of *S. macularis* showing the peripheral body divided and separated to opposite sides of the nucleus and away from the nucleolus.

(e) Nuclei of *S. macularis* in germ tube migrating apart after division. Note the peripheral body at the pointed leading end of the nucleus and the nucleolus at the other end.

(f) Nucleus in germ tube of *E. polygoni* showing the nucleolus and nucleolar body in the middle of the elongated nucleus.

(g) Nucleus in germ tube of *E. polygoni* showing a meshwork of chromatinic strands connected to a deeply stained granule.

(h - k) Nuclei of *S. macularis* and (l) nucleus of *E. polygoni* showing strands of chromatin material in what may be the prophase stage of division. All magnifications X 3,000
Figure 67. Powdery mildew conidia and germ tubes fixed with acetic acid-alcohol, hydrolyzed with NHCl and stained with Giemsa to show dividing nuclei.

(a and b) Dividing nuclei of *S. macularis* showing two groups each of chromatinic strands. In (a) the strands seem to be branching off from a longer strand. In (b) each group is in the form of a triangle and has a dense granule at its apex (arrows).

(c and d) Dividing nuclei of *E. cichoracearum* showing two groups each of chromatinic material with a third group between them. In (c) what appears to be the peripheral granules are at the extremities, the "chromosomes" are attached to them and the nucleolar granules are in the middle (arrows).

(e) Dividing nucleus of *S. macularis* showing a group of 8 chromatinic bodies arranged in a circle around a central granule.

(f - h) Dividing nuclei of *S. macularis* showing (f and g) bars of chromatin material in parallel arrangement in the germ tube; (h) Two groups of chromatin material in germ tube, each attached to a common thread with a granule at its end (arrows).

(i - j) Dividing nuclei of *E. polygoni* showing groups of chromatin material. In (i) the unstained area appears to be the nucleolus. In (j) the two groups are almost completely separate. X 3,000
Figure 68. Cells of powdery mildew fixed with acetic acid-alcohol hydrolyzed with NHCl and stained with Giemsa to show dividing nuclei.

(a) Nucleus of *S. macularis* in tip of germ tube apparently undergoing anaphase movement. Note the trailing strand between the two groups.

(b and c) Nuclei in conidia of *S. macularis* in anaphase stage of division. Two chromatic masses are shown each with a few finger-like strands bridging the gap between the two masses.

(d) Dividing nucleus in germ tube of *S. macularis*. Two chromatinic masses are shown, their expanded ends oriented in opposite directions as if sliding apart.

(e) Dividing nucleus in conidium of *E. polygoni* apparently in the anaphase stage. Two darkly stained granules are shown on opposite sides with more lightly stained strands connecting them to granules of chromatinic material in the middle. The lightly stained area shown by the arrow is believed to be the area of the nucleolus.

(f - h) Two nuclei stage in (f) germ tube, (g) conidium of *S. macularis*; (h) conidium of *E. cichoracearum*. X 3,000
Figure 69. Proposed sequence in stages of division of nucleus of _S. macularis_.

(a – c) Division of the peripheral granule and subsequent separation of the two granules to opposite poles of the nucleus.

(d – e) Separation of the chromatin material into two groups each strand of a group attached to a common strand with a granule.

(f) Contraction of the chromatin material into two small solid blocks lined up side by side.

(g and h) Separation of the two blocks accompanied by the trailing behind of the free ends of chromatin strands.

(i and j) Formation of two nuclei from the two separated chromatin groups.

(k) Further migration of the nuclei and the formation of a cross wall to form two new cells.

X 3,000
Figure 70. Section of nucleus of germinated conidium of *S. macularis* showing chromatinic bodies in the nuclear matrix. The body shown by the arrow is paired and may be newly duplicated chromatids. The nuclear membrane shows signs of having begun to disintegrate. X 29,300
Figure 71. Section of conidium of *S. macularis*
showing a body of uncertain identity
outside the nucleus. The body has the
appearance of a nucleolus but is outside
the nuclear membrane. It is too large
in relation to the size of the nucleus
to be the peripheral body. Fine fibre-
like strands can be seen extending from
it and have apparently began to in-
vaginate the nuclear membrane. X 21,200
CHAPTER V

HAUSTORIA AND HOST INFECTION

i. Appressorium formation

The powdery mildews are obligate parasites and the survival of the developing mycelium is entirely dependent on the successful establishment of itself upon the host from which it must derive all its food.

With the limited supply of food stored in the conidium it is essential that contact with the host cytoplasm be made as early as possible after germination of the conidium.

When mildew conidia are germinated on glass slides the germ tubes grow rapidly at first producing usually three to four cells after which growth slows down almost completely and in a short while they shrivel and die.

Small appressorial swellings are formed on glass slides but they are produced under such conditions only when the germ tubes have reached their maximum length. The tip of the tube usually branches into two short branches each one swelling slightly at the tip. On the surface of the host, appressoria are produced very early after the germination
of the conidia. In *E. polygoni* appressoria are generally produced on the second cell of the germ tube which cell may form an appressorium directly or it may first branch into two with each branch forming an appressorium from which a penetration peg will develop to form the first haustorium. Figure 72 shows germ tubes of *E. polygoni* developing appressoria on epidermal strips of clover leaf. One or two swellings on the side of the appressorium develop into hyphal branches which continue the growth of the young mycelium.

Apparently, haustoria may be formed from the hyphae without the formation of appressoria. Just behind the growing tip of the hyphae a wedge-shaped swelling develops on the underside that is in contact with the host and the penetration peg grows out of the tip of the wedge (Fig. 73).

ii. Host penetration

Both light and electron microscopy show that the mycelium is closely and firmly attached to the host surface by a thick coating of mucilage. If one attempts to pull the mycelium from the leaf surface with a needle or a pair of sharp forceps the host cuticle tends to become detached with the hyphae.

Electron micrographs show that the mucilage sheath is
thicker on the underside of the hyphae than elsewhere and that it is closely fixed to the host cell (Fig. 37). It was also noted earlier that germinating conidia have a thicker mucilage sheath than ungerminated ones.

At the point of penetration the firmly attached hypha forms a swelling, the lower wedge-shaped portion of which presses downward on the epidermal cell wall. This often causes a depression in the wall which deepens as the wedge swells into it. The tip of the wedge elongates and becomes more pointed and finally punctures the cuticle (Figs. 74-76).

Sections through the infection peg and host cell show that there is no difference in the staining reaction of the cuticle at the point of penetration and elsewhere, but that there is a distinct difference in the cellulose layer (Figs. 75 and 76). Electron micrographs of sections of material fixed with osmic acid show that the area around the penetration peg stains deeply and is much more dense to electrons than the rest of the wall which is normally electron transparent. The darkly stained electron-dense ring begins immediately below the cuticle in the cellulose wall and is continuous along the length of the peg until it enters the lumen of the cell.

The penetration peg itself stains differentially, the central portion being transparent to electrons whereas the cytoplasm along the wall is very dense and of similar
appearance to the dark area of the wall around the peg. The wall of the penetration peg which separates the two electron-dense areas is transparent to electrons (Fig. 78).

Figure 79 shows that the electron-dense area precedes the tip of the penetration peg and there is a space between this area and the point of the peg. This darkly stained area presents a diffuse appearance rather than being bounded by a well-defined limiting line, thus indicating the diffusion of an osmophilic substance into the tissue in advance of the peg.

The peg does not generally penetrate the wall at right angles to the surface, and it is very unusual to get a section that shows the haustorium together with the whole length of the peg. Sections through the whole path of the peg usually show it to be curved indicating resistance to pressure.

iii. Reaction of the host to penetration

At the point of penetration the host wall becomes considerably thickened by the accumulation of additional wall material across the path of the peg. Figure 79 shows that the thickening of the wall precedes the advance of the peg. By the time the peg breaks through into the lumen of the cell the host wall at this point is five to six times thicker than normal (Figs. 77 to 79).

This thickening forms a collar that surrounds the
penetration peg and projects into the cell lumen. The host collar is not simply a swelling of the original wall. It consists of newly formed material deposited on the inside of the wall at the point of penetration. In the electron micrographs (Figs. 75 and 79) the outline of the original wall can be seen along the base of the swollen collar. They show the original wall to be of similar thickness as elsewhere. Whereas the true wall consists of parallel layers and gives a stratified appearance, the material of which the collar consists is amorphous and shows no layering.

iv. Development of the haustorium

As soon as the penetration peg emerges from the surrounding collar and becomes free in the host cell, it begins to enlarge. The protrusion of the host wall collar and penetration peg into the cell lumen causes the host plasma membrane to become invaginated and surround the swollen end of the peg as well as the protruding collar. The peg does not puncture the plasma membrane. Figures 78, 79 and 86 show that the plasma membrane is continuous with the membrane that lines the host collar and surrounds the entire haustorium. This membrane is unbroken throughout. As the haustorial vesicle continues to enlarge, a nucleus migrates into it through the penetration peg. At the neck of the haustorium next to the host collar, a septum is formed which cuts off the body of the haustorium
from the peg, and the haustorial vesicle becomes an independent cell (Fig. 78). No pore has been observed in this septum but it is assumed that it is perforated since an unperforated septum would be a serious barrier to the passage of food material from the haustorium to the superficial mycelium which it feeds.

The haustorial vesicle enlarges into a pear-shaped structure about ten to eleven microns long and six to eight microns wide, from which branches grow out, more or less randomly in S. macularis (Fig. 81), but apparently from the distal end in E. polygoni (Figs. 82 and 83). The number of branches growing out from the central body is usually small, not more than three having been observed in any one section. But the branches themselves become profusely branched so that the central body of the haustorium is surrounded by a tangled mass of hyphae, which overlap one another in a random fashion (Figs. 79 to 84). However, there is always a space between the hyphal branches and the central body which seems to float in a medium of similar appearance as its own cytoplasm. This medium also fills the spaces between the hyphae and the entire sac that surrounds the haustorium.

The turgor pressure of the host cell surrounding the haustorium is evidently greater than that of the inside of the haustorial sac, for the surrounding membrane, instead of being flush and smooth, fits closely into all the indentations caused by the ramifying hyphal branches (Fig. 80).
There are no septae in the hyphal branches emerging from the central body so that the entire structure, consisting of the central body and all its branches, constitutes a single cell with a single nucleus.

v. **Fine structure of haustorium**

The central body of the haustorium is surrounded by a wall 0.15 microns thick and of similar structure and electron density to the inner wall surrounding the rest of the fungus. The mucilaginous layer is completely absent from both the central body and its branches.

Occupying a central position in the body of the haustorium, is a large and conspicuous nucleus about 5 microns in diameter or more than two-thirds the diameter of the cell (Figs. 80, 82 and 83). This nucleus migrated into the haustorium through the penetration peg which is slightly less than 1.5 microns in diameter — a good illustration of the capacity of the nucleus to vary its shape. The structure of the nucleus is identical with that previously described for nuclei in the conidia.

In some cases numerous small vacuoles occur throughout the haustorium, both in *S. macularis* (Fig. 81), and in *E. polygoni* (Fig. 82). These vacuoles are not very similar to those found in the conidia and hyphae. They are much smaller, more numerous, contain darkly stained particles of various sizes and become more empty-looking as the
haustorium gets older.

The haustorial cytoplasm is moderately dense to electrons. Endoplasmic reticulum is present but not abundant. On the contrary, mitochondria are numerous, in the central body as well as in the branches (Fig. 80).

Although elongated and spherical sections of mitochondria appear to be equally abundant, the constancy of their diameters clearly indicates that the spherical sections are cross sections of elongated types. Hence, the haustorial mitochondria appear to be predominantly of the chondriocont type. Their structure is similar to those previously described for the conidia.

Lipid bodies are surprisingly abundant in the haustoria. They tend to be quite large, some of them about half the diameter of the hyphal branches. Similar bodies have been observed in the host cytoplasm surrounding the haustorium.

The entire haustorium is surrounded by a single membrane (Figs. 80 - 84), which was previously shown to be the invaginated host plasma membrane. It encloses a fluid-like substance of cytoplasmic appearance which is sometimes of similar electron density to the fungal cytoplasm but is often more transparent to electrons. No mitochondria or any other organelles typical of living cytoplasm have ever been found in this surrounding fluid.

The enveloping membrane forms a distinct separating boundary between the host cytoplasm and the contents of the haustorial sac, the two standing out in strong contrast
owing to their different electron densities.

Cells in which the haustorium has been well established have a markedly increased amount of dense cytoplasm which fills the cell so that no vacuoles are apparent (Figs. 30 and 81). Others in which the haustorium appears to be newly introduced have an optically empty appearance as do the neighbouring uninfected epidermal cells in which most of the cell is vacuole (Fig. 79).

Bordering the membrane that surrounds the haustorium, generally on the inside of the sac but occasionally on the outside, are randomly dispersed electron dense particles. This material is discontinuous, loosely distributed and structureless in appearance. It is absent along much of the surrounding membrane and does not form a distinct encapsulation layer.

Hence, the only barrier that separates the haustorium from the cytoplasm of the host cell is the single membrane which surrounds the haustorium. In haustoria of E. polygoni the enclosing membrane tends to have a more continuous deposit of dense material and the sac is generally more loosely fitted around the haustorium (Figs. 82 and 83).

vi. Host-parasite relations

The mycelium of the three species of mildew examined here is entirely superficial and the haustoria, which are the only part of the fungus to enter the host cell, are confined to the epidermal cells.
Apart from the increase in the cytoplasmic contents of infected cells, no apparent changes have been observed in neighbouring epidermal cells or in underlying mesophyll cells. However, infected leaves tend to ripen and fall off more quickly than healthy leaves.

The distribution of haustoria in the cells varies with the different mildews. Haustoria are particularly numerous in sunflower where in heavily infected areas, on the average, one in every four cells contains an haustorium and frequently two haustoria are found in one cell. They are similarly abundant in clover, in which two haustoria frequently occur in one cell. In strawberry, haustoria are far less numerous, averaging about one in twenty cells. Two haustoria have not been observed in one strawberry cell but two adjacent cells have occasionally been seen to be infected. In clover, the upper epidermis of the leaf is most often infected but the lower surface is also susceptible. In sunflower both surfaces of the leaf are generally infected and the stem and leaf petiole are equally susceptible. In strawberry, the upper surface of the leaf is rarely infected except in very young leaves on stolons that are hanging down in shaded areas. Also, the petiole of infected strawberry leaves usually remains free from infection. Stolons that are hanging down are readily infected near the tip but older parts are not infected.

There is also a marked difference in susceptibility between the two species of strawberry, Fragaria ovalis.
and F. chiloensis which served as hosts to S. macularis.

Although the two species were grown on the same bench in the greenhouse, one could always find mildew on the lower surface of the leaves of F. ovalis but only very occasionally on F. chiloensis chiefly when new plants were potted and were growing rapidly. As soon as the plants were sprayed with miticides the mildews disappeared and F. chiloensis ceased to be infected except after repotting.

While examining sections of leaf epidermis in the electron microscope in search of haustoria it was observed that there was a very marked difference in the thicknesses, not only of the cuticle but of the cell wall of the upper and lower epidermal cells of F. ovalis. Measurements in the electron microscope showed that the cell wall of the lower epidermis of the leaf was only 0.4 microns thick whereas that of the upper epidermis was 3.3 microns thick. Following up this observation measurements were also made of the thicknesses of the epidermis of the stolon and of both leaf surfaces of F. chiloensis.

As a means of comparison, Figure 85 shows electron micrographs of the upper and lower epidermis of the leaves of both species as well as of epidermis taken from stolons growing on top of the bench and of those hanging down. All micrographs were magnified 7500 times. The marked differences in their wall thicknesses can be seen immediately. The measurements which include the cuticle
### Table A. Differences in cell wall thickness of "E. crassipes" of strawberry

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>WALL THICKNESS (in microns)</th>
<th>LOWER EPIDERMIS</th>
<th>UPPER EPIDERMIS</th>
<th>STOLON ON BENCH</th>
<th>STOLON HANGING</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. octaethis</td>
<td>1.0</td>
<td>2.05</td>
<td>3.3</td>
<td>0.4*</td>
<td></td>
</tr>
<tr>
<td>P. chiffonensis</td>
<td>1.4</td>
<td>2.7</td>
<td></td>
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</tr>
</tbody>
</table>
are summarized in Table V.

This evidence indicates that not only the cuticle thickness but the differences in the cell wall thickness may be significant factors in preventing infection of strawberry by mildew.

The upper epidermis of leaves of *F. ovalis* was repeatedly inoculated with conidia of *S. macularis* in an attempt to get them infected. Examination of these leaves under the light microscope showed that the conidia germinated in large numbers but, except in cases in which very young leaves were used, infection did not take place.

After examining numerous sections of upper epidermis of *F. ovalis*, two haustoria were found in adjoining cells as shown in Figure 86. Their appearance was not at all like normal healthy haustoria. The branches were small and lacking turgidity and the whole structure appeared necrotic. Whether or not the unhealthy appearance of these haustoria was due to premature death of the parent hyphae or to some defence reaction of the host cell is not known.
Figure 72. Light micrograph of germinated conidia of *E. polygoni* on clover leaf epidermis. Note the appressorial swellings on the first cell of the germ tube. X 1,800

Figure 73. Light micrograph of the tip of a germ tube of *E. polygoni* showing a wedge-like swelling which appears to be pressing down on the host surface. Apparently haustoria may be produced from the hyphae without the formation of an appressorium. X 8,100
Figure 74. Section of the epidermis of a leaf of the strawberry showing a penetration peg of *S. macularis*. The peg has not yet penetrated the host. Note the difference in electron density of the area surrounding the peg and the rest of the cell wall. X 12,800

Figure 75. Section of an epidermal cell of strawberry leaf containing an haustorium of *S. macularis*. Part of the penetration peg is shown. Note that the area of high electron density begins immediately below the host cuticle. X 4,100
Figures 76 and 77. Sections of epidermal cell of strawberry leaf showing the penetration peg of *S. macularis*. The cellulose wall is highly electron dense around the peg, starting just below the host cuticle (Fig. 76). The electron density of the cuticle does not change at the point of penetration. In Figure 77 the dense area precedes the tip of the penetration peg and there is an area of low electron density at the tip of the peg. Figure 76. X15,200. Figure 77. X 22,000
Figure 78. Section of an haustorium of *S. macularis* in an epidermal cell of strawberry leaf. The host wall forms a collar around the penetration peg. Outside the collar is a membrane that is continuous around the haustorium. A septum cuts off the penetration peg from the body of the haustorium. X 34,100
Figure 79. Section of an haustorium of *S. macularis* in an epidermal cell of strawberry leaf. The haustorium consists of a pear-shaped central body and numerous branches. The whole structure is enveloped in a membrane which is continuous along the host collar and around the inside of the host wall. X 8,100
Figure 80. Section of an haustorium of *S. macularis* in epidermal cell of strawberry. The central body contains a nucleus surrounded by numerous mitochondria. The enveloping membrane folds into the narrow spaces between the haustorial branches. The branches appear to be bathed in a fluid that fills the haustorial sac. X 18,300
Figure 81. Section of epidermal cells of strawberry stolon showing an haustorium of *S. macularis* in one cell. The infected cell is filled with dense cytoplasm whereas the adjoining cells are much less dense. Note the branching of the haustorium from the end and side. X 6,500
Figure 82. Section of haustorium of *E. polygoni* in epidermal cell of clover leaf. The haustorial branches arise from the end of the central body. The haustorium is surrounded by a membrane with scattered electron-dense deposits chiefly on the outside. Note the numerous small vacuoles. X 10,000
Figure 83. Section of haustorium of *E. polygoni* in epidermal cell of clover leaf. Note the nucleus in the central body with the strands joined to the nucleolus. Note also the branches at the end of the central body. X 10,00
Figure 84. Section of epidermal cell of clover leaf showing haustorial branches of *E. polygoni*. The peg is to one side of the haustorium. Note the numerous branches. X 11,800
Figure 85. Sections of epidermal cells of leaves and stolons of two species of strawberries showing the differences in cell wall thicknesses.

(a) Upper epidermis of leaf of *F. ovalis*
(b) Upper epidermis of leaf of *F. chiloensis*
(c) Lower epidermis of leaf of *F. ovalis*
(d) Lower epidermis of leaf of *F. chiloensis*
(e) Epidermis of old part of stolon of *F. ovalis* lying on bench
(f) Epidermis of young part of stolon of *F. ovalis* hanging in shade.

X 7,500
Figure 86. Section of two epidermal cells from the upper epidermis of *F. ovalis* infected by *S. macularis*. The haustoria in both cells appear to be dead. X 4,000
CHAPTER VI

DISCUSSION

In order to understand how an organism functions it is necessary to know something of its structure. Functionally, the powdery mildews are, in a number of ways, quite different from the majority of fungi. Their obligate parasitism, their superficial mycelium, their ability to germinate in the absence of an external supply of water and their failure, in most cases, to germinate in the presence of free water, are features not generally found among fungi.

These peculiar characteristics exhibited by the powdery mildews, along with the conflicting reports that often result from these peculiarities, have elicited the expression of the opinion of many workers, that detailed study of the structure of the mildews be undertaken (20, 34). It was partly in answer to this need that this study was carried out.

As one would have expected, the findings reveal that certain differences in structure accompany the evident differences in function of powdery mildews as compared with other fungi, particularly in the case of the conidia.
In a recent symposium on the spore, Gregory (47), in a suggested definition of the spore, mentioned among other things, that spores were characterized by small water content and a lack of vacuoles. Here the powdery mildew conidia are strikingly uncharacteristic. This study has shown that powdery mildew conidia are highly vacuolated structures, more than 50% of the cell being taken up by vacuoles. With regard to their water contents, Yarwood (116) and Jhooty and McKeen (64) had already shown that from 53 to 70% of the weight of conidia of S. macularis and E. polygoni is water.

The evidence given here that mildew conidia consist largely of water containing vacuoles, is of importance in that it gives adequate explanation of the mildews' capacity to carry their water and to germinate in the absence of any external source of water. Since the spores contain a high water content it is unnecessary to postulate a high internal osmotic pressure for water absorption from air.

Since some powdery mildew conidia germinate at zero relative humidity it would be difficult to conceive of water being absorbed from the air at such high water tensions even if the osmotic pressure was 60 to 90 atmospheres as proposed by Brodie (20). An osmotic pressure range of 14 to 19 atmospheres, as obtained for these mildews, is much more consistent with the known water relations of the cell. These figures are also quite close to those of Jhooty and McKeen
who obtained pressures of 17 to 21 atmospheres.

The gross disparities in the osmotic pressures obtained by different authors for powdery mildew conidia may be due to two factors, namely, differences in the length of time during which the conidia are left in the plasmolyte and differences in interpretation of incipient plasmolysis as related to these cells. Jhooty and McKeen left the conidia in sucrose solution 24 hours and obtained a maximum of 60% plasmolysis in two hours. Brodie reported that no plasmolysis of either *E. polygoni* or *E. graminis hordei* occurred in sucrose solutions, although they shrank considerably in strong sucrose solutions. He did not state how long they were left in the solutions. It is clear that a high percentage of powdery mildew conidia remain unplasmolyzed for a long time even in very concentrated solutions of sucrose. However, the wrinkling of the cell wall and contraction of the vacuoles as observed in conidia suspended in such solutions, indicate that they lose water. The honeycomb-like structure of powdery mildew conidia allows them to lose much water without a simultaneous withdrawal of the plasma membrane from the cell wall. If the whole conidium shrinks by the withdrawal of water, plasmolysis will not be evident until dehydration becomes excessive. The extent of water loss from the conidium will depend upon the efficiency of its insulation. Complimentary changes are observed when conidia are suspended in pure water. Some slowly absorb
water, the vacuoles enlarge, burst and form larger ones and the thin cell walls finally break.

If the conidia lose water by exosmosis, it follows that the cell sap is hypotonic to the surrounding solution. The criterion for determining the osmotic pressure of the cells should, therefore, be the point of isotonicity beyond which obvious changes are observed in the cell such as shrinking of the vacuoles and wrinkling of the cell wall.

The fact that some conidia remain unplasmolyzed and unshrivelled even in solutions of excessively high concentrations is most probably due to the impermeability of their outer covering. These results agree with those of Brodie in that conidia are less readily plasmolyzed in solutions of sucrose than in salt solutions of similar osmotic strength. This may be due to the ionic nature of the salt solutions as pointed out by Conway (35).

Yarwood (116) suggested in 1950 that the capacity of powdery mildew conidia to germinate at low humidities was due to their high water content and their efficient system of water conservation. In 1966 Somers and Horsfall (106) placed the emphasis on the second of these proposals by saying that water retaining power and not absolute water content was the important factor. This work shows that powdery mildew conidia are constructed not only to contain a high percentage of water but also to retain this water.

The close correlation between the percentage of water contained in the conidia and the percentage of vacuolar space
to spore volume strongly suggest that most of the water in the conidia is contained in the vacuoles. Indeed, the repeated observation of bursting vacuoles accompanied by a flow of fluid in the cell, the successful staining of the vacuoles with neutral red and the high degree of plasmolysis of which the conidia are capable, provide ample evidence that they are largely comprised of water-containing vacuoles. As for their ability to conserve water, it was shown that an insulating mucilagenous layer of wall material surrounds the conidia. The evident presence of fatty material in this layer enables it to serve efficiently as a barrier against water loss. The effectiveness of this protective coat is limited by its thickness particularly at the ends of the conidia where the abscission scar is apt to be left uncovered in those conidia which were dislodged prematurely. If conidia are left in warm dry air many of them shrivel and die before they germinate. Hence the ability of the conidia to germinate at low humidity depends upon their ability to retain the water contained in the vacuoles.

Retention of water is achieved, not only by the encapsulation of mucilage but by the nature of the internal structure of the cell itself. The vacuole, instead of being one central cavity, consists of several separate compartments each having a protective membrane. There are indications that the vacuolar membrane is of very limited permeability. Experiments with various dyes including neutral red, a well
known vacuolar stain, show that dyes did not enter the vacuoles until the membrane was ruptured. At the same time, globular bodies inside the surrounding cytoplasm were deeply stained, thus showing that the dye did penetrate the cell.

Goldacre (44) showed that when cells of Neurospora were emersed in neutral red solution the dye went immediately into the vacuole. He quoted Guilliermond as saying: "That penetration of the vacuolar membrane occurs during accumulation is an experimental fact. The vacuole is the first site of accumulation of free ions." This author further shows that dyes such as methylene blue, Janus green and neutral red penetrate and stain cell vacuoles as a vital process. High concentrations kill the cell and consequently stain the whole protoplast.

The reverse process occurs in mildew conidia. The above mentioned dyes stain the cytoplasm but not the intact vacuole. It is therefore evident that the vacuolar membrane of powdery mildew conidia is different from that of other cells in that it does not permit the passage of free ions.

Electron micrographs show the vacuolar membrane to be more dense to electrons than the membranes of the endoplasmic reticulum and the typical unit membrane structure seen in the endoplasmic reticulum has not been resolved in the vacuolar membrane.

The delimitation of the vacuoles into several separate
compartmentalized by a membrane of limited permeability provides a much more efficient water retaining mechanism than would be possible in a vacuole of a single cavity with usual membrane permeability. As a further insurance against depleted water supply, powdery mildew conidia germinate almost immediately after abscission so that they do not dry out before establishment on the host.

It is worthy of note that conidia of *E. polygoni* produce germ tubes within three hours of incubation on dry glass slides. These conidia have end walls which are considerably thinner than the side walls and could therefore be expected to lose water more rapidly than those of *S. macularis* which has an equally thick wall all around the cell. Conidia of *S. macularis* take an average of nine hours to germinate and some as many as twelve to fifteen hours at 20°C.

These findings, therefore, confirm those of Yarwood, Jhooty and McKeen and other workers that powdery mildew conidia contain water adequate for their germination at conditions of high water stress. They further show that the water is contained within vacuoles, which, along with the outer insulating of the cell wall, help to prevent loss of water from the cell.

However, it is not to be expected that the contents of the vacuoles is only water. Electron microscopy reveals that granular material is often present in the vacuoles.
In *S. macularis* and *E. cichoracearum*, this material is usually sparsely distributed but in *E. polygoni* it is quite dense. The fact that the osmotic pressure of the conidia is in the order of 14 - 19 atmospheres makes it evident that the vacuolar sap must contain soluble material of an osmotic nature. Several authors have reported the isolation of various compounds from plant cell vacuoles. According to Buvat (26) vacuoles accumulate various metabolic products in the form of true solutions of crystalloids or colloids. Matile (69) listed several enzymes that had been localized in vacuoles isolated from rootlets of corn seedlings. In a more recent publication (70) Matile and Wiemken reported the presence of hydrolytic enzymes in isolated vacuoles of yeast cells. In the case of corn the enzymes were bound to the membranes of the vacuoles whereas in yeast they were believed to be located in the vacuolar sap. On the basis of these findings Matile and Wiemken concluded that the yeast vacuole is a lysosome.

This author believes that the vacuoles of powdery mildews contain, in addition to water, important reserves of food and possibly of intermediate compounds which can be utilized in the synthesis of new cytoplasmic structures during germination.

As obligate parasites, powdery mildew conidia must store in their conidia all the food reserves, enzymes and
and other materials necessary for germination and subsequent growth, independent of an external food source until the young mycelium is successfully established upon the host. It is known that mildew conidia will germinate and produce long germ tubes on dry glass slides. They have never been successfully grown, however, on artificial media. As shown in this work the conidia contain surprisingly little cytoplasm, evidently less than 50% of the cell both by weight and volume. Yet, these conidia produce three to four germ tubes upon germination all of which are turgid and even after the mycelium is established, the conidium can still be seen to be unshrivelled.

It was pointed out that at germination most of the cytoplasm moves out of the conidium into the germ tube and that the conidium, now highly vacuolated, retains a nucleus and is cut off from the germ tube by a septum. Following this, new germ tubes are produced, all of which are supplied with cytoplasm from the conidium. This can only be possible if new cytoplasm is being synthesized from material in the vacuolated cell and the retention of a nucleus suggests that the parent conidium is still metabolically active. The vacuolar sap may, therefore, be considered as a solution of nutrient material or possibly a colloidal suspension from which the cell may draw needed nourishment and building material for continual synthesis.

In this connection, the previously mentioned association
of the myelinoid bodies with the vacuoles is of particular interest. The occurrence of myelinoid bodies in association with vacuoles or vesicles in fungi has been reported by several authors (23, 61, 67, 118). Their function has been variously interpreted as being concerned with the morphogenesis of mitochondria (Linnane et al, 1962), "the mobilization of cellular reserves" (Buckley et al, 1966), "mitochondrial role, membrane synthesis and nucleoid partitioning" (Zachariah and Fitz-James, 1967). Greenawalt (46) found these bodies most abundant in spores of Neurospora crassa when they were grown in a medium containing 10 - 20% sucrose. They were especially abundant during the early stages of germination. He noted that the myelinoid bodies were frequently associated with large vesicles within which varying amounts of electron-opaque material were contained.

Linnane and his co-workers claimed that these membrane systems contain some of the enzymes normally associated with mitochondria. In all the micrographs seen by the author, the myelinoid bodies enclose or partly enclose a central cavity transparent to electrons most often with dispersed electron-dense particles. One of Linnane's micrographs shows a vacuolated body projecting into the cell vacuole and containing very dense particles. This body could also be the basal end of a myelinoid body similar to those observed in the mildews and in other fungi.
Although one cannot say with certainty what the function of these myelinoid bodies is, it is likely that they have a synthetic function. The large number of folds that make up this system of membranes provide a greatly increased surface area for enzyme activity. The membranes are bathed by the vacuolar fluid and some micrographs show that the enclosed cavity is continuous with the vacuole. Also, the membrane of the myelinoid body is continuous with the vacuolar membrane.

Material from the vacuole may therefore pass through the myelinoid membranes along which synthesis of new material takes place to become incorporated in the cytoplasm with which the membrane is continuous. It is the opinion of the author that these bodies have a synthetic function.

Relevant to this point of view are observations on vacuole formation in developing mildew conidia and germ tubes. Formation of the vacuoles begin with the appearance of small electron transparent areas in the surrounding dense cytoplasm. Inside these clear areas are small, membrane-bound bodies with amorphous electron dense inclusions (Figs. 35 & 36). The vacuoles increase in size from the tip of the tube backwards and as they do so the enclosed membrane-bound bodies gradually disappear. The small vacuoles coalesce into larger ones and at some stages the vacuolar membrane appears to be incomplete. Possibly the
the electron-dense vesicles inside the vacuole contain enzymes which break down complex components of the cytoplasm into simpler compounds, some of which are utilized as sources of energy. Unused material may enter into solution or colloidal complexes to form the vacuolar fluid, and a membrane is formed around this fluid phase to delimit it from the more viscous cytoplasm.

It is possible that a similar predigestion of material takes place in the mature conidia preparatory to germination, since mildew conidia begin germination immediately after abscission. These xenospores, a term suggested by Gregory (45), have no resting period and must have everything ready to begin their new life at the time of dispersal. In this context, the conclusion of Matile (69) that "The vacuoles of higher plant cells represent organelles in which the processes of intracellular digestion take place" may have significance in the fungi as well.

The method of vacuole formation here described is similar to that suggested by Muhlethaler as reviewed by Buvat (26). According to these authors the vacuoles arise at the separation of an aqueous phase in the cytoplasmic ground substance and later become surrounded by the vacuolar membrane. The rapid utilization of food reserves with the accompanying increase in water of respiration during germination and germ tube growth provide the necessary conditions
for the development of vacuoles as described above.

Allen, in his review paper - "Metabolic Aspects of Spore Germination in Fungi" (8) - noted that there was as yet (1965) no information concerning the changes in spore constituents occurring in powdery mildew conidia. Also, that there was very little information concerning changes in fine structure during germination of airborne spores. It is hoped that this work will provide some useful information in both.

One surprising change reported here, occurring in mildew conidia during germination, was the accumulation of what appeared to be glycogen. Since this compound was not observed in unincubated conidia it would have to be synthethized early in the germination process. The presence of glycogen in fungi is of usual occurrence. Zaloka (119) reported that after osmic tetroxide and other usual cytological fixations, glycogen was unevenly distributed in bigger or smaller clumps in Neurospora hyphae. He also claimed that glycogen forms the main reserves of carbohydrate for endogenous respiration in fungi. In the mildews, particles resembling glycogen aggregates were occasionally seen in sections of hyphae but not in unincubated conidia. They were, however, quite abundant in germinated conidia.

A possible explanation is that the conidia utilize
their lipid reserves during the early stages of germination and convert their simple sugars to glycogen for storage. This could be later converted to glucose for the continued metabolic activity which has already been shown to continue in the conidia long after germination begins. Electron micrographs show that most of the lipid bodies in the conidia move out into the germ tube and the amount of lipid in the tube decreases as the germ tube elongates.

Allen (8) has shown that actively germinating sporangia of Phytoothora infestans show an increase in glycogen accumulation and that Blastocladiella spores utilize glucose rapidly and convert it to glycogen during the early stages of germination. The same author reported a decrease in lipids and an increase in carbohydrates occurring in spores of P. graminis during germination and indicates that during incubation of most spores fatty acids and polyalcohols were primarily used.

Another very obvious change occurring in germinating conidia is the marked increase in ribonucleoproteins. These tend to be most dense at the point of entry to the germ tube and in the germ tube itself. Accompanying this is an equally marked increase in the endoplasmic reticulum. This is not surprising as the synthetic processes of the cell are greatly increased at this stage.

Conidia that were fixed before the first nuclear
division show an aggregation of membranes and very dense cytoplasm next to the nucleus. These findings are of interest when compared with those of Cantino et al. (27) who found no endoplasmic reticulum in Blastocladiella spores and those of Moyer and Storck who as reported by Allen (8), think that the reticulum is lacking in young mycelia. Young mycelia of powdery mildews are rich in endoplasmic reticulum. There are also very definite changes in the number, sizes and shapes of mitochondria as previously noted. This is in agreement with the findings of Hawker and Hendy (52) and Hawker and Abbott (54) for spores of Rhizopus and Botrytis. There are strong evidences that the mitochondria multiply by fission. Dumbbell-shaped mitochondria were very frequently observed in incubated conidia. Pairs of partly separated mitochondria were common, some with extremely attenuated isthmuses, others with squared ends almost touching each other as if partitioned off by an invaginated membrane.

Evidence was also given that some mitochondria were formed from membranes of the endoplasmic reticulum swelling into loops and developing cristae from the inside of the loop.

Publications advocating both of these views on mitochondrial multiplication are found in the literature (54, 57, 67, 80, 91, 102). To express Robertson's view in his own words, "It now seems that mitochondria are formed when cytoplasm pushes into a cavity bounded by an internal
membrane, which then pinches off and separates from the continuous system." (91). Micrographs quite similar to that shown by Robertson in support of this view have been shown in this work on the mildews (Fig. 39). It is conceivable that if membrane-bound cavities derived from the endoplasmic reticulum, can pinch off into separate units, existing mitochondria can also pinch off to form new ones. The rapidity with which mitochondria increase in germinating conidia would also seem to support this explanation of their method of multiplication. Hawker (57) believes that, in *R. stolonifer*, new mitochondria are formed by the rounding and splitting off of the lobes of large convoluted forms. She cited Ekundayo as showing that anaerobic conditions prevented the division of mitochondria in germinating spores.

Moore (80) has shown that karyochorisis — the division of cells and cellular organelles by the invagination of their membranes — is a general phenomenon among living organisms. He produced evidence to show that nuclei, mitochondria and chloroplasts replicate by this method. His excellent electron micrograph of a mitochondrion of *Blastocladiella* showing centripetal invagination of the outer membrane to complete the division is convincing, and gives added support to the present findings. On the contrary, no evidence could be found in support of the view
that mitochondria were formed from myelinated membrane systems (67) or from granules in the cytoplasm which act as precursors or preformed subunits of mitochondria (50).

The three species of powdery mildew conidia here examined contain both mitochondria and endoplasmic reticulum during all the stages of their development and the evidences seem convincing that new mitochondria are formed from the proliferation of both of these units. The membranes that make up the endoplasmic reticulum are identical in structure to those that surround mitochondria. It should not, therefore, be surprising if these membranes give rise to mitochondria.

Another point of interest relative to germination is the formation of the germ tube. Electron microscopy shows that the germ tube is formed from the original spore wall, the inner layer of which stretches and thins out at the neck while the outer layer, obviously a newly secreted coat, becomes a thick protective layer at this delicately thin area of the germ tube wall. Allen (8) and Hawker (57) have shown that spores of species of *Rhizopus* and *Cunninghamhamella* and *Gilbertella persicaria* lay down a new inner wall which becomes continuous with the germ tube at germination. The spore wall of *Rhizopus* is single layered while that of *Cunninghamhamella* is double layered. Spores of
Botrytis cinerea also have a double layered wall but they do not form a new wall at germination. The germ tube is formed from the inner layer of the spore wall. This is also true of Penicillium frequentans.

Mildew conidia are not resting spores and their walls are identical with that of the mycelium, therefore it is not surprising that the layered structure of the wall does not change during germination. The wall at the tip of the germ tube always shows an amorphous, ill-defined structure and how elongation is achieved has not been determined. Also, since no water is absorbed during germination, the conidia do not swell as is general for germinating spores. The strong phototropic response of the germ tubes - particularly of E. polygoni - was peculiar in that the response was a positive one, whereas Carlile (29) claims that light causes negative phototropism of germ tubes by stimulating growth. He cited Jaffe and Etold, 1912, as showing that spores of B. cinerea, exposed to unilateral light, produced germ tubes from the far side of the spore. The opposite was found to be true in S. macularis and E. polygoni, conidia of which produced germ tubes on the illuminated side. However, Carlile did say that illumination of half the spore resulted in the germ tube being produced on the illuminated side. How this was done was not explained. It would be of interest to in-
vestigate this factor in mildew conidia.

Septation in fungal hyphae has long been used as one of the bases of taxonomy. The septum in the Ascomycetes is characteristically a simple iris-like ingrowth of the lateral walls, tapering off gradually toward a central pore. The septum as described here for three species of powdery mildews, is atypical. It is thickest in the centre and the pore is formed between two oppositely facing concavities in the centre of the septum. This is a step further in advance toward the more complex dolipore of the Basidiomycetes and if this type of septation is found to be unique among Ascomycetous fungi, it may be of significance in the phylogeny of the powdery mildews.

So far, the powdery mildews have been shown to differ from other fungi in a number of ways, and it is apparent that they differ even more significantly in the mode of division of their nuclei. There is, of course, no general pattern of nuclear division among fungi, although present trends in the literature seem to indicate that somatic nuclear division is amitotic in the majority of fungi. Yet, Robinow has clearly shown that mitosis occurs in *Basidiobolus rararum* (96) and several other authors describe some sort of mitosis to occur in other fungi with conventional metaphase plates and spindles (49, 109).
In 1964 Moore (80) proposed the term karyochoris is for the type of division he observed in *Cordyceps militaris* in which division occurs by invagination of the nuclear membrane. This is different from the process described by Robinow for members of the Mucorales in which the chromatin material separates into two groups inside the nucleus followed by elongation and constriction to effect division into two daughter nuclei.

Somatic nuclear division in the powdery mildews does not appear to follow any of these patterns. Although distinct strands of chromatin are clearly visible in stained material, a metaphase plate of separately paired chromosomes has not been observed, nor has an extra-nuclear spindle. Hence, this type of division cannot be considered conventionally mitotic. On the other hand division is not accompanied by invagination or constriction like the stretched-taffy effect frequently described for some fungi. The behaviour of the chromosomes during nuclear division varies from the pattern of classical mitosis in their constant attachment to the peripheral granule, their contraction into compact groups at metaphase and their separation at anaphase apparently without the aid of an extra nuclear spindle. The process appears to be somewhat similar to that described by Robinow and Caten (97) for *Asperillus nidulans*. Their report shows that mitosis begins
with the division of a granule similar to that described here and the development of a gradually lengthening fibre between the daughter nuclei. They suggested that the fibre is the equivalent of a mitotic spindle. However, in the mildews the chromatin material seems to be constantly attached to the peripheral granule. This fact was well illustrated by Harper; who observed this conspicuous granule in *Erysiphe* and *Phyllactinia* (48). He claimed that it constitutes a point of attachment for the elements of the nucleus and determines a definite polar organization on the part of the chromatin and of the nucleus as a whole. He showed that the chromosomes are attached to this body by achromatic fibres and that following division of this "central body" the two centres separate to opposite sides of the nucleus to form the poles of the spindle. Colson (32) also claimed that the first sign of spindle formation in *Phyllactinia* is the division of the small lateral granule.

The term lateral granule used here by Colson is the same as Harper's central body. The author avoids the use of the term central body since the same term is used by Robinow, Bakerspigel and other workers in reference to the nucleolus. Also, the term central body is inappropriate since this body always occupies a peripheral position somewhat opposite to the nucleolus. The term peripheral body, consequently, is suggested.
The presence of this peripheral body is not peculiar to powdery mildew nuclei. Bakerspiel (10) also observed a similar densely stained granule in nuclei of Neurospora crassa and he noticed that this body was usually seen to be doubled at the time of separation of the bar of chromatin, each half separating to a sister nucleus. He also quoted Singleton as saying "A tiny heterochromatic region can frequently be made out at one side of the nucleus with suggestions of chromosome strands extending from it". Careful study of other Ascomycetes may reveal a more general occurrence of this body. Its behaviour, as described by Harper and others and as observed in this study, strongly implies a role akin to that of a mitotic spindle i.e., that of equally distributing the chromatin material of the nucleus to the daughter nuclei.

Workers who have studied nuclear division in the ascus of other powdery mildews found conspicuous extra nuclear spindles. No such spindles were seen in the dividing somatic nuclei studied in this work. As previously noted, however, nuclear division in somatic fungal cells may differ from that obtained in the sexual stages. In two recent reports on nuclear behaviour on the vegetative hyphae of N. crassa Wilson (111) and Wilson et al (110) claimed that a spindle occurs only between separating chromatids.
Aist and Wilson (4) recently reported similar findings for *Ceratocystis fagacearum*. They found nuclear division in this fungus to be unlike classical mitosis with spindle fibres appearing only between separating chromatids. They also observed bars of chromatin material lining up at metaphase as was described for the mildews. Reference was made to the fact that the blocks of chromatin formed at metaphase were later seen to orient themselves on either sides of the nucleolar region and the electron micrograph shown in Figure 60 shows what appears to be fine spindle-like fibres radiating from a body appearing like the nucleolus.

Robinow (96) showed that the spindle in *Basidiobolus* originated from the nucleolus. The possibility therefore exists that an intranuclear spindle develops from the nucleolus and extends between the two chromatin masses thus pushing them apart. The previous separation of the chromosomes into two groups could have been effected by the division and subsequent migration of the two peripheral bodies to which the chromosomes are always attached, or independent movement of the peripheral bodies could accomplish both processes. Electron micrographs of anaphase—telophase could clarify this but attempts to obtain such micrographs were unsuccessful.

The rare occurrence of anaphase and telophase nuclei in specimens prepared for both light and electron microscopy
indicates that the final stages of division are exceedingly rapid. This is not unusual. Robinow (92) reported that in some Mucorales division is accomplished in two to four minutes and Moore (80) showed that metaphase or early anaphase in *Puccinia* was probably only a few seconds, not more than about one cell in 5000 - 10,000 having been found in this stage. The rapidity with which this final stage of division is accomplished tends to suggest that conventional mitosis is not involved. The reference made by Wilson (111) to the presence of a centriole in *N. crassa* is interesting. He observed that migrating nuclei have a head or centriole and a tail which is the nucleolus. The author described migrating mildew nuclei as having the peripheral granule at the leading end and the nucleolus at the opposite end (Fig. 66e). Evidently the body referred to by Wilson as the centriole in *N. crassa* is equivalent to the peripheral body of the mildews. Electron microscopy has shown the centriole of the cells examined to have a characteristic structure. No body having such a structure has been seen in any of the large number of sections of powdery mildews examined. It is, therefore, unlikely that a centriole is present in cells of these fungi.

According to Hashimoto et al (51) the retention of the nuclear membrane during cell division is characteristic of the Ascomycetes. This conclusion cannot be confirmed
in the case of the powdery mildews here examined. Light microscopy seems to indicate from the diffuse arrangement of the chromosomes at prophase that the nuclear envelope is not present and some electron micrographs show nuclei of germinated conidia with the nuclear membrane absent at least in part (Figs. 29 and 70).

With regard to the Giemsa-positive bodies located in the nucleolus it was pointed out that they form a part of the chromatinic structure of the nucleus. However, they have not been proven to be DNA positive. The reaction of the nuclei to the Feulgen stain was very slight. Histone tests were strongly positive and it is clear that these nuclei contain a high proportion of heterochromatin which seems to form a matrix into which the DNA-bearing material is embedded. It is therefore very likely that much of the nuclear contents which are stained with Giemsa and other nuclear stains and appear as chromosome strands, do not consist of DNA. These nucleolar bodies, as well as other deeply staining strands in the nucleus, may or may not be chromatinic in nature. They may contain a DNA core embedded in a heterochromatin matrix. Robinow (94) noticed that in Lipomyces lipofer, during the greater part of mitosis, the nucleolus remains in contact with one or two of the chromosomes.

Brandham (17) observed similar chromosome strands
embedded in the nucleolus of *Closterium siliqua*, two in haploid nuclei and four in diploids. He referred to them as nucleolar organizer chromosomes.

Buvat (26) stated that several cytologists recognized before electron microscopy that chromatinic masses, similar to chromocentres, remain applied against the nucleoli in resting nuclei. He went on to say that these nucleolar chromocentres were not genuine nucleolar organizers since it was shown by McClintock (1934) that they were not indispensable for the genesis of the nucleolus.

It has been pointed out in this study that in *E. cichoracearum* these nucleolar bodies are disconnected from the other chromatin masses and do not appear to form a part of the daughter nuclei. Evidently, these structures are heterochromatic masses and not entirely DNA.

In summary, the division of powdery mildew nuclei involves stages similar to those occurring in ordinary mitosis but lacking an extra-nuclear spindle and discretely resolvable chromosomes on the metaphase plate. Segregation of the chromosomes is achieved by the division and subsequent migration of a peripheral body to which the chromosomes are attached by heterochromatic strands. Metaphase groups are aligned parallel to the long axis of the cell and their separation is at right angles to it. This form of division shows some similarities to those described for other plant pathogenic fungal nuclei by Aist and Wilson (3, 4),
Brushaber et al (22) and by Robinow and Caten (97).

The frequent association of lipid bodies with the nucleus, as shown here, was previously reported by Buckley et al (24) as occurring in germinating spores of Rhizopus stolonifer. No explanation can be offered for this relationship.

With regard to the method of penetration of host cells by plant pathogenic fungi, opinions vary from total enzymic dissolution of both cuticle and cellulose wall (28, 113) to its being totally mechanical (88).

Between these two extremes are those who believe that penetration of the cuticle is mechanical whereas penetration of the cellulose layer is both chemical and mechanical (13, 34, 72). The author is of the latter opinion.

No evidence was found for chemical lysis of the cuticle. In 1937 Woodward (113) working on another powdery mildew, Podosphaera leucotricha, reported that chemical dyes showed an enlargement of the passage through the cuticle. Electron microscopy has not confirmed this in the case of the three species examined by the author. The passage made through the cuticle by the penetration peg was always of the same diameter as the peg. Also, there was no difference in electron density of the cuticle at the point of penetration and elsewhere.

Experiments by Brown and Harvey (21) showed that
extracted juice of parasitic fungi did not dissolve the cuticle of their hosts. Wood (112) asserted in 1960 that no one has demonstrated that plant pathogens are able to degrade the cuticle chemically. However, the same author cited indirect evidences that this is possible.

It is the opinion of the author that penetration of the cuticle of strawberry, clover and sunflower by powdery mildews is purely mechanical. Woodward's main 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 if penetration was purely mechanical. He could not find evidence of such attachment in the mildews (113). Corner also failed to find a method of attachment although he claimed that cuticular penetration was evidently mechanical (34).

Clear evidence has here been given that the mycelium of powdery mildews is firmly attached to the host cell by means of the mucilaginous coat that surrounds the hyphae (Fig. 37). Nour (83) also showed the effectiveness of this means of attachment in preventing conidia from being washed off their hosts by rain or direct watering.

That fungal hyphae can penetrate relatively hard substances by mechanical pressure was clearly demonstrated by Brown and Harvey (21) experimenting with artificial membranes, and Blackman and Welsford (13) demonstrated the
same process with *Botrytis cinerea* in *Vicia faba* leaves. The latter workers claimed that the mucilaginous coating held the spore to the leaf even before penetration. Pressure for penetration was achieved by the close attachment of the hypha to the leaf and by extension in length, being anchored at the basal end. Flentje (43) also 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 some hosts resulted in failure to infect the host. Here then, is a second important function of the mucilaginous sheath that surrounds the fungal wall. It provides the necessary attachment of the fungus to its host, so that adequate pressure can be developed for puncturing the cuticle of the host.

Further evidences indicate that chemical dissolution is involved in the penetration of the cellulose wall. The intense electron density of the wall immediately surrounding the penetration peg in contrast to the rest of the wall which is of low electron density, clearly indicates a difference in the chemical nature of the wall surrounding the peg. The diffuse pattern of the deeply stained area also suggests the diffusion of a chemical opaque to electrons. The production of additional wall material by the host to form a collar around the penetration peg may also
be considered as suggesting that chemical reactions are involved. Electron micrographs show that the collar is produced in advance of the penetrating peg indicating the diffusion of a stimulating substance which induces the host to react in this way. However, the possibility that such a callosity could have been formed as a reaction to wounding cannot be ruled out. Penetration of the cellulose layer is most likely both chemical and mechanical. The tortuous path often taken by the peg is indicative of the fact that some mechanical resistance is encountered, necessitating the application of pressure by the fungus.

Findings on the development and structure of the haustoria are in agreement with those of Smith (105) and McKeen et al (72). The finger-like processes arising from the ellipsoidal body do not arise exclusively from the ends in *S. macularis* as shown by Hirata (58) and by McKeen, Smith and the author (72) to be true for *E. cichoracearum*. However, in *E. polygoni* they do seem to grow only from the ends. The claim made by Dekhuijzen (37) that the central body of the haustorium of *Sphaerotheca fuliginea* is connected with the sheath membrane by finger-like protrusions is not supported by these findings. The finger-like projections are branches of the central body and are not connected with the sheath membrane.

Berlin and Bowen (11) reported that haustoria of *Albugo candida* do not contain nuclei. They attributed this
to the fact that the nucleus could not pass through the narrow penetration peg. The invalidity of this explanation is clearly shown in the powdery mildews, haustoria of which possess a conspicuous nucleus occupying about two-thirds of the central body. The nucleus must necessarily have migrated into the haustorial vesicle through the penetration peg, thus illustrating its capacity to stretch. The cutting off of the haustorium from the penetration peg by a septum converts the haustorium into an independent cell complete with its own nucleus. This nucleated cell should function more effectively in the active absorptive processes involved in feeding the fungus.

Berlin and Bowen (11) and Ehrlich and Ehrlich (40) showed the haustoria of *A. candida* and *P. graminis* respectively to be surrounded by an encapsulation of considerable thickness. In the powdery mildews examined here, no such encapsulation was observed. The haustoria were surrounded by a single membrane which was continuous with the host plasma membrane along the host wall, the host collar and around the haustorium. Small amounts of amorphous electron opaque deposits lined the enveloping membrane, chiefly on the fungal side, but sometimes on both sides. This material was always discontinuous, completely absent from parts of the membrane and never formed a discrete continuous capsule around the haustorium.
It is the authors opinion that the surrounding membrane is derived from the host plasma membrane and that the loose electron-dense deposits are waste products chiefly of fungal origin. This opinion is in agreement with that of Hirata and Kojima (59). These authors believe that the membrane of the sac is the invaginated host membrane but is transformed into a thicker membrane to serve as a protective device for the cell against the diffusion of the constituents of dead haustoria.

It is clear that some changes must take place in the host plasma membrane as it enlarges with the growing haustorium. That the membrane must either stretch or grow is evident from the fact that it is not pulled away from the cell wall, an inevitable result if stretching or growth did not occur. The fact that the enveloping membrane is no thinner than the plasma membranes and is most often thicker, suggests either that stretching did not occur or that additional material was added to the membrane. If the membrane grew around the haustorium, the stimulation to additional growth could also cause additional thickening of the membrane by the deposition of new material into it. Hirata and Kojima (59) claimed that thickening of the membrane is due to the addition of calcium.

The evidences provided here by electron microscopy do not support the view of Capperali (28) that the sheath is a secondary membrane of pectic material which is secreted
by the haustorium. Only one membrane is present and it is continuous with the host membrane. Nor can the author agree with Smith (105) that the sheath consists of disintegrated cellulose from the ingrowth of the host wall. If this were so, the deposits would all have to be inside the membrane, but they may be seen to be on both sides of it. Also, if they were derived from the ingrowing cell wall they should be absent from the end of the haustorium distal to the penetration peg. The opinion was expressed by Ehrlich and Ehrlich (40) that the sheath is either partly of fungal origin or is a special membrane produced by the host. Support for this claim was drawn from the fact that Hirata and Kojima succeeded in pulling the haustorium from the host with its sheath intact. Recently, Dekhuijzen (36) also isolated the intact haustorium and sac from cucumber leaves. This is an interesting fact but does not prove that the membrane is of separate origin from the host plasma membrane.

As was previously pointed out, the host membrane must enlarge considerably to accommodate the haustorium so that it forms a sac within a sac. The increased turgor pressure of the surrounding cytoplasm causes the membrane to invest the haustorial branches very closely, folding in and out between the branches. It would be difficult if not impossible to pull the haustorium out of this tightly-fitting investment without breaking the membrane at the narrow neck at
the collar. Even with high speed centrifugation as used by Dekhuijzen one would expect this to take place.

Whatever the nature or origin of the surrounding membrane, its structure must be such that it allows the passage of nutrient material from the host to the haustorial branches. If the membrane serves a protective role, free diffusion of material would be unlikely. The greater density of the fluid inside the haustorial sac in comparison with the surrounding cytoplasm, and the increased accumulation of cytoplasm inside the infected cell, denote that active absorption is taking place.

With regard to possible host resistance, the author is of the opinion that differences in cell wall thicknesses are important. Several authors have shown that excessively thick cuticle layers may prevent penetration by fungal parasites (63, 74, 88). 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. The author believes that the thickness, not only of the cuticle, but of the cellulose wall itself is of even greater importance. It was shown that the upper epidermal wall of F. ovalis is of similar thickness to that of F. chiloensis both of which are considerably thicker than the lower epidermis of F. ovalis. Of the two species of strawberry, F. ovalis is by far the more susceptible to S. macularis, but only on the lower epidermis.
The host was shown to react to penetration by laying down additional wall material in advance of the penetration peg. To overcome this reaction the fungus would have to penetrate more rapidly than the host builds up callus material. If this host reaction begins as soon as the peg punctures the cuticle and continues to do so in advance of the penetration peg, a very thick cell wall may delay penetration long enough for the germ tube to die before the peg emerges into the cell.

If powdery mildew conidia cannot use external sources of food or water at germination, their survival will depend upon early establishment upon the host. A thick cell wall could, therefore, be a serious barrier to infection, particularly as these fungi grow only under dry conditions. That the formation of such callosities prevent fungal infection in many plants, is shown by Akai (5). He showed that swelling begins to take place in the cell wall on pea leaves before the penetration of the cuticle by Botrytis cinerea. Hence, the advance protection as outlined above is possible. Akai also showed that some fungi fail to penetrate the callosity into the cell. This may easily be the reason for the failure of S. macularis to infect old leaves of either species of strawberry or the upper surface of the more susceptible species, F. ovalis.
Finally, the peculiar reaction of powdery mildew conidia to even very dilute solutions of the detergent sodium laurel sulphate may offer a clue to a simple method of control. In such a solution the conidia almost immediately lose their vacuolate structure and appear granular and structureless. They soon swell up with the absorption of water and burst. Conidia so treated never germinate. It is possible that the spraying of mildew infected plants with a dilute solution of this detergent may prove effective against infection.
LITERATURE CITED


