

# 11

## Developmental Ultrastructure of Hyphae and Spores

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### I. Introduction

Much of the ultrastructural work in the cereal rusts has its foundations in the excellent light microscope work of earlier times, particularly that of Allen (1923, 1928, 1932a,b, 1933a,b, 1934), Rice (1927), and Ruttle and Fraser (1927). Since the pioneering electron microscopic

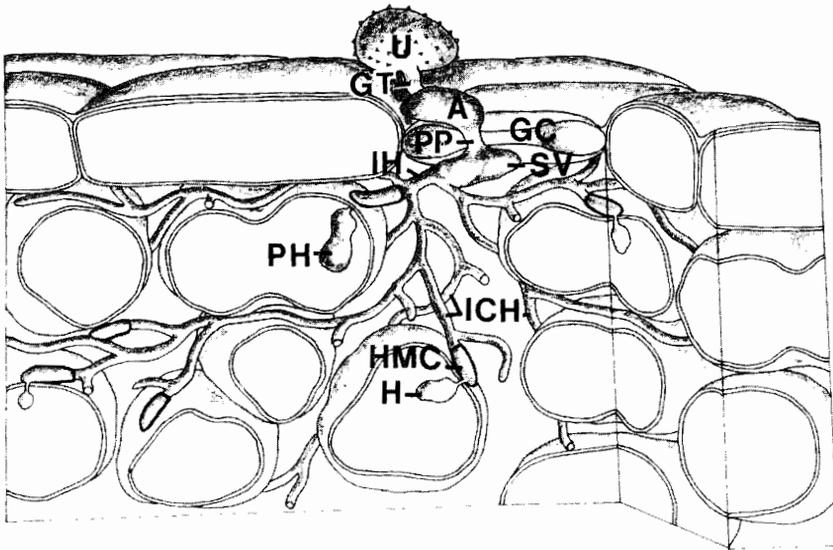
work in the rusts by Ehrlich and Ehrlich (1961, 1962, 1963), Moore (1963a,b), and Moore and McAlear (1961), there have been steady improvements in the processing of tissue, and a great number of details have emerged. A review by Littlefield and Heath (1979) has provided a comprehensive view of structure in the rusts. However, work since 1979 in ultrastructural cytochemistry has revealed additional details. In this chapter the basic fungal structures as they pertain to the cereal rusts will be reviewed and supplemented with cytochemical data where possible, with emphasis on research on *Puccinia coronata* or *P. graminis* f. sp. *tritici* conducted in our own laboratory. The following section deals primarily with dikaryotic parasitic growth. With a few possible exceptions, structural features of hyphae in axenic growth do not differ substantially from those in parasitic growth. Also, there is little in the literature to indicate that the hyphal protoplasts of the various rusts in either the dikaryotic or monokaryotic state are substantially different.

## II. Intercellular Hyphae

Following infection structure development and formation of the primary haustorium, intercellular hyphal growth begins with branching of the infection hypha proximal to the primary haustorium mother cell (Fig. 1). Figure 1 is a generalized illustration of an approximately 60-hr-old rust fungal colony in a cereal leaf. Growth and branching of the hyphae continue, with haustorium formation, until the mycelium has extensively ramified through an area of leaf tissue. Initial colony growth tends to occur to one side of the substomatal vesicle, thus the colonies are often somewhat asymmetric with respect to the point of infection.

### A. CYTOPLASMIC CONTENTS

The constituents of the mycelial protoplasts and their appearance vary depending on the physiologic state of the cell. Figure 2 is representative of a young hyphal cell near the edge of an advancing parasitic colony. The cytoplasm is typically dense with closely packed ribosomes, endoplasmic reticulum (ER), mitochondria, vacuoles (some with electron-dense inclusions), multivesicular bodies, and storage products either as lipid or glycogen. The majority of the ribosomes occur free in the cytoplasm, although where ER occurs, ribosomes may



**Fig. 1.** A diagrammatic representation of a young rust fungal colony, about 60 hr old, in a cereal leaf. The sequence of development is germination of the urediospore (U), formation of an appressorium (A) from the germ tube (GT) over a stomate, penetration past the guard cells (GC) via a penetration peg (PP), formation of a substomatal vesicle (SV), growth of an infection hypha (IH), formation of the primary haustorium (PH), then branching and growth of intercellular hyphae (ICH), and formation of additional haustoria (H). The haustorium mother cells (HMC) are indicated in bold outline. (Drawn by Dr. J. Chong.)

also be attached to it. The hyphal tip cell apex, despite the importance of this region in fungal growth, has not been studied in detail in any of the cereal rusts. However, the structure of this zone appears to be characteristic in a wide range of fungi. [See Howard (1981) for an analysis of the hyphal tip in *Fusarium acuminatum* using freeze-substitution, a method that appears to result in improved structural preservation of this fragile zone.] The most characteristic feature of the hyphal apex in the fungi is a zone generally free of organelles but containing an accumulation of apical vesicles, which are associated with hyphal tip growth. In the rusts these have been illustrated in hyphae of *Melampsora lini* (Coffey, 1975) and in germ tubes of *Uromyces phaseoli* var. *vignae* (Littlefield and Heath, 1979) and *Gymnosporangium juniperi-virginianae* (Mims, 1977). A possibly unique type of apical body in penetrating haustoria of *P. coronata* or *P. graminis* f. sp. *tritici* is discussed by Harder and Chong in Chapter 14 of this volume, Section IV,D,1.

Membranes organized into an easily recognized Golgi body have not been found in the rusts. In most septate fungi, functional Golgi sites probably exist mainly as single cisternae (Beckett *et al.*, 1974). It was suggested (Littlefield and Heath, 1979) that a similar single-cisternal arrangement applies to the rusts. The Golgi bodies in other organisms are derived from the ER (Morré and Mollenhauer, 1974); thus it would be difficult to differentiate regions of smooth ER that may have specialized Golgi function.

Microbodies are typically found in the cytoplasm of rust fungal hyphae. The distribution, morphology, and function of microbodies in plant pathogenic fungi have been reviewed by Maxwell *et al.* (1977). The term *microbody* refers to small cytoplasmic bodies, approximately 0.2–1.5  $\mu\text{m}$  in diameter (Maxwell *et al.*, 1977), bound by a single membrane. They may contain either amorphous or crystalline substances, which are concentrations of enzymes. Catalase is a common constituent of microbodies of higher organisms, and the DAB test for catalase (Frederick and Newcomb, 1969) is frequently used to identify microbodies.

In the rusts, microbodies of either amorphous or crystalline contents may be found (Fig. 16). They are most frequently associated with septa, although they may also be found elsewhere in the cytoplasm. Although they conform to a morphological definition of microbodies, tests for catalase in the crystal-containing microbodies of *Puccinia helianthi* (Coffey *et al.*, 1972) or amorphous-content microbodies of *U. phaseoli* (Mendgen, 1973) have been negative.

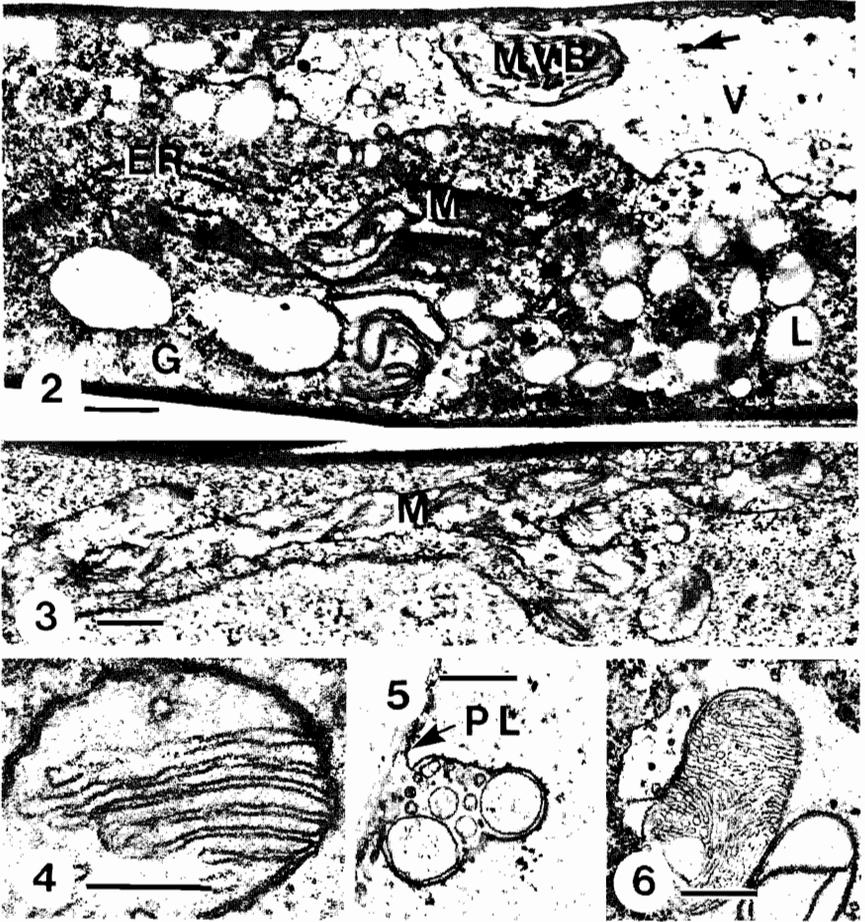
The mitochondria in the intercellular hyphal cells are irregularly shaped, generally filiform, and may be lobed or branched (Fig. 3). Where series of sections are available to trace the conformation of mitochondria, many of those that appear as single bodies in individual sections are actually lobes or branches of much larger structures. This type of mitochondrial structure is reminiscent of the single giant, branched mitochondrion per cell of the yeast *Saccharomyces cerevisiae* (Hoffmann and Avers, 1973). However, series of sections through the cells show that there are at least several mitochondria per cell in the rusts.

The cristae normally occur as an invagination of the inner membrane of the mitochondrial envelope and are typically arranged in parallel platelike stacks (Fig. 4), which is characteristic of most higher fungi. This arrangement of cristae has been consistent in all growth phases of the cereal rusts examined. The matrix of the mitochondria is variable in electron density and may contain mitochondrial ribosomes. Coffey *et al.* (1972) reported considerable variation with respect to

density of the matrix, amorphous inclusions, and conformation of cristae of mitochondria of *P. helianthi* or *M. lini*, depending on location (haustorial or hyphal) or whether growth was parasitic or axenic. These variations have not been noted with consistency in any of the cereal rusts except for conformational changes in the haustorium mother cells (see Harder and Chong, Chapter 14, this volume).

Microtubules are common components of rust fungal cytoplasm. They are most frequently encountered where there appears to be movement of organelles, such as locations where there is hyphal branching or where there are pseudosepta (see Section II,C,2) around which organelles are in an apparent stage of migration (D. E. Harder, unpublished). In an analysis of organelle movement in *U. phaseoli* var. *vignae*, I. B. Heath and Heath (1978) indicated that microtubules were most commonly associated with nuclei and mitochondria, and that the microtubules were involved in the control of the position of these organelles. The latter conclusion was strengthened through experiments using antimicrotubule agents (Herr and Heath, 1982). In *U. phaseoli* var. *vignae*, most of the microtubules in the hyphae were located in the peripheral region of the cytoplasm, were oriented in the direction of cytoplasm movement, were usually less than 2  $\mu\text{m}$  long (although some were up to 8  $\mu\text{m}$  long), and were probably anchored to microfilaments in the cytoplasm. Although a similar detailed analysis has not been conducted in any of the cereal rusts, observations of *P. coronata* and *P. graminis* f. sp. *tritici* (D. E. Harder, unpublished) indicate similar associations with organelles and orientation of microtubules. For a more detailed discussion of fungal microtubules see Staples and Macko, Chapter 9, this volume.

Various configurations and aggregations of membrane-bound vesicles are frequently encountered in hyphal cells. These conform to the definition of a lomasome (Moore and McAlear, 1961), where the vesicles occur between the fungal wall and plasmalemma (Fig. 5). However, no function has been ascribed to these structures, and they have gained little prominence in the recent literature. Perhaps they are stress-related artifacts of preparation procedures. A second type of multivesicular body occurs within the cytoplasm, and these are usually an aggregation of tubules or vesicles within a membrane-bound body (Fig. 6), or they consist of concentric rings of membrane that resemble myelin figures. Again, no function for these bodies is known, and their existence in living cells is also not certain. However, similar membrane configurations have frequently been found near the base of haustorial bodies (J. Chong and D. E. Harder, unpublished). Coffey *et al.* (1972) considered similar bodies in *M. lini* to be artifacts, but they



Figs. 2–6. Some cytoplasmic components of intercellular hyphae of *Puccinia coronata*. ER, Endoplasmic reticulum; G, glycogen; L, lipid; M, mitochondrion; MVB, multivesicular body; PL, plasmalemma; V, vacuole. (All figures are from D. E. Harder, unpublished.) Fig. 2. A hyphal cell from near the colony edge, which is representative of the appearance of the protoplast of this type of active, growing cell. The arrow points to electron-dense (probably polyphosphate) granules. ( $\times 17,300$ ; bar,  $0.60\ \mu\text{m}$ ). Fig. 3. An elongated, branched, and lobed mitochondrion ( $\times 18,500$ ; bar,  $0.50\ \mu\text{m}$ ). Fig. 4. A parallel array of platelike mitochondrial cristae ( $\times 8000$ ; bar,  $0.20\ \mu\text{m}$ ). Fig. 5. A multivesicular body located between the plasmalemma and hyphal cell wall, defined as a lomasome ( $\times 25,000$ ; bar,  $0.40\ \mu\text{m}$ ). Fig. 6. A multivesicular body located within the cytoplasm ( $\times 22,100$ ; bar,  $0.45\ \mu\text{m}$ ).

have also been seen in freeze-substitution preparations (D. E. Harder and K. Mendgen, unpublished), thus they may have a functional role, perhaps in the synthesis of plasma membranes. A third type of multivesicular body is a group of small vesicles enclosed within a large vesicle. These are most frequently found in apparently physiologically active cytoplasm, and they aggregate in particular near the poles of mitotic nuclei (see Fig. 12). No specific function for these bodies is known, although they resemble the multivesicular bodies in *Mucor rouxii*, which were reported to resemble chitosomes (Bracker *et al.*, 1976).

Large vacuoles are the most prominent in older cells of the mycelium, which presumably reflects the loss of synthetic activity of these cells. However, in the cytoplasm of young active cells there are also smaller vacuoles that frequently contain electron-dense inclusions (see Fig. 2). In haustoria or haustorium mother cells of *P. coronata*, similar inclusions were concluded to be composed mainly of polyphosphate (Chong, 1981). However, Heath and Heath (1979) found no phosphate in similar-appearing inclusions in "vacuole precursor vesicles" in infection structures of *U. phaseoli* var. *vignae*, although possibly the phosphate was extracted during processing. The latter vesicles were considered (Heath and Heath, 1979) to be involved in one of two pathways leading to vacuole formation.

## B. NUCLEI AND NUCLEAR DIVISION

### 1. Interphase Nuclei

*a. Morphology.* The dikaryotic hyphal cells of the cereal rusts usually contain two roughly oval-shaped nuclei of somewhat variable diameter. In the intercellular hyphae the nuclei exist in the "expanded" form (*sensu* Savile, 1939). The chromatin is typically dispersed and is not visible in the electron microscope. Each nucleus contains a prominent nucleolus.

There are exceptions to the normal oval-shaped nuclei. In some axenically cultured (Fig. 7) and parasitic hyphal cells, elongated dumbbell-shaped nuclei may be found. These show no evidence of mitosis and are frequently associated with pseudosepta (see Section II,C). Observations of multinuclear cells in the hyphae of the rust fungi may in part be due to the halves of the dumbbell forms appearing as discrete nuclei in the light or electron microscope.

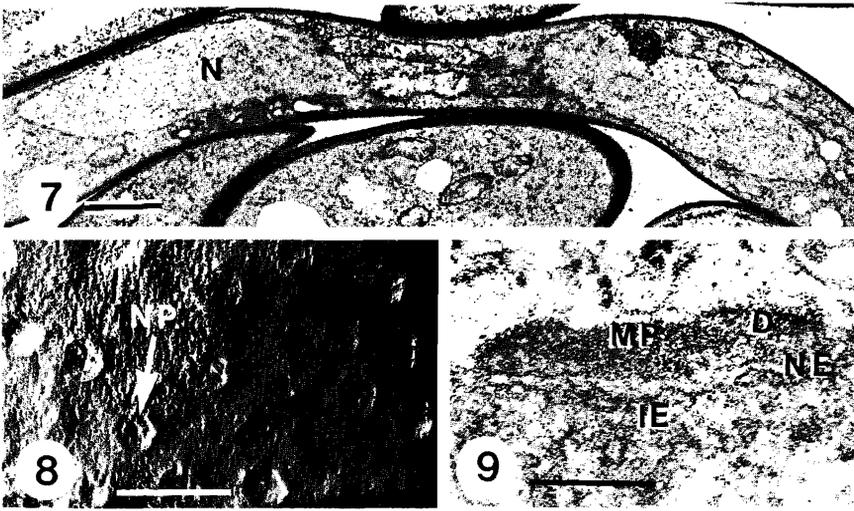
*b. Nucleoli.* In physiologically active cells the nucleoli are prominent, occupying up to 60% of the nuclear volume (Harder, 1976a). In

parasitic growth of those cereal rusts examined, the nucleoli are structurally typical of those of most eukaryotic organisms. The nucleolar matrix is composed of fibrillar and granular regions, interspersed with lacunar spaces. The lacunae appear as meandering channels through the nucleolus (Harder, 1976a), and these are continuous with the nucleoplasm and a larger central lacunar space. In axenic culture the nucleoli are typically more compact and lack the clear differentiation of fibrillar and granular regions. The granular component is generally more prominent (also see Manocha, 1971). The granularity of the nucleolus generally reflects synthetic activity (Smetana and Busch, 1974), which may be of relatively greater importance in axenic culture than in parasitic growth.

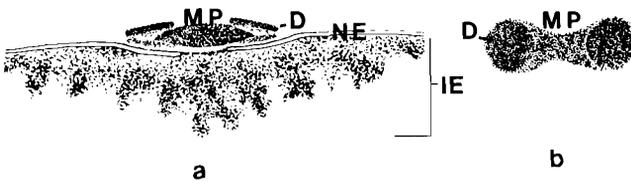
*c. The Nuclear Envelope.* The nuclear envelope in the rusts is consistent with that of other eukaryotic organisms; it is composed of a double membrane, the outer of which is continuous in places with the ER (*P. coronata*; D. E. Harder, unpublished) and contains nuclear pores. The freeze-etch preparation in Fig. 8 shows the nuclear pores of *P. coronata* to be complex structures, with strands of material radiating from a central granule to the pore boundary. In cross section the pores measure about 65 to 75 nm in diameter. The nuclear pore structure in the rusts appears to be consistent with the model of pore structure proposed for higher plants (Gunning and Steer, 1975).

*d. Nucleus-Associated Organelle.* A characteristic body, referred to mainly as the nucleus-associated organelle (NAO) (Girbardt and Hadrich, 1975) or spindle-pole body (SPB) (Aist and Williams, 1972), is associated with the rust fungal nuclei. Structurally this body is relatively consistent throughout the Uredinales and is a constant feature of nuclei at all growth stages of the rust fungi. Although NAO and SPB are used synonymously in the current literature, the use of either term has functional implications. The only known function of this body is as a microtubule organizer during nuclear division (McLaughlin, 1981), although Heath (1981) has argued for other possible functions. Until the latter aspect is resolved, either the SPB or NAO designation is equally valid. The term NAO, although not necessarily favored over SPB, will be used here to remain consistent with recent reviews (Heath, 1978, 1981; Littlefield and Heath, 1979).

An interpretation of the structure of the NAO of *P. coronata* is illustrated in Fig. 10 [for illustrations of other rusts see Heath and Heath (1976) and O'Donnell and McLaughlin (1981c)]. A longitudinal perpendicular section through a NAO of *P. coronata* is shown in Fig. 9.



**Fig. 7.** A nonmitotic, dumbbell-shaped nucleus (N) in an axenically cultured hypha of *Puccinia graminis* f. sp. *tritici* ( $\times 9200$ ; bar,  $1.10 \mu\text{m}$ ). [From Harder, 1976b. Reproduced by permission of the National Research Council of Canada.] **Fig. 8.** A freeze-etch replica of a nuclear envelope of *Puccinia coronata*. A central granule is evident in most of the nuclear poles (NP), and the granules are joined to the pore margins by threadlike processes ( $\times 70,000$ ; bar,  $0.21 \mu\text{m}$ ). (From D. E. Harder, unpublished.) **Fig. 9.** An interphase nucleus-associated organelle (NAO) of *Puccinia coronata*. A disk (D) lies at an inclined angle on either side of a middle piece (MP). An associated bilayered intranuclear element (IE) subtends the NAO inside the nuclear envelope (NE) ( $\times 85,500$ ; bar,  $0.20 \mu\text{m}$ ). (From Harder, 1976a. Reproduced with permission of the National Research Council of Canada.)



**Fig. 10.** A diagrammatic interpretation of the side [a] and top [b] views of the interphase nucleus-associated organelle of *Puccinia coronata*. D, Disk; MP, middle piece; IE, intranuclear element; NE, nuclear envelope. The broken line across the pore in the NE indicates that this pore may or may not be present.

The NAO characteristically lies in a depression of the nuclear envelope. An apparent pore in the nuclear envelope, located centrally underneath the NAO, has been observed in several *Puccinia* spp. (Harder, 1976a; Wright *et al.*, 1978), but this has not been seen in other studies (Heath and Heath, 1976; O'Donnell and McLaughlin, 1981c). This apparent pore may be due to sensitivity of this region of the nuclear envelope to tissue-processing procedures, or it may represent a particular stage of the nuclear cycle. The interphase NAO basically consists of two roughly circular, probably several-layered disks lying at an inclined angle on a middle piece. The tapered ends of the middle piece are inserted into the layers of the disks. The disks consist of an electron-dense upper layer and one or more diffuse lower layers. The disk layers become more distinct during mitosis (Fig. 12). A bilayered hemispherical structure subtending the NAO occurs in the nucleoplasm. This structure, designated the intranuclear element (McLaughlin, 1981), consists of an amorphous region immediately inside the nuclear envelope, subtended by a zone of loosely organized strands of material. In several instances a thread-like connection has been observed to extend from the latter zone to the nucleolus (Harder, 1976a). A function for the intranuclear element has not been established.

## 2. Mitosis

In the cereal rusts, mitosis has been studied ultrastructurally in only two species (*P. coronata*, Harder, 1976a,b; *P. striiformis*, Wright *et al.*, 1978), thus the picture of mitosis in these rusts is sketchy. Heath (1978, 1980) has reviewed mitosis in fungi, including the Uredinales.

As a prelude to mitosis in the rusts just mentioned, the nucleus becomes deformed and variable portions of the nucleolus, along with some of the nucleoplasm, are ejected into the cytoplasm (Harder, 1976a; Wright *et al.*, 1978). Similar conclusions were reached for other rusts through light microscopy (Craigie, 1959; Saville, 1939). However, Heath and Heath (1976) indicated that in *U. phaseoli* var. *vignae*, nucleolar ejection was later, beginning during anaphase and completed by telophase. The timing of nucleolar ejection may be variable. Figure 11 illustrates a nucleus of *P. coronata* in metaphase, and serial sections had shown the adjacent nucleolus to be completely detached from the parent nucleus. Nucleoli in a stage of ejection, but still attached to the nucleus, have been found no later than early metaphase (D. E. Harder, unpublished). Thus in *P. coronata*, nucleolar ejection appears to be completed by metaphase. Regardless of the timing of nucleolar ejection, the portion of the nucleus involved in mitosis is smaller than the normal interphase nucleus, and the reduction in size appears to be

brought about largely by expulsion of the nucleolus, along with variable amounts of nucleoplasm.

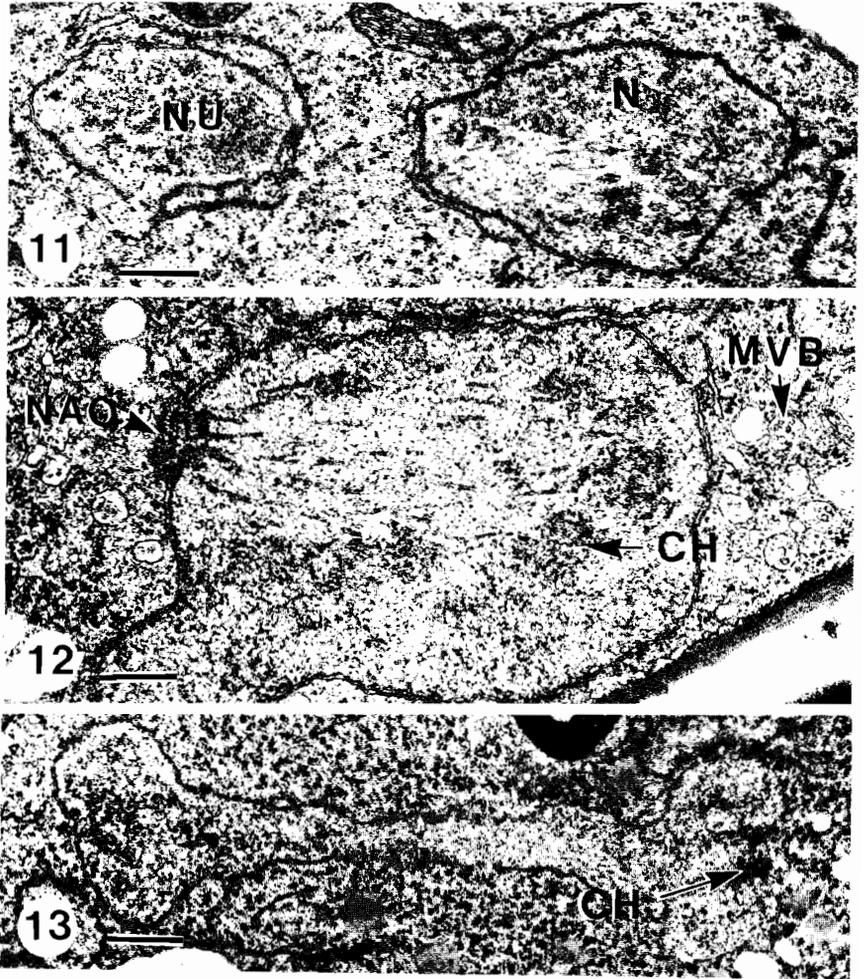
The onset of mitosis is indicated by separation of the disks of the NAO, these disks becoming positioned at the poles of the mitotic spindle [*U. phaseoli* var. *vignae*, Heath and Heath, 1976]. Division of the NAO has not been traced in the cereal rusts, although it presumably is similar to that in *U. phaseoli* var. *vignae*. In *P. coronata* the mitotic polar disks are enlarged and the disk layers are more distinct as compared to their interphase state. During metaphase, a number of multivesicular bodies aggregate in the cytoplasm adjacent to either mitotic pole (Fig. 12). Despite the consistency of the occurrence of these bodies (see also Heath and Heath, 1976; Wright *et al.*, 1978; O'Donnell and McLaughlin, 1981a,b,c), no clear function for them has been established (see also Section II,A).

The mitotic spindle of the rusts contains both chromosomal and pole-to-pole tubules (most clearly documented for *U. phaseoli* var. *vignae*, Heath and Heath, 1976). During metaphase the chromosomes become somewhat condensed, but they are not sharply contrasted in the electron microscope. There is no condensation of chromosomes into a metaphase plate; rather, they become arranged around the periphery of the spindle (Fig. 12). In the cereal rusts clearly identifiable kinetochores have not yet been found. However, the chromosomes are presumably attached to the spindle microtubules at a kinetochore-equivalent region. At telophase, longer, straighter tubules can be seen that pass from the NAO into the constricted portion of the nucleus [Harder, 1976b]. These are the pole-to-pole tubules, which probably function to push the halves of the dividing nucleus apart.

Telophase is marked by elongation of the nucleus and constriction in the middle to assume a dumbbell form (Fig. 13). The chromatin has aggregated at the poles around the periphery of the spindle. At all stages of mitosis until telophase, the nuclear envelope remains intact. At late telophase the central portion of the nuclear envelope together with the spindle tubules appear to disintegrate to allow formation of the daughter nuclei.

### C. CELL WALLS AND SEPTA

The hyphal walls of the rusts appear to be bilayered [Littlefield and Heath, 1979], although this is not apparent in most preparations for electron microscopy. Differentiation of layers within the wall is most evident at the point of septation (see Fig. 16), where the electron-translucent middle septal lamella ends in a discrete line along the



**Figs. 11–13.** Mitosis in *Puccinia coronata*. [From Harder, 1976b. Reproduced with permission of the National Research Council of Canada.] Fig. 11. A nucleolus [NU] that has completely separated from the metaphase nucleus [N] ( $\times 20,600$ ; bar,  $0.50 \mu\text{m}$ ). Fig. 12. A metaphase nucleus. The chromosomes (CH) are arranged around the periphery of the spindle, the tubules of which originate in the nucleus-associated organelle (NAO). Note the multivesicular bodies (MVB) in the cytoplasm adjacent to either mitotic pole ( $\times 27,500$ ; bar,  $0.36 \mu\text{m}$ ). Fig. 13. A dumbbell-shaped telophase nucleus. The chromatin (CH) has aggregated at either pole, and the nuclear envelope appears to be partially disorganized in the middle of the constricted portion of the nucleus ( $\times 14,700$ ; bar,  $0.68 \mu\text{m}$ ).

outer layer of the periclinal wall and an inner wall layer is continuous with the septal wall layers. At times a third but less discrete outer layer is evident, which has been interpreted as a covering, with possibly protective or other functions (see Littlefield and Heath, 1979). This layer is also continuous around the haustorium mother cell and apparently is involved in adhesion of the haustorium mother cell to the host wall. This material is removed after protease treatment (see Harder and Chong, Chapter 14, this volume, Section IV,E), indicating a proteinaceous content.

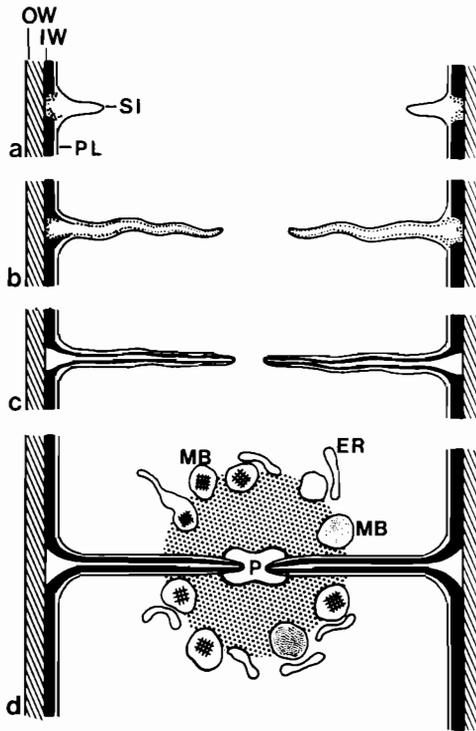
The hyphae of the rusts are compartmentalized by cross walls, or septa. Two major types of septa have been identified in the rusts; the typical hyphal septa that normally form following conjugate nuclear division, and more unusual "pseudosepta." These are discussed in turn in the following subsections.

### 1. Typical Septa

The formation and structure of septa is shown diagrammatically in Fig. 14. The first indication of septum formation is an invagination of the plasmalemma (the septal initial), with an electron-lucent zone appearing within the invagination and extending partially into the inner wall layer (Fig. 14a). Septal growth continues by centripetal invagination of the plasmalemma, accompanied by deposition of wall material within the invagination (Fig. 14b). Following invagination of the plasmalemma, the wall material condenses and two electron-dense lamellae form within the invagination. These lamellae are continuous with the inner layer of the periclinal wall and are separated along the length of the septum by an electron-lucent zone, to form two independent walls (Figs. 14c, 17). Near the centers of perforate septa the walls taper to a point to form the periphery of the central pore.

The pores of mature septa in the Uredinales normally have associated with them a characteristic structure, the *septal pore apparatus* (see Coffey *et al.*, 1972; Harder, 1976b; Heath, 1975). The pore apparatus consists of a membranous diaphragm that bounds both sides of the pore and contacts the plasmalemma somewhat beyond the apices of the septal walls to form a pulley-like shape (Figs. 14d, 15). On either side of the pore there is usually an organelle-free hemispheric zone of diffuse material (Figs. 14d, 16). Microbodies containing either crystalline inclusions or amorphous material (Figs. 14d, 16) occur around the periphery of the diffuse zone. The pores are often occluded with an electron-dense substance (Fig. 16). Where the occlusion occurs, it acquires the pulley shape of the membranous diaphragm around the pore.

In the intercellular hyphae of *P. coronata* the diameters of the septal



**Fig. 14.** A diagrammatic representation of the formation of a typical septum in a rust fungus. (a) Initially the plasmalemma (PL) invaginates to form a septal initial (SI), and the inner layer of the hyphal wall (IW) dissolves at the SI. The outer wall layer (OW) remains intact. (b) The septum then grows inward centripetally, and wall material is deposited within the invaginated plasmalemma. (c) The septum has completed inward growth, leaving a central pore. The wall material has formed two electron-dense lamellae that are continuous with the inner layer of the longitudinal hyphal wall. (d) A mature septum with septal pore apparatus. A diaphragm, which in cross section is pulley-shaped, surrounds the central pore (P). A zone of amorphous material occurs on either side of the pore, which in turn is surrounded by microbodies (MB) that contain either crystalline (crosshatched) or amorphous (dotted) material. The microbodies are probably derived from the endoplasmic reticulum (ER). [Drawn by Dr. J. Chong.]

pores vary, ranging from 23 to 66 nm. However, in the haustorium mother cell septum the pore is much smaller, about 9.5 nm in diameter (Fig. 18). Smaller diameter pores are also found in the septa delimiting the base of spores (see Sections V,B and VI).

## 2. Pseudosepta

The pseudosepta (Ehrlich *et al.*, 1968) are characterized by the occurrence of all layers of the longitudinal wall across the septum (see Fig.

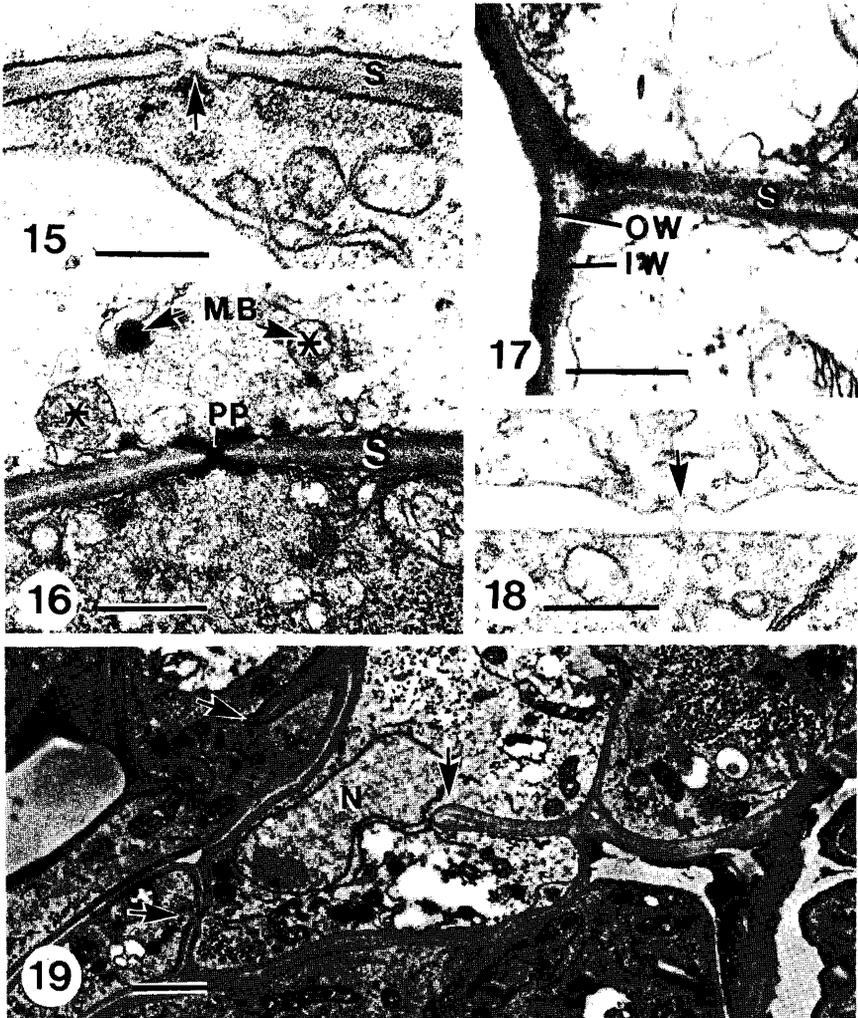
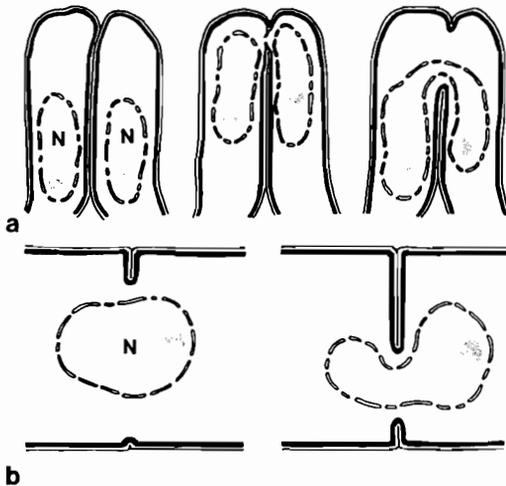


Fig. 15. A nonoccluded septal pore (arrow) in a hyphal septum (S) of *Puccinia coronata*. Note the pulley-shaped diaphragm that surrounds the pore ( $\times 60,000$ ; bar,  $0.25 \mu\text{m}$ ). (From Chong, 1981.) Fig. 16. A hyphal septum (S) of *Puccinia coronata* with septal pore apparatus. In this septum the central pore is occluded with a pore plug (PP). Note the diffuse amorphous zone on either side of the pore and the microbodies (MB) with either crystalline (densely staining) or amorphous (asterisk) contents ( $\times 33,600$ ; bar,  $0.45 \mu\text{m}$ ). [From D. E. Harder, unpublished.] Fig. 17. Part of a mature hyphal septum (S) of axenic *Puccinia graminis* f. sp. *tritici*. Note the continuity of the septal lamellae with the inner layer (IW) of the hyphal wall. The outer wall layer (OW) is continuous across the end of the septum ( $\times 32,000$ ; bar,  $0.50 \mu\text{m}$ ). (From D. E. Harder, unpublished.) Fig. 18. A septal pore (arrow) in a haustorium mother cell septum. The pore is smaller than those in intercellular hyphae, and there is no pore apparatus ( $\times 52,900$ ; bar,  $0.28 \mu\text{m}$ ). (From Chong, 1981.) Fig. 19. A number of cells of *Puccinia coronata* near the base of a telial sorus, which are compartmentalized by pseudosepta (arrows). Note the nucleus (N) associated with one of the septa ( $\times 8200$ ; bar,  $1.20 \mu\text{m}$ ). (From D. E. Harder, unpublished.)

20), an acentric pore of variable diameter, and absence of a pore apparatus. They have also been designated as partial septa (Littlefield and Bracker, 1971a) or infolded-wall septa (Rijkenberg and Truter, 1975). Figure 19 shows several hyphal cells near the base of a teliosorus of *P. coronata* to be partially compartmentalized by pseudosepta.

The pseudosepta could arise either through an infolding of the longitudinal hyphal wall or through dissolution of a section of the walls of anastomosing hyphae. Structurally it is not easily determined if the ends of the septa are the result of growth or degradation. Figure 20 illustrates the latter two possible modes of development of these septa.

Of particular interest is the possibility of anastomosing hyphae. The pseudosepta are especially prominent in the layer of multinucleate cells near the aecial base of *P. sorghi* (Rijkenberg and Truter, 1975), and are also common in the closely packed pseudoparenchymatous cells at the bases of sori. These conditions would provide the greatest opportunity for hyphal anastomoses. The pseudosepta also occur, but to a lesser extent, in hyphae of axenic growth or in hyphae near the leading edge of colonies (D. E. Harder, unpublished). Frequently associated with pseudosepta are nuclei in an apparent stage of migration (Fig. 19). These nuclei are usually in an elongated dumbbell form and may have two nucleoli (D. E. Harder, unpublished). These configurations suggest



**Fig. 20.** Illustration of two possible modes of pseudoseptum formation. (a) The common wall between two anastomosing hyphae partially dissolves, allowing mixing of the cell contents of both hyphal cells and possible fusion of nuclei (N). (b) Asymmetric invagination of the longitudinal walls of a hyphal cell to form a pseudoseptum. (Drawn by Dr. J. Chong.)

hyphal anastomoses and possible nuclear fusions. A suggested mechanism for providing genetic variation in the rusts is somatic recombination. Although this concept has not gained very wide experimental support, there are a number of reports in the literature that indicate the operation of such a mechanism. The previous observations may provide a structural basis for somatic recombination.

### III. Pycnia

The pycnium is the structure in which the gametes of the rust fungi are produced. Except for *Puccinia striiformis*, *P. kuehnii*, and *P. melanocephala*, in which the sexual stage is unknown, the cereal rust discussed in these volumes are macrocyclic heteroecious fungi, in that their sexual stage occurs on an alternate host. Following teliospore germination and meiosis, infection of an alternate host by haploid basidiospores results in the establishment of a monokaryotic hyphal colony, from which the pycnia arise. The monokaryotic colonies appear essentially similar to the dikaryotic colonies except for a lesser preponderance of haustoria relative to hyphal growth in the monokaryotic state (see Harder and Chong, Chapter 14, this volume, Section VII).

#### A. MORPHOLOGY

Hiratsuka and Cummins (1963) differentiated 11 types of pycnial structures in the rusts. Of these, the cereal rusts are represented by their type 4: a subepidermal, determinate structure having a strongly convex hymenium (i.e., flask-shaped) and bounded by well-developed periphyses. An individual pustule derived from a single basidiospore may contain a number of pycnia [*P. graminis* f. sp. *tritici*, Craigie, 1927b]. Multi-pycnial infections have also been observed for *P. coronata* (D. E. Harder, unpublished). The morphology of a typical pycnium is illustrated in Fig. 21.

#### B. CELL TYPES

Lining the base of the pycnium is a closely packed layer of *pseudo-parenchymatous* (= hymenial) cells. These cells give rise to the *pycniosporophores*, *paraphyses* (= periphyses, or sterile hyphae), and

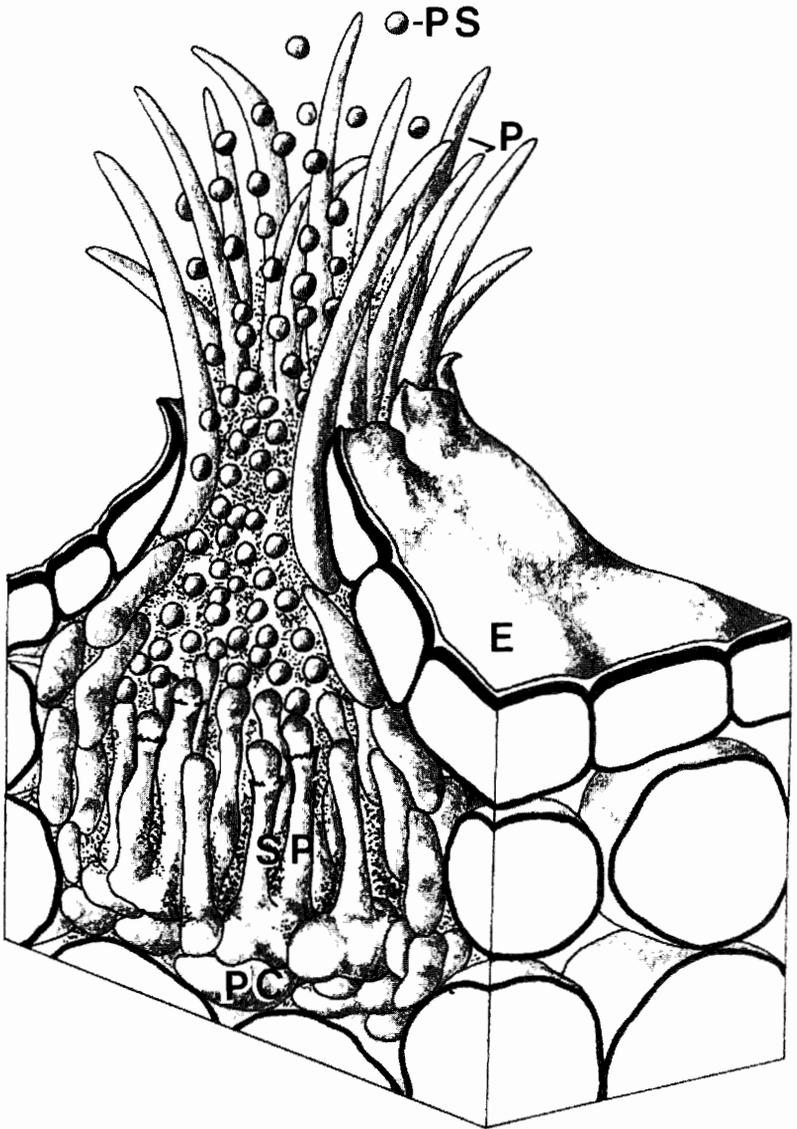


Fig. 21. A diagrammatic representation of a pycnium of *Puccinia coronata*. A layer of pseudoparenchymatous cells (PC) lines the base of the pycnium, from which are derived the pycniosporophores (SP) and the paraphyses (P). The pycniospores (PS) are produced by the pycniosporophores. E, Host epidermis. [Drawn by Dr. J. Chong.]

probably the *flexuous* (i.e., receptive) *hyphae*. The flexuous hyphae are not illustrated in Fig. 21 because of insufficient structural information. The pycniosporophores produce the *pycniospores*. Interspersed among the pycniosporophores are sterile cells of indeterminate origin or fate. These cells are marked by dense protoplasts and variously shaped electron-lucent inclusions; see Section V,A for a further description of this type of cell.

Each mononucleate pseudoparenchymatous cell may give rise to one or more pycniosporophores. The pycniosporophores in turn may be branched, forming a candelabra-like structure. The pycniosporophores are uninucleate and elongated, forming a palisade of closely packed, somewhat intertwined cells near the base of the pycnium. There are no unusual features that distinguish the protoplasts of these cells from most other hyphal cells of the monokaryotic thallus. The paraphyses are elongated robust cells that arise from the pseudoparenchymatous cells at the sides of the pycnial cavity. In a mature pycnium the paraphyses flare upward and outward, surrounding an opening in the pycnium, the *ostiole*. In the early stages of pycnium formation the paraphyses are aggregated into a somewhat pointed structure (Buller, 1950; Gold *et al.*, 1979) that apparently functions to rupture the host epidermis. Ultrastructurally the protoplasts of the paraphyses are much like other cells of the pycnium, except that the nuclei are usually somewhat elongated and microtubules are very prominent adjacent to and parallel with the walls. The pycnial paraphyses of *P. coronata* do not contain any of the unusual inclusions that occur in the uredial paraphyses of the same fungus (see Section V,A).

There are as yet no transmission electron microscopic illustrations of the flexuous hyphae in the rusts, although they have been shown by scanning electron microscopy in pycnia of *P. recondita* (Gold *et al.*, 1979), *M. lini* (Gold and Littlefield, 1979), and *Gymnosporangium clavipes* (Kozar and Netolitzky, 1975). In the *Puccinia* spp. the flexuous hyphae occur intermixed among the paraphyses; approximately 20 such hyphae per pycnium were reported to occur in *P. graminis* (Buller, 1950). The flexuous hyphae can be distinguished from the paraphyses by being less erect and less pointed at their apices.

### C. PYCNIOSPORE ONTOGENY

Among the cereal rusts, pycniospore formation has been described ultrastructurally for *P. sorghi* (Rijkenberg and Truter, 1974a) and *P. coronata* (Harder and Chong, 1978), and in several noncereal rusts by

Codron (1981), Mims, *et al.* (1976), and Metzler (1981). Figure 22 is a diagrammatic summarization of pycniospore formation in *P. coronata*, and may be referred to in the following description of pycniospore development.

The pycniospores are produced successively in chains from the pycniosporophores; a single pycniosporophore gives rise to a large number of pycniospores. An understanding of the mechanism of pycniospore formation is contingent on understanding the cell wall relationships during spore formation. The pycniosporophore walls are composed of two layers: a broad outer layer and a relatively narrower inner layer. The formation of the first pycniospore is marked by a swelling of the pycniosporophore apex. Both wall layers extend around the swelling apex. The formation of successive spores essentially recapitulates the first spore in that a complete bilayered wall is synthesized to envelop each forming spore. During the swelling process, mitosis occurs, after which one nucleus migrates into the pycniospore and the other re-

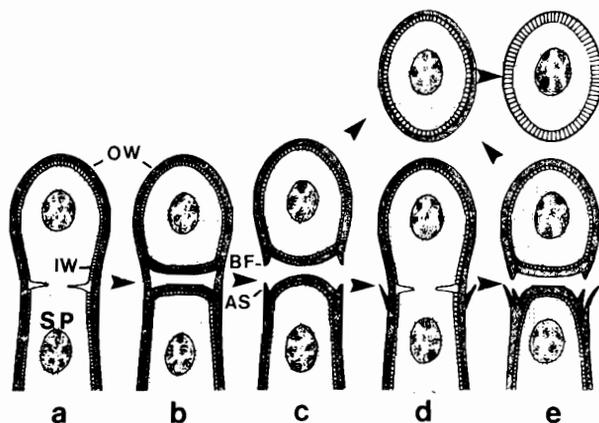


Fig. 22. A diagrammatic interpretation of pycniospore formation in *Puccinia coronata*. The sequence of the development of the first spore is from a to c, and repeat spore formation from d to e. (a) After nuclear division and swelling of the pycniosporophore (SP) apex, septum formation has begun by invagination of the plasmalemma and breakdown of the inner wall layer (IW) within the invagination. (b) Both inner and outer wall (OW) layers have grown across the septum, but the outer layer of the periclinal wall is intact. (c) Spore release occurs by rupture of the outer wall layer, leaving a basal frill (BF) on the immature spore and an annular scar (AS) on the pycniosporophore. (d) and (e) Repeat stages of (a) and (b), respectively, showing formation of the second spore from the pycniosporophore and showing the beginning of accumulation of annular scars, at the same vertical level, to form a collar at the pycniosporophore apex. Maturation of the pycniospore is marked by thickening of the inner wall layer and disappearance of the outer wall layer [above (d) and (e)].

mains in the pycniosporophore. Following nuclear migration, septation occurs (Fig. 22a) to delimit the pycniospore. During septation only the inner layer of the periclinal wall is initially disrupted. At maturity the septum is composed of two bilayered walls separated by an electron-lucent lamella (Fig. 22b). The septal wall layers are continuous with the respective inner and outer wall layers of the pycniosporophore and immature pycniospore. Pycniospore secession then occurs by rupture of the outer layer of the periclinal wall (Fig. 22c). The rupture of this wall initially leaves a remnant, often seen as a basal frill, on the young pycniospore. The outer wall layer of the immature pycniospore then gradually becomes thinner (Fig. 22c) and is replaced by the thickening inner layer, until at maturity the inner layer comprises the bulk of the pycniospore wall (Fig. 22c). In Fig. 22d the process begun in Fig. 22a, involving the same pycniosporophore, is repeated. The secession of pycniospores leaves pronounced wall remnants (annular scars) on the pycniosporophore walls. Each succeeding pycniospore is formed by extension of the bilayered pycniosporophore wall from inside the base of the most recent annular scar. The walls of all succeeding pycniospores originate at about the same locus on the pycniosporophore. In this way, repeated pycniospore secession leads to a buildup of a series of concentric annular scars to form a thickened collar at the pycniosporophore apex.

#### IV. Aecia

The aecium is the fruiting structure that produces the aeciospores following dikaryotization and reinitiates the dikaryotic life cycle phase. The aecia typically form on the undersides of the leaves shortly after pycnium development. A single aecial pustule consists of multiple aecia.

##### A. MORPHOLOGY

Five major morphological types of aecia in the rusts have been defined (Cummins, 1959). The aecia of all of the cereal rusts are typified by the "acidoid" type. This type of aecium is a somewhat cylindrical to trumpet-shaped structure bounded by a single layer of peridial cells, and originates subepidermally. The aeciospores are produced within the confines of the peridium. The structure of the aecium is reconstructed in the drawing in Fig. 23.

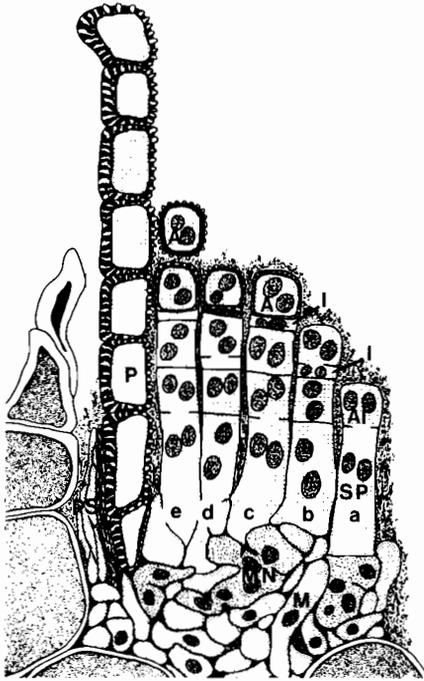


Fig. 23. Diagrammatic representation of a cereal rust aecium. At the base of the aecium are monokaryotic (M) and multinuclear fusion cells (MN). The latter cells give rise to the dikaryotic aeciosporophores (SP) and peridial (P) cells. Outside the peridium is an aggregation of crushed prosenchymatous cells (PS). The sequence of aeciospore formation is shown from a to e. (a) Division of the aeciosporophore to form an aeciospore initial (AI). (b) Formation of additional aeciospore initials and division of the first aeciospore initial to form an immature aeciospore (A) and an intercalary cell (I). (c) Formation of more aeciospore initials, and secondary wall and ornament formation in the aeciospore. (The number of aeciospore initials formed is probably variable.) (d) Maturation of the aeciospore and disorganization of the intercalary cell. (e) Release of the aeciospore and continued aeciospore formation.

## B. CELL TYPES

The following descriptions of cell types in aecia are from ultrastructural studies of *P. sorghi* (Rijkenberg and Truter, 1974b, 1975), *P. recon-dita* (Gold *et al.*, 1979), *P. graminis* f. sp. *tritici* (Holm and Tibel, 1974), and *P. coronata* (D. E. Harder, unpublished).

The sides of the aecium are bounded by a cylinder consisting of a single layer of dikaryotic *peridial cells*. These cells are characterized by a unique wall structure (Fig. 24). The walls to the outside of the aecium are considerably thicker than the inward-facing walls. The walls are

highly differentiated into a uniformly electron-dense portion and electron-lucent, branched, dagger-shaped processes. In the outward-facing wall the processes extend from near the plasmalemma through the wall to the outer surface, but not extending beyond it. In the inner wall facing the aecial cavity, the primary wall materials surrounding the processes disintegrate, partially exposing them. These then form irregularly spaced and shaped ornaments, or *clavae* (Gold *et al.*, 1979), over the surface of this wall.

The differential thickness of the peridial walls has been suggested (Littlefield and Heath, 1979; Savile, 1954) as a possible mechanism of opening and closing the aecium in response to changes in humidity. The thin inner wall may respond more rapidly to changes in humidity; during periods of low humidity the inner wall would contract to close the aecium, and vice versa during periods of high humidity.

Near the base of the peridium but external to it are a number of fungal cells in various stages of disintegration and compaction. These cells, designated *prosenchymatous* cells in aecia of *P. sorghi* (Rijkenberg and Truter, 1974b), have no apparent function in the aecial complex.

Rijkenberg and Truter (1974b) differentiated three types of cells in the closely packed, intertwining stroma of hyphae at the aecial base. These were (1) uninucleate, often vacuolate cells, (2) multinucleate *fusion cells*, and (3) degenerate cells with large vacuoles and granular inclusions. The origin or fate of the latter cells was not followed, and they may be degenerating uninucleate or other cells; thus they may not be a distinct cell type. A fourth cell type, the binucleate *sporophores*, arise from the multinucleate cells at the base of the aecium. The sporophores give rise to *intercalary* (= *disjunctor*; Holm and Tibell, 1974) cells and the *aeciospore*.

### C. DIKARYOTIZATION

The dikaryotization process in the rusts, despite its importance in the life cycle, is poorly understood. Virtually all of the information available has been obtained from light microscopy (for reviews, see Lamb, 1935; Buller, 1950). The initial phase of dikaryotization, the fusion of (+) and (-) mating types, occurs in the pycnium, but the formation of dikaryotic cells apparently occurs first at the base of the aecium.

The light microscopic studies as reviewed by Buller (1950) have shown several variations in the fusion of (+) and (-) mating types. Of those pertinent to the cereal rusts, the following variants have been

documented: (a) fusion of (+) and (-) basidiospore-derived hyphae (*P. graminis* f. sp. *tritici*, Craigie, 1927a,b), and (b) fusion of (+) or (-) pycniospores, respectively, with (-) or (+) flexuous hyphae (*P. graminis* f. sp. *tritici*, Craigie, 1927a,b, 1933). In addition, Cotter (1960) and Garrett and Wilcoxson (1960) demonstrated (c) the fusion of aeciospore or urediospore germ tubes with flexuous hyphae of *P. graminis* f. sp. *tritici*. Although variants (a) and (c) just described could conceivably add to the pool of nuclei for later reassortment in the aecium, they probably are not significant in nature.

The fusion of pycniospores with flexuous hyphae is well documented by light microscopy. In most of the rusts studied, a fusion tube of variable length or diameter forms between the pycniospore and the flexuous hypha. The only electron micrograph available is a scanning micrograph of apparent fusion in *Melampsora lini* (Littlefield and Heath, 1979). In *P. graminis* f. sp. *tritici*, the fusion tube is reduced to a slightly raised papilla on the flexuous hypha, through which the passage of the pycniospore nucleus was observed (Savile, 1939). In the cereal rusts studied by Buller (1950), fusion could occur at any point along the flexuous hypha. In the latter work tropisms between the flexuous hyphae and pycniospores, which induced branching or bending of the former to the pycniospore, were indicated. Although Buller (1950) indicated that in the main only one fusion occurs between a flexuous hypha and a pycniospore, each pycnium contains of a number of flexuous hyphae; thus multiple fusions can occur within a single pycnial sorus.

The stages of the dikaryotization process following fusion are the least well understood. One criterion used by Craigie and Green (1962) to trace the fate of the pycniospore nucleus of *P. graminis* f. sp. *tritici* was that the latter nuclei are in a compact "unexpanded" form, whereas those of the haploid thallus are "expanded." Using this criterion, the pycniospore nuclei were traced to cells of the protoaecium, where they required about 20 to 25 hr to arrive. How the nuclei arrive at the protoaecium and details of their postarrival fate are not known. Craigie and Green (1962) indicated that the pycniospore nuclei do not undergo mitosis during their migration, although Rijkenberg and Truter (1975) indicated that these nuclei underwent mitosis soon after arrival. The cells at the base of the aecium are mainly either uni- or multinucleate, and arise by cell fusion and/or nuclear division (Rijkenberg and Truter, 1975). Allen (1934) showed multinucleate cells in the protoaecium of *P. sorghi*, this condition presumably occurring before the arrival of a pycniospore nucleus. The next known development is that the dikaryotic primary aeciosporophores arise from the multinucleate fusion

cells (Rijkenberg and Truter, 1974b). It was suggested by the latter authors that perhaps the injection of a pycniospore nucleus into a multinucleate cell is necessary to begin the final phase of dikaryotization. Each multinucleate cell gives rise to several sporophores, thus an assortment of compatible mating-type nuclei must occur at this stage to form the stable dikaryon. However, no details of this process are known.

#### D. AECIOSPORE FORMATION

##### 1. Ontogeny

The formation of successive aeciospores from the sporophores is shown diagrammatically in Fig. 23. This diagram is reconstructed from *P. recondita* (Gold *et al.*, 1979), *P. sorghi* (Rijkenberg and Truter, 1974b, 1975), and *P. graminis* f. sp. *tritici* (Holm and Tibell, 1974). The primary aeciosporophore may divide to form secondary aeciospores [not shown in Fig. 23]. Following mitotic nuclear divisions, the two daughter nuclei migrate to the distal end of the sporophore, followed by septation to form an aeciospore initial. In *P. graminis* f. sp. *tritici* (Holm and Tibell, 1974), several aeciospore initials are cut off to form a chain of these cells. The aeciospore initials, beginning first with the uppermost one, undergo a further division to form the aeciospore and a usually wedge-shaped intercalary cell. In this way continuous chains of aeciospores are produced. The intercalary cells then disintegrate to release the mature aeciospores.

##### 2. Aeciospore Ornamentation

The aeciospores of all of the cereal rusts are covered with ornamental processes (see Fig. 25). Littlefield and Heath (1979) differentiated the two most common types of ornaments as either coglike or annulate knobs. The coglike knobs are somewhat cylindrical and flattened at the apex, whereas the annulate knobs appear as irregular stacks of disks. All of the cereal rusts that have been studied were judged to have the coglike ornaments. However, these two types of ornaments may not be distinctly different but may represent variations in the degree of differentiation of the individual disks. Both immature and mature (Fig. 26) aecioscope ornaments of *P. coronata* show lateral striations that correspond to irregularities in their sides, indicating a stacked-disk arrangement. The aecial ornaments of the cereal rusts, although previously defined as coglike, are probably built up by the stacking of disks.

With two known exceptions, the aeciospores of the cereal rusts are covered with only the coglike processes. The exceptions are *P. graminis* f. sp. *tritici* (Fig. 25) [Holm *et al.*, 1970] and *P. poarum* (Henderson *et al.*, 1972), which in addition have large refractile granules interspersed among the coglike processes.

The process of aeciospore wall ornament development is interpreted diagrammatically in Fig. 27. The ornaments begin to form within the primary wall of the immature aeciospore shortly after intercalary cell formation. They first appear as electron-lucent areas against the plas-

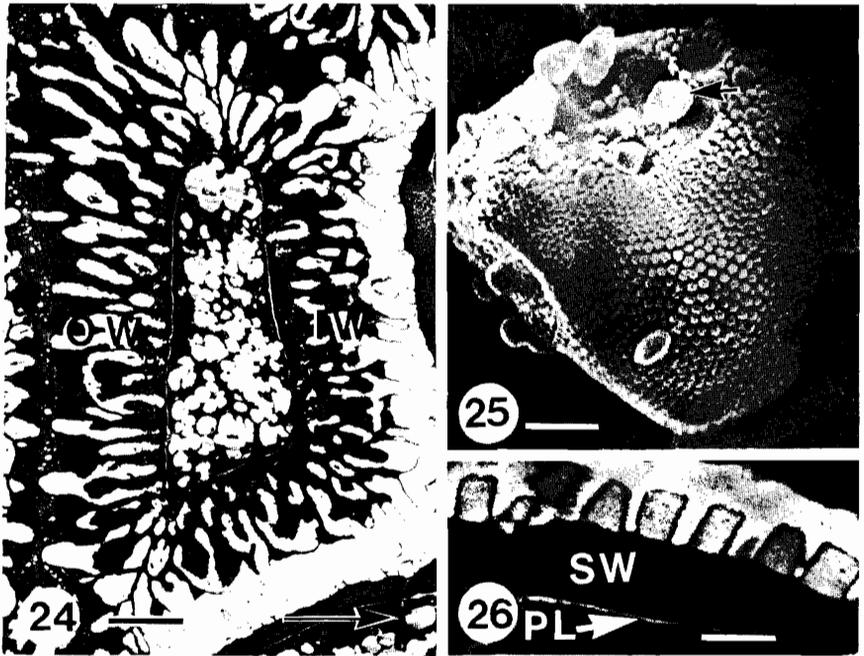
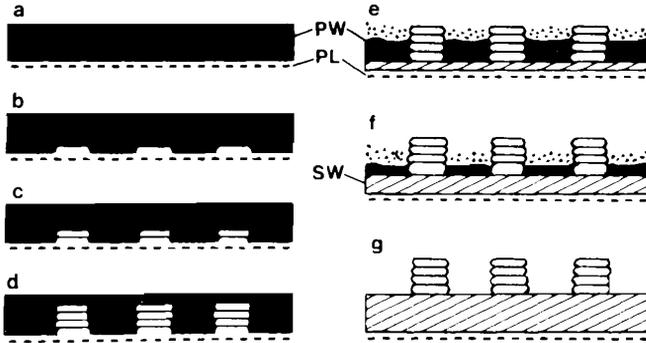


Fig. 24. An aecial peridial cell of *Puccinia coronata*. The cell wall [OW] facing the outside of the aecium is thicker than the wall [IW] facing the aecial cavity (arrow). The wall consists of an electron-dense matrix through which occur electron-lucent, branched processes. The processes extend beyond the surface of only the inward-facing wall ( $\times 4200$ ; bar,  $2.40 \mu\text{m}$ ). (From D. E. Harder, unpublished.) Fig. 25. A scanning electron micrograph of a mature aeciospore of *Puccinia graminis* f. sp. *tritici*. Note the small coglike ornaments and the larger refractile granules (arrow) ( $\times 3000$ ; bar,  $3.30 \mu\text{m}$ ). (From Holm *et al.*, 1970. Reproduced with permission of the editor, *Svensk Botanisk Tidskrift*.) Fig. 26. Wall ornaments located on the surface of the secondary wall (SW) of a mature aeciospore of *Puccinia coronata*. PL, Plasmalemma. This micrograph was overexposed to reveal the probable stacked-disk construction of the ornaments ( $\times 12,500$ ; bar,  $0.80 \mu\text{m}$ ). (From D. E. Harder, unpublished.)



**Fig. 27.** Diagrammatic representation of ornament formation in *Puccinia* spp. (a) Primary wall (PW) with plasmalemma (PL). (b) First disk formed in the primary wall against the plasmalemma. (c) and (d) Formation of successive disks until the mature size of the ornament is attained. (e)–(g) Dissolution of the primary wall and formation and thickening of the secondary wall (SW). At maturity, the ornaments rest on the surface of the secondary wall. [Drawn by Dr. J. Chong.]

mallema and extend into the wall. The primary wall continues to thicken, and at the same time the ornaments grow outward, presumably by periodic addition of new material at their bases against the plasmalemma. After the ornaments have attained their full extension the primary wall begins to disintegrate. Subsequently, a secondary wall forms that intervenes between the ornaments and the plasmalemma. The secondary wall continues to thicken, and at the same time the primary wall dissolves away from around the ornaments, leaving them exposed and attached to the surface of the secondary wall.

## V. Uredia

### A. MORPHOLOGY AND CELL TYPES

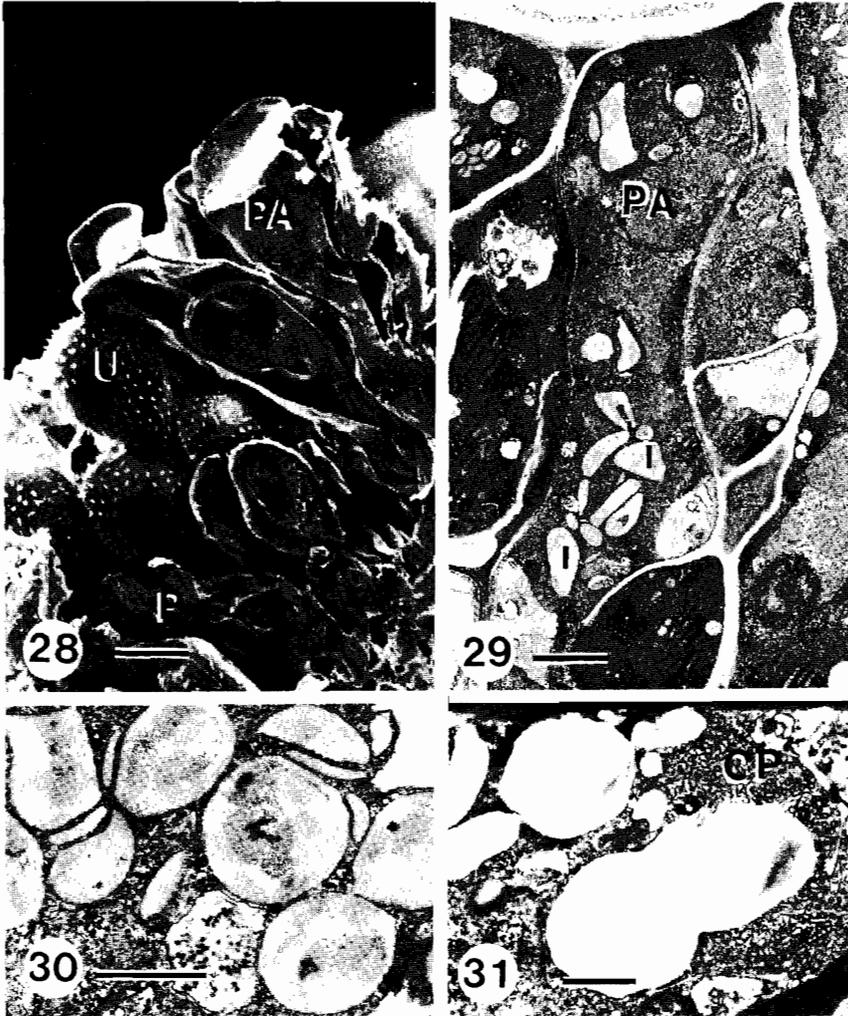
The uredia of the cereal rusts are not bound by a defined layer of cells, thus they are morphologically not discrete. The first phase of uredium development is marked by an aggregation of fungal cells in an intercellular space underneath the epidermis. These cells, the *sporogenous* (i.e., basal) cells, become closely packed and form the base of the uredium. The sporogenous cells are somewhat elongated hypha-like cells, enlarged at the spore-forming end. The protoplasts of these cells are characteristic of those of the intercellular hyphae. The sporogenous cells give rise to the *pedicels* (i.e., stalk cells) and *uredio-*

spores. The uredia of some rusts contain accessory cells such as *paraphyses* and/or sterile interstitial cells. Of the cereal rusts, only the uredia of *P. coronata* contain paraphyses.

Figure 28 is a scanning micrograph through a uredium of *P. coronata* showing the urediospores, paraphyses, and pedicels or interstitial cells. The paraphyses tend to predominate at the margin of the uredium, although they also may occur within the uredium. The paraphyses in Fig. 28 appear somewhat collapsed, probably largely because of dehydration during processing. The paraphyses, along with the interstitial cells, contain unusual inclusions in their cytoplasm. Figure 29 is a longitudinal section through a young paraphysis cell of *P. coronata*, which contains irregularly shaped electron-lucent inclusions. In contrast to an earlier conclusion (Harder, 1976c), similar inclusions have subsequently been found in uredia of *P. recondita*, *P. graminis* f. sp. *tritic*, and *P. graminis* f. sp. *avenae* (Fig. 30), which are not paraphysate. Cells with similar inclusions are also found in pycnia of *P. coronata* (D. E. Harder, unpublished) and *Gymnosporangium juniperi-virginianae* (Mims *et al.*, 1976), buffer cells in telia of the latter fungus (Mims, 1977), and various cells in uredia of *Melampsora lini* (Hassan and Littlefield, 1979). The common factor in all of the cells with these inclusions is that they are sterile cells in the various fruiting bodies of the rusts. In the case of buffer cells or paraphyses, they perhaps add mechanical support. However, the composition of the inclusions is not known, and they also do not occur in the pycnial paraphyses of *P. coronata* (D. E. Harder, unpublished). Many of the cells in which they are found appear to have no traceable function; they are isolated cells occurring interspersed in the sporogenous tissue, and they are frequently degenerative. Mature uredia of *P. coronata* contain numerous cells of this type. The inclusions in these cells continue to grow; they coalesce (Fig. 31), and eventually the cells collapse. These peculiar cells appear to be of wide occurrence in the fruiting tissue of the rusts; in the case of the nonparaphysate uredia of the cereal rusts, they may represent aborted paraphysis-type cells. A similar situation also could apply to *P. coronata*, except that some of these cells, particularly at the uredial margins, develop into paraphyses.

## B. UREDIOSPORE ONTOGENY

Urediospores have been defined morphologically as always borne singly on pedicels that arise from successive new growing points on a sporogenous cell (Kunholtz-Lordat, 1943). This definition essentially



**Fig. 28.** A scanning electron micrograph near the margin of *Puccinia coronata*. Note the paraphyses (PA), urediospores (U), and smaller cells (P), which are either pedicels after the release of urediospores or interstitial cells ( $\times 1000$ ; bar, 10.0  $\mu\text{m}$ ). (From Takahashi and Furuta, 1973. Reproduced with permission from Dr. N. Hiratsuka, The Tottori Mycological Institute.) **Fig. 29.** A young paraphysis cell (PA) in a uredium of *Puccinia coronata*. Note the irregularly shaped inclusions (I) in this cell ( $\times 4200$ ; bar, 2.40  $\mu\text{m}$ ). **Figs. 30 and 31.** Inclusions similar to those in Fig. 29, in interstitial cells in uredia of (Fig. 30) *Puccinia graminis* f. sp. *avenae* and (Fig. 31) *P. coronata*. The cytoplasmic membranes in these cells frequently form a finely membranous network or may appear as a "crochet pattern" network (CP, Fig. 31) (Fig. 30:  $\times 30,000$ ; bar, 0.50  $\mu\text{m}$ . Fig. 31:  $\times 13,300$ ; bar, 0.75  $\mu\text{m}$ ). [From D. E. Harder, unpublished.]

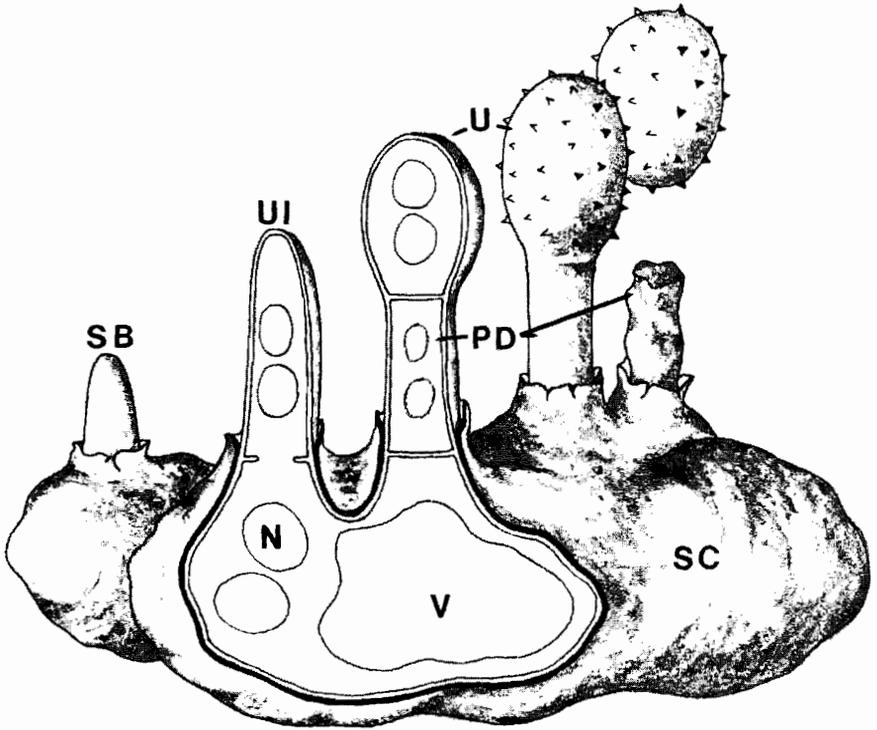


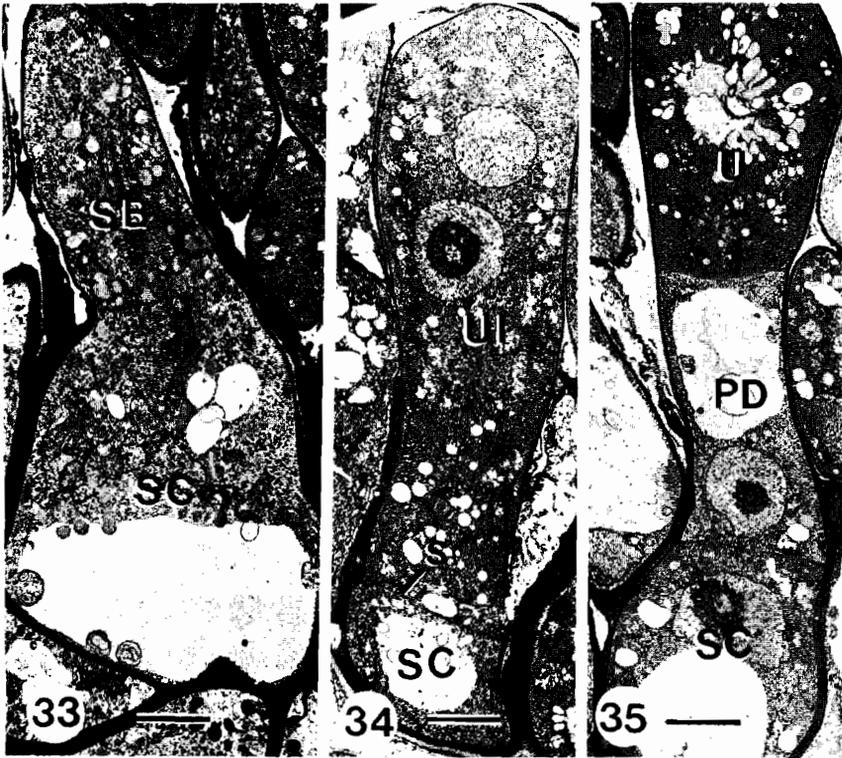
Fig. 32. A diagrammatic partially cutaway illustration of urediospore formation in *Puccinia coronata*. The urediospores (U) are produced successively from new growing points on a sporogenous cell (SC). Shown is the successive formation of a spore bud (SB), urediospore initial (UI), pedicel (PD), and urediospore. N, Nucleus; V, vacuole. (Drawn by Dr. J. Chong.)

describes the mode of urediospore formation in most rust fungi, including *Puccinia*. The succession of urediospores from a sporogenous cell defines them as sympoduloconidia (Hughes, 1970). The successive formation of urediospores from a sporogenous cell is illustrated in Fig. 32. Of the cereal rusts, urediospore formation has been studied ultrastructurally in *P. coronata* (Harder, 1976c), *P. sorghi* (Rijkenberg, 1975), and *Physopella zaeae* (Heath and Bonde, 1983).

Urediospore formation is initiated by the outgrowth of a *spore bud* from the swollen end of a sporogenous cell (Fig. 33). The spore bud is formed by evagination of the inner wall layer through a rupture in the outer wall layer of the sporogenous cell. The relatively thin wall of the spore bud is continuous with the inner layer of the sporogenous cell. Conjugate nuclear division then occurs, the spore bud elongates, and

separation occurs to delineate the *urediospore initial* from the sporogenous cell (Fig. 34). During continued growth of the urediospore initial, the number of lipid droplets in this cell increases. A second nuclear division then occurs, followed by nuclear migration and septation to partition the *pedicel* and immature *urediospore* (Fig. 35).

The nuclei in the pedicels remain smaller than those in sporogenous cells or intercellular hyphae [Harder, 1976c]. These correspond to the "unexpanded" nuclei in pedicels of *Uromyces fabae* (Savile, 1939). The smaller size of these nuclei appears to be brought about by their



Figs. 33–35. Urediospore formation in *Puccinia coronata*. (Figs. 33 and 35 from Harder, 1976c. Reproduced by permission of the National Research Council of Canada. Fig. 34 from D. E. Harder, unpublished.) Fig. 33. A spore bud (SB) in a stage emerging from a sporogenous cell (SC) by outgrowth of the inner wall layer of the SC ( $\times 5100$ ; bar, 2.0  $\mu\text{m}$ ). Fig. 34. The stage next to that in Fig. 33; septum (S) formation has begun, to divide the spore bud from the sporogenous cell (SC), to form the urediospore initial (UI) ( $\times 4100$ ; bar, 2.40  $\mu\text{m}$ ). Fig. 35. The urediospore initial has divided to form the pedicel (PD) and immature urediospore (U) ( $\times 3500$ ; bar, 2.90  $\mu\text{m}$ ).

failure to grow to normal ("expanded") size rather than by expulsion of part of the nucleus as during mitosis.

The young urediospores rapidly grow to mature size, accompanied by increased density of the cytoplasm, disappearance of vacuoles, increased accumulation of lipid droplets, wall thickening, and spine development. The septal wall separating the urediospore and pedicel is thickened only on the urediospore side of the septum. On the pedicel side, the septal wall remains approximately as thick as that in the intercellular hyphae. A channel extends through the thickened portion of the cross wall to a septal pore (*P. graminis* f. sp. *tritici*, Ehrlich and Ehrlich, 1969; also see an equivalent channel and pore in a teliospore, Fig. 41). The septal pores at the spore bases appear to be smaller than those in intercellular hyphal septa, although insufficient sections have been examined to obtain reliable measurements. These pores also do not possess a septal pore apparatus.

### C. UREDIOSPORE MORPHOLOGY

#### 1. Protoplasts

The urediospore protoplasts are dense and contain most of the usual cellular constituents, that is, nuclei, mitochondria, endoplasmic reticulum, vesicles, ribosomes, and storage material. The mitochondria are more rounded and more compact than those in intercellular hyphae. The urediospores are typically packed with lipid droplets, which is their major storage product. Glycogen has been reported to occur in *P. graminis* f. sp. *tritici* (Ehrlich and Ehrlich, 1969) and *P. recondita* (Salako, 1981), but it was not found in urediospores of *P. coronata* (Harder, 1976c).

There are conflicting reports concerning the absence or presence of nucleoli in nuclei of mature urediospores or in germ tubes (see M. C. Heath and Heath, 1978, and references). Nucleoli were found in all growth phases, including mature urediospores, of *Uromyces phaseoli* var. *vignae* (M. C. Heath and Heath, 1978). In *P. coronata* the nucleolus in the most mature urediospore in which it could be found was a fibrillar ring-shaped structure with a large central lacuna (D. E. Harder, unpublished). A similar configuration was found in a mature urediospore of *P. graminis* f. sp. *tritici* (Mitchell and Shaw, 1969) or in a germ tube of *U. phaseoli* var. *vignae* (M. C. Heath and Heath, 1978). This type of nucleolus has been associated with presumed decrease in nucleolar function (Smetana and Busch, 1974). M. C. Heath and Heath (1978) attributed the inability to find nucleoli, or reports of their re-

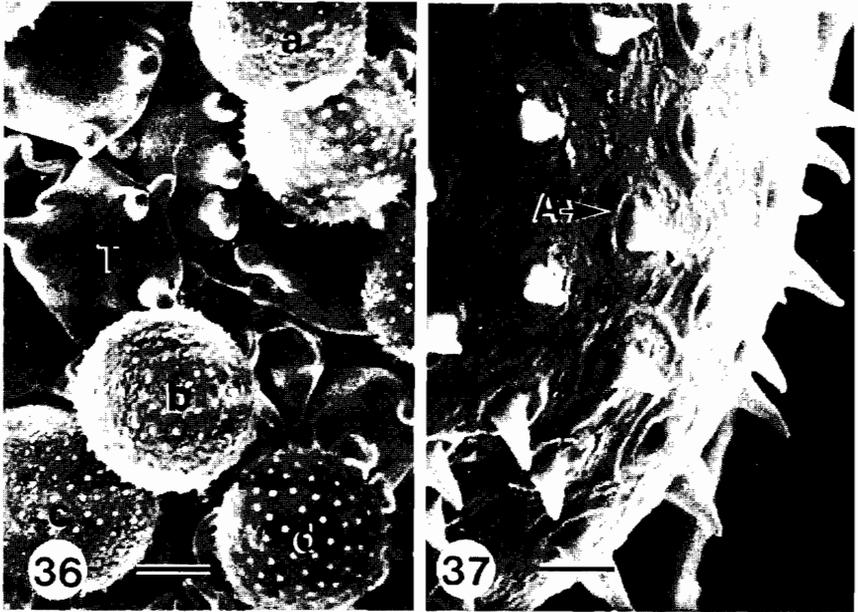
duced size in mature urediospores, as possibly due to insufficient numbers of sections of any one sample being examined. One further problem is that little is known about the effects of the conventional processing procedures on the protoplasts of mature urediospores. The thick walls and dense protoplasts of these spores make structural preservation by chemical means very difficult. The nucleoli that have been shown in mature urediospores appear to exist in a modified fibrillar form. There may be variation in levels of preservation or contrasting by various workers, contributing to the inconsistency in the literature.

## 2. Walls and Ornaments

The mature urediospore walls consist entirely of secondary wall material, the primary walls having dispersed during spore maturation (see later and Fig. 38). The walls of hydrated spores are  $\sim 1.0$ – $1.5$  nm thick and consist of several layers. Earlier reports (Ehrlich and Ehrlich, 1969; Thomas and Isaac, 1967; Williams and Ledingham, 1964) had indicated a three-layered wall: a thin pellicle-like outer layer, a relatively narrow middle layer, and a broad inner layer. However, the resolution of wall layers appears to depend on the processing methods used. With freeze-etch (*Melampsora lini*, Littlefield and Bracker, 1971b) or several histochemical treatments (*P. graminis* f. sp. *tritici*, Rohringer *et al.*, 1984), the broad inner zone may be resolved into at least two layers, indicating a four-layered urediospore wall.

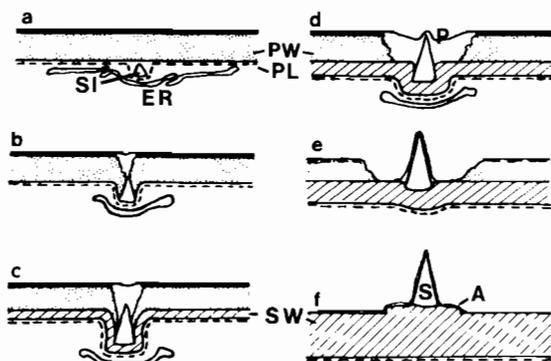
The urediospores of the *Puccinia* spp. are echinulate, with minor variations in surface morphology among some species (Brown and Brotzman, 1979). The spines are normally slightly bent at the tip and are located on the surface of the spore wall, surrounded by a somewhat raised annulus (Fig. 37).

Spine development in *P. graminis* f. sp. *tritici* was first described by Thomas and Isaac (1967). A correlative scanning and transmission electron microscope study of *P. spargenoides* (Amerson and Van Dyke, 1978) has provided the most comprehensive view of spine development in the rusts. With minor variations, the ontogeny of urediospore spines appears essentially similar in most of the rust fungi. Figure 36 is a scanning electron micrograph showing urediospores in several stages of development (labeled a–d, from youngest to oldest). Spine development is illustrated diagrammatically in Fig. 38. Spine initials first become evident at about the time that secondary wall formation occurs along the pedicel–urediospore septum; they appear as an electron-lucent area just beneath the primary wall (Fig. 38a). There appears to be a concentration of endoplasmic reticulum around the inner periphery of



**Fig. 36.** A scanning electron micrograph of part of a mature uredium of *Puccinia coronata*. Various stages in sequence of spine emergence may be seen in spores a–d. Teliospores (T) are developing in this uredium ( $\times 1300$ ; bar,  $7.70\ \mu\text{m}$ ). (From Takahashi and Furuta, 1973. Reproduced with permission by Dr. N. Hiratsuka, The Tottori Mycological Institute.) **Fig. 37.** A scanning electron micrograph of a mature urediospore of *Puccinia coronata*. The spines are surrounded by a raised annular ring (A) ( $\times 10,000$ ; bar,  $1.0\ \mu\text{m}$ ). (From Takahashi *et al.*, 1978. Reproduced by permission from Dr. R. Kawashima, Agricultural Research Center, Japan.)

the spore at this stage (*P. coronata*; D. E. Harder, unpublished). Most reports indicate the persistence of endoplasmic reticulum at the base of developing spines. As the spine begins to lengthen, some primary wall material is deposited toward the base of the spine, but the wall disperses at the tip of the spine (Fig. 38b). Subsequently, secondary wall material is formed that invaginates into the spore around the base of the spine (Fig. 38c). Further development is marked by thickening and straightening of the secondary wall and disintegration of the primary wall radially from the spine, until the spine is fully exposed on the surface of mature spore wall (Fig. 38d–f). The pellicle remains intact during this process, and eventually covers the mature spine. In scanning micrographs the surfaces of immature urediospores are wrinkled (evident in Fig. 36b), which is likely due to the partial disintegration of the primary wall. The dissolution of the primary wall leaves polygonal



**Fig. 38.** Diagrammatic illustration of the successive stages of urediospore spine (S) formation. (a) Formation of a spine initial (SI) between the plasmalemma (PL) and primary spore wall (PW). Endoplasmic reticulum (ER) is prominent in this region. The primary wall consists of a relatively broad inner layer and a thin electron-dense pellicle-like outer layer. (b) Lengthening of spine, dissolution of the primary wall at the spine apex, and some growth of the primary wall toward the base of the spine. A pellicle (P) remains intact across the dissolved portion of the primary wall at this and all subsequent stages. (c) and (d) Further growth of the spine, continued dissolution of the primary wall radially from the spine, and development of a secondary wall (SW) layer. The thickening of the secondary wall layer pushes the spine through the opening in the primary wall. (e) The spine has attained its mature length, the primary wall has nearly dissolved away, and the secondary wall continues to thicken. (f) A mature spine (S) on a somewhat raised annular ring (A) on the surface of the secondary wall. The layers of the secondary wall are not shown in this diagram. The pellicle remains continuous around the spine.

ridges between the spines of *P. coronata* (Corlett, 1970), some evidence of which remains in Fig. 37.

## VI. Teliospore Ontogeny

Details of teliospores and their structure are covered in Chapter 12 by Mendgen, in this volume. Hence, in this chapter only teliospore ontogeny will be described. The ultrastructure of teliospore development in several *Puccinia* spp. has been studied by Bennett *et al.* (1978), Harder (1977), and Mims and Thurston (1979).

At various stages in the development of infection the teliospores begin to form alongside the urediospores in the urediosorus. The following description of teliospore ontogeny is from observations of *P. coronata* (Harder, 1977). The teliospore-bud stage is indistinguishable

from the comparable urediospore stage. The succeeding stages of teliospore initial, pedicel, and primary spore cell (single teliospore cell stage) formation are also comparable to urediospore formation (comparable stages of teliospore and urediospore formation are respectively illustrated in Figs. 39 and 35). The main feature that distinguishes a teliospore at this stage is thickening of the spore wall at the distal end of the primary spore cell [Fig. 39] and an accumulation of glycogen in

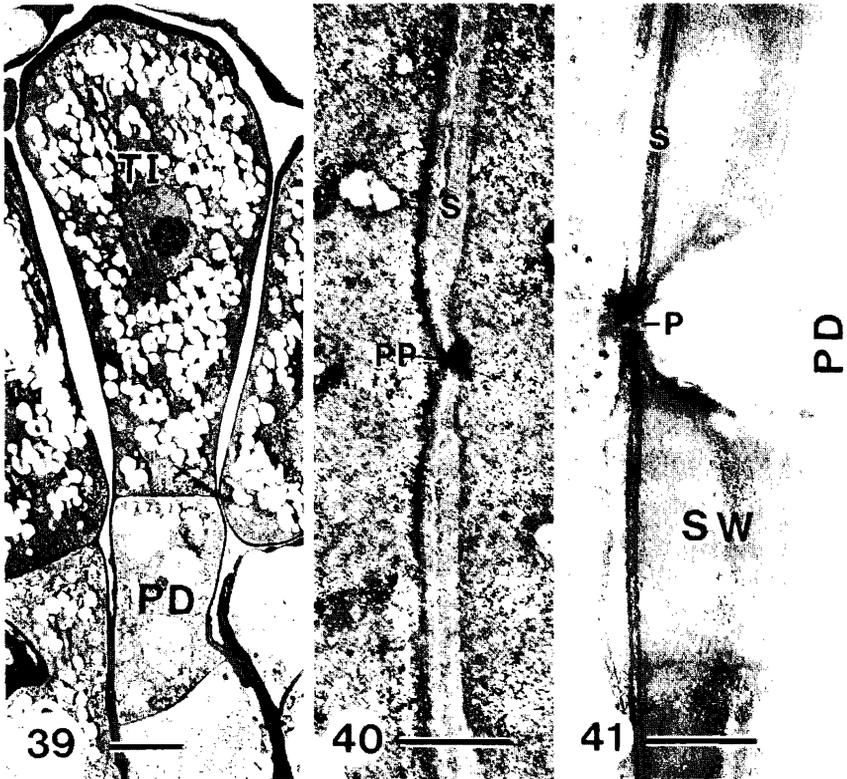


Fig. 39. A stage of teliospore formation of *Puccinia coronata* comparable to that of urediospore formation in Fig. 35. Shown are the pedicel (PD) and teliospore initial (TI). The teliospore initial will undergo one further division to form the two-celled teliospore ( $\times 3400$ ; bar,  $2.90 \mu\text{m}$ ). (From Harder, 1977. Reproduced with permission of Academic Press, New York.) Fig. 40. The septum (S) dividing the two cells of a nearly mature teliospore of *Puccinia coronata*. A pore plug (PP) occludes the septal pore, and ( $\times 27,500$ ; bar,  $0.55 \mu\text{m}$ ). (From D. E. Harder, unpublished.) Fig. 41. The septum (S) and wall between the teliospore and pedicel (PD) in *Puccinia coronata*. Secondary wall (SW) formation has occurred mainly on the pedicel side of the septum. This septum has a small pore (P) at the base of a channel in the secondary wall, which at this stage appears partially occluded ( $\times 3500$ ; bar,  $0.43 \mu\text{m}$ ). (From D. E. Harder, unpublished.)

the sporogenous and spore tissue. There is relatively much less glycogen in the uredial tissue of *P. coronata*, but it is not known if a comparable distribution occurs in other rusts.

Further teliospore development is marked by elongation of the primary spore cell, conjugate nuclear division, and septation to form the final two-celled structure of the teliospore. The septum dividing the two cells is perforate with an electron-dense occlusion in the pore (Fig. 40). There is no septal pore apparatus surrounding these pores. At this stage the wall has not appreciably thickened at the proximal end, but has thickened further at the distal end to form the "crown" for which *P. coronata* is named. Wall thickening at the base of the spore first occurs along the pedicel–teliospore cross wall. This thickening occurs first as patches along the septum, which coalesce and thicken until mature-wall thickness is attained. There is considerable thickening of the cross wall before there is much thickening of the wall of the lower part of the spore. The cross-wall thickening occurs predominantly on the pedicel side, which distinguishes it sharply from the urediospore–pedicel cross wall, where the thickening occurs only on the spore side. The cross wall at the base of the teliospore is perforate, with a channel extending through the thick secondary wall (Fig. 41). In the latter figure there is a moderately electron-dense, somewhat diffuse occlusion in the pore.

One of the major unknowns of teliospore formation is the precise timing and mode of diploidization. Mature teliospores are highly resistant to ultrastructural processing procedures, thus little is known of their detailed structure. Beyond a certain stage of maturation, membranes appear to be poorly preserved (Harder, 1977). As the teliospores approach maturity, the nuclei of each pair in both cells of the teliospore become closely appressed to one another and appear ready for fusion, but because of poor preservation of membranes, actual fusion has not been observed with certainty.

*Note Added in Proof*

Recently published information on the tropical rust fungus *Physopella zae* (Heath and Bonde, 1983) has shown that several successive urediospores are produced from the same site on a sporogenous cell.

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