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Structure and Physiology of Haustoria

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I. Introduction

Haustoria in the fungi were first mentioned by De Bary (1863), and the first detailed description for the rusts was provided by Ward (1882). Bushnell (1972) defined a fungal haustorium as "a specialized organ which is formed inside a living host cell as a branch of an extracellular

(or intercellular) hypha or thallus, which terminates in that host cell, and which probably has a role in the interchange of substances between host and fungus." The absorption of nutrients has generally been considered its main function, although Rice (1927) already questioned this concept. Since that time, work with the electron microscope has largely elucidated haustorial structure. However, for the rusts, direct evidence for the nutrient absorption role of haustoria is still lacking.

The morphology of haustoria and their relationships with their hosts have recently been thoroughly reviewed (Bracker and Littlefield, 1973; Littlefield and Heath, 1979). The physiology and possible function(s) of haustoria have been reviewed by Bushnell (1972). The basic structure of haustoria in dikaryotic rust infections is relatively uniform. Rather than making another comprehensive review on this topic, in this chapter we will detail the structure of two cereal rust fungi, *Puccinia graminis* f. sp. *tritici* and *P. coronata* on the basis of our own published or unpublished material. Research results obtained since the recent reviews will be emphasized, and where applicable, enhancement or modifications of earlier interpretations will be made.

II. Methodology and Interpretation

In the literature a variety of ultrastructural descriptions may pertain to given biological structures. These discrepancies may be due to variations in the stage of development of a structural component or to the methods used in preparing tissues for electron microscopy. To assess more reliably the occurrence and relationship of structures, a variety of processing methods should be employed. Many of the descriptions used in this chapter are the result of specific procedures to reveal particular components. The various methods used to elucidate the structure and composition of the parts of the haustorial apparatus are outlined, with their interpretations, as follows:

1. *Conventional processing* (Glt/OsO₄-UA/PbC). Tissue fixation is with glutaraldehyde (Glt) and osmium tetroxide (OsO₄) followed by staining with uranyl acetate/lead citrate (UA/PbC). Variations of the procedure may involve omitting the OsO₄ fixation or omitting the PbC stain.

2. *Periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) staining* (Thiery, 1967). When used with proper controls, this method is specific for polysaccharides with vicinal hydroxyl groups (glycogen and starch are common examples).

3. *Periodic acid–chromate–phosphotungstate (PACP)* (Roland *et al.*, 1972). This stain enhances the electron density of the plant cell plasmalemma.

4. *Subtractive methods used in conjunction with conventional or specific stains.* Specific cellular components may be removed by enzymatic (e.g., protease or cellulase) digestion or lipid solvent extraction. The presence or absence of these components then is tested for by various staining procedures.

5. *Lectin (WGL, Con A)–colloidal gold markers.* A wide variety of plant lectins bind with more or less specificity to particular cell components. The lectins may be conjugated with colloidal gold particles; then the lectins, which bind to cell components, can be detected in the electron microscope. Wheat germ lectin (WGL) has been used to detect chitin (Horisberger and Rosset, 1977) in fungal material and concanavalin A (Con A) to assay for α -linked glucose or mannose in polysaccharides (Horisberger and Vonlanthan, 1977), which results in relatively nonspecific detection of a variety of carbohydrates.

6. *Energy-dispersive X-ray (EDX) analysis.* This method is useful to detect mineral elements (atomic number of 11 or higher in the periodic table) where they occur in sufficient concentration in cellular components.

7. *Freeze-etch.* Rapidly frozen specimens are fractured and etched to reveal details of membrane topography, composition, or organization. One of the major advantages is that tissues undergo a minimum of chemical alteration and are not extracted. If freezing damage can be avoided, this method is a reliable indicator of structure with a minimum of artifacts. Freeze-etch may be combined with histochemistry, as in the detection of membrane sterols in the host–pathogen interaction with the polyene antibiotic filipin (Harder and Mendgen, 1982).

The interpretation of results using the various methods just described may be quite subjective. The subtractive methods such as enzyme digestion or solvent extraction depend on the absence or reduction of staining intensity as compared to untreated controls. If controls are rigorously applied and observations are made repeatedly, conclusions regarding the likelihood of the existence and location of a chemical component may be made.

III. Terminology and Definitions

Considerable inconsistency exists in the literature in designating component parts of the haustorial apparatus. In this chapter the termi-

nology as outlined by Bushnell (1972) and modified by Littlefield and Heath (1979) will be followed. The term haustorium itself has been the subject of controversy in reference to dikaryotic and monokaryotic infections. In monokaryotic infections the haustoria morphologically differ substantially from those in dikaryotic infections, and more closely resemble mycelial hyphae. In this chapter the terms D- and M-haustoria as used by Littlefield and Heath (1979) are used to designate the intracellular structures of dikaryotic or monokaryotic infections, respectively. Detailed comparisons between the D- and M-haustoria will be made. (See 1 in Note Added in Proof.)

The terms used, their definitions, and abbreviations for D-haustoria (Fig. 1) are as follows:

1. *Collar*: an irregularly occurring deposition of material between the host plasmalemma and host cell wall at the penetration site (Figs. 38–41). The collar may extend around the haustorial neck up to the base of the haustorial body. The collar is not a part of the haustorial apparatus.

2. *Extrahaustorial matrix (EH matrix)*: a region of varying dimensions and density that occurs between the haustorial body wall and the EH membrane.

3. *Extrahaustorial membrane (EH membrane)*: an extension of the host plasmalemma that surrounds the entire intracellular haustorium.

4. *H Haustorial body*: the irregularly shaped bulk of the haustorium that begins where the neck expands at its distal end.

5. *H Haustorial body wall*: the fungal cell wall enclosing the haustorial body.

6. *H Haustorial neck*: the constricted portion of the haustorium originating inside the host cell wall and extending to the base of the haustorial body.

7. *H Haustorium initial*: the postpenetration finger-like projection into the host cell. After swelling at its distal end to form the haustorial body, it becomes the haustorial neck (Figs. 2, 15, and 19).

8. *H Haustorium mother cell (HMC)*: a slightly swollen, terminal cell of an intercellular hypha that attaches to a host cell and gives rise to the haustorium. It is found only in dikaryotic infections.

9. *HMC septum*: the septum that delimits the HMC from the penultimate hyphal cell (Fig. 2).

10. *Neck ring*: an electron-dense portion of the neck wall that occurs approximately midway along the haustorial neck. Where the neck ring is composed of more than one "ring," the composite is the neck ring, and each portion is designated as a band.

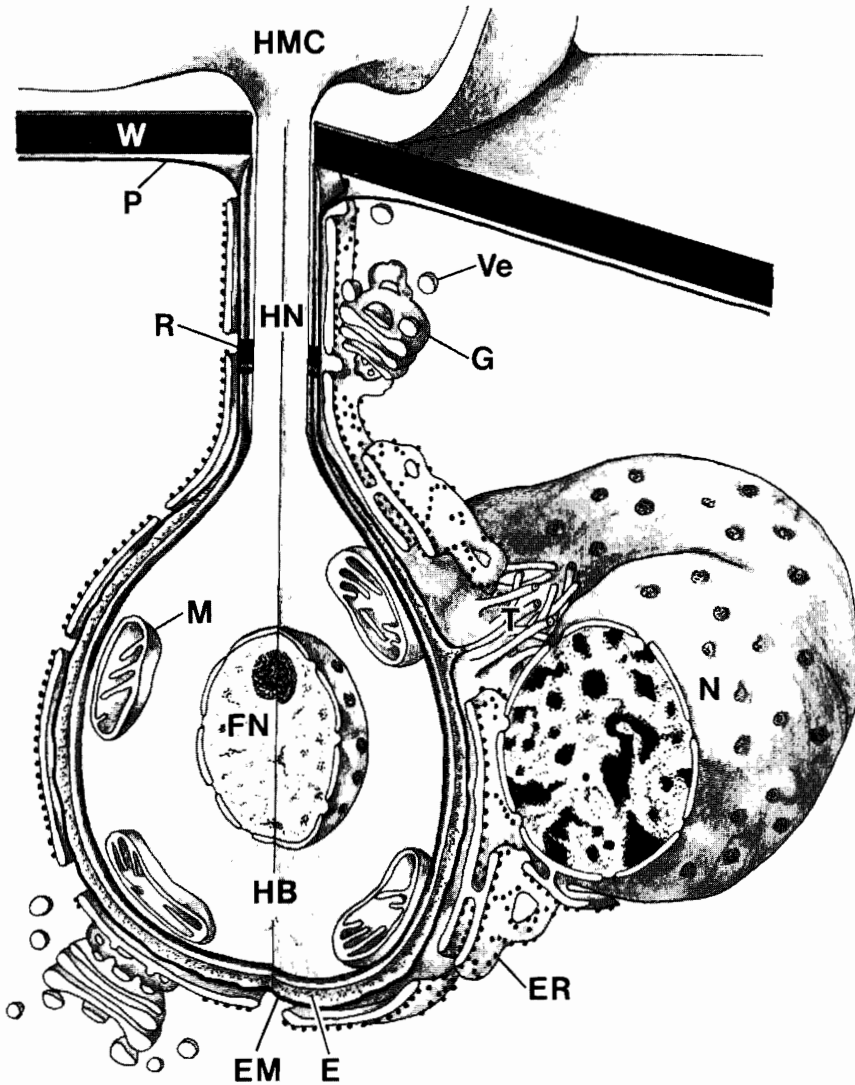


Fig. 1. Diagram of an invaded host cell cut open at the site of penetration to show the three-dimensional structure of a mature D-haustorium of *P. coronata*, and its association with the host cell organelles involved. The structures are not drawn to scale, and some are illustrated by only a few examples (e.g., Golgi bodies, vesicles, ribosomes). E, Extrahaustorial (EH) matrix; EM, extrahaustorial (EH) membrane; ER, endoplasmic reticulum; FN, fungal nucleus; G, Golgi body; HB, haustorial body; HMC, haustorial mother cell; HN, haustorial neck; M, mitochondrion; N, host nucleus; P, plasmalemma; R, neck ring; T, tubule complex; Ve, vesicle; W, host cell wall.

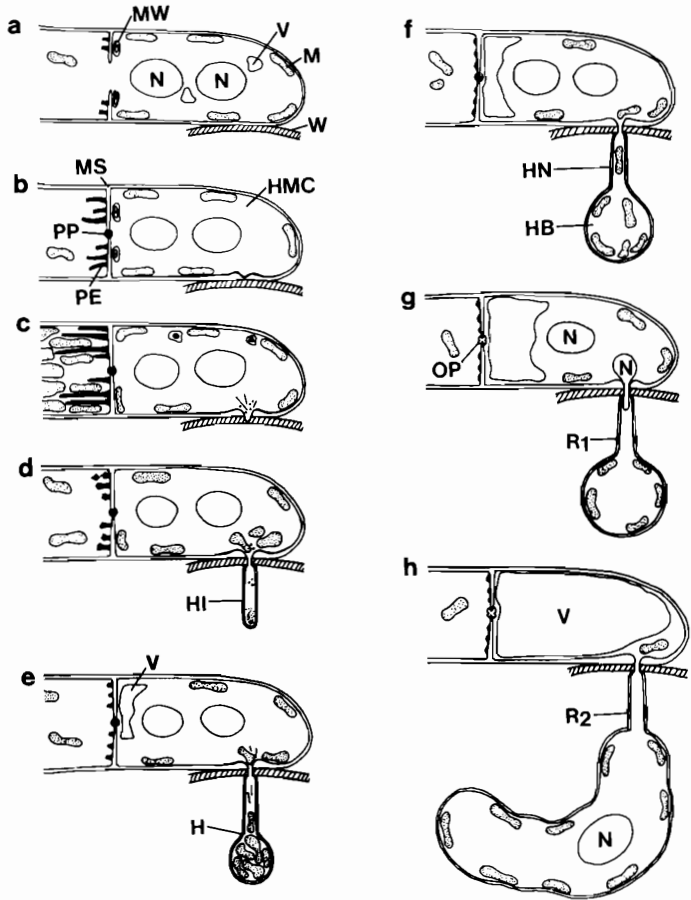


Fig. 2. A diagrammatic, chronological (a-h) representation of the events of D-haustorium development and the correlated state of the septal pore apparatus of the haustorium mother cell septum. H, Haustorium; HB, haustorial body; HI, haustorium initial; HMC, haustorium mother cell; HN, haustorial neck; M, mitochondrion; MS, haustorium mother cell septum; MW, membranous whorl; N, nucleus; OP, open septal pore; PE, plasmalemma elaboration; PP, plugged septal pore; R₁, neck ring with one band; R₂, neck ring with two bands; V, vacuole; W, host cell wall.

11. *Neck wall*: the fungal cell wall extending along the haustorial neck.

12. *Penetration peg*: the narrowest portion of the haustorium that passes through the host cell wall (Fig. 11).

Similar terms to those above are applied to the M-haustorium where applicable (see Fig. 37).

IV. Dikaryotic Haustoria

Figure 1 is an interpretation of a mature haustorium of *P. coronata* and is intended as a reference for haustorial structure in this fungus and its relation to the host cell. However, rust haustoria undergo numerous structural changes during their formation, therefore any one description of a haustorium is valid only for the point in its development when it was sampled. Thus the following discussion traces the structure of the haustorium from differentiation of the HMC through to maturity. The various stages of haustorium formation are illustrated in the drawing in Fig. 2.

A. HAUSTORIUM MOTHER CELL (HMC) DIFFERENTIATION

The induction of HMC differentiation is discussed in Staples and Macko (Chapter 9, this volume). In this section we will deal with specialized morphological features of the HMCs. Although the cytoplasmic contents of the HMCs do not differ from those of intercellular hyphal cells, the mitochondria undergo a change in conformation and distribution. In both *P. coronata* (Fig. 3) and *P. graminis* f. sp. *tritici* (Chong, 1981), the mitochondria are uniformly distributed around the periphery of the HMC protoplasts, are compact, and have a flattened apparently ovoid form, oriented with the flat face parallel to the HMC wall. Compare this conformation to the randomly distributed, irregular filiform mitochondria in the intercellular hyphal cells (see Harder, Chapter 11, this volume, Section II,A,4). The nuclei in the HMCs also are more compact and more regularly oval-shaped (Fig. 3) than those in the intercellular hyphae. Compact nuclei were also reported in HMCs of *Uromyces fabae* (Savile, 1939) and *U. phaseoli* var. *vignae* (Heath and Heath, 1978).

The HMCs and hyphal cells of both *P. graminis* f. sp. *tritici* and *P. coronata* can be clearly differentiated on the basis of their walls and septa (Chong, 1981). After UA/PbC (Fig. 4) or PA-TCH-SP staining, the HMC walls are thicker and have more layers than do the hyphal walls. Of the layers of the hyphal walls that are continuous with the outer layers of the HMC walls, the lightly staining outermost layer is probably not a rigid structural part of the wall, but a mucilaginous coating substance (Fig. 4). This layer also apparently serves to affix the HMC to the host cell wall. It is susceptible to protease digestion, and after treatment with protease, the HMC becomes detached from the host cell (see Fig. 24). The HMC septa are also composed of more layers than the hyphal septa. The wall layer that is adjacent to the fungal

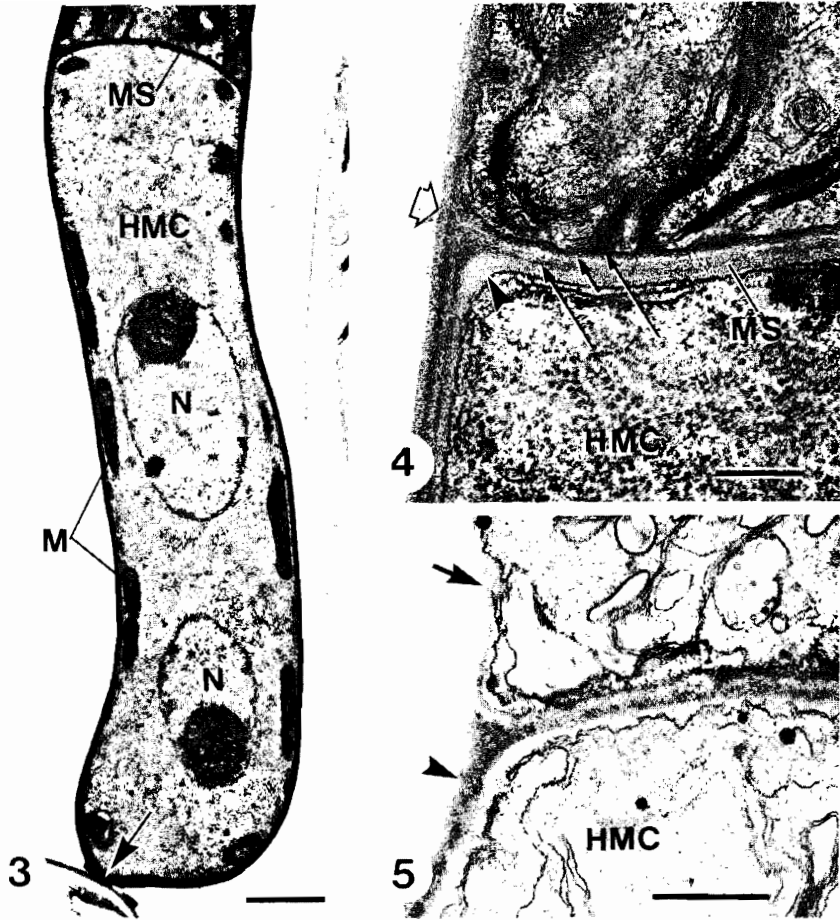


Fig. 3. A section taken from one of a series of sections of a young haustorium mother cell (HMC) of *Puccinia coronata*. Host wall penetration had begun (arrow), but the haustorium had not yet formed. Mitochondria [M] are densely stained and are located around the periphery of the cell adjacent to the plasmalemma. The nuclei [N] are ovoid and compact. MS, Haustorium mother cell septum (Glt/OsO₄; UA/PbC) ($\times 10,300$; bar, 1 μm). (From Chong, 1981.) Fig. 4. A section showing part of a haustorium mother cell (HMC) of *Puccinia coronata*. The HMC wall is multilayered and is thicker than the hyphal wall, which has only two layers. These two layers are continuous with the outer layers of the HMC wall. The HMC septum (MS) is composed of four layers. The two electron-opaque layers (long arrows) are continuous with the periclinal wall (open arrow). A third, more lightly stained lamella (short arrow) separates the two electron-opaque layers and ends at the periclinal wall. The fourth lightly stained layer (arrowhead) is continuous around the rest of the HMC (Glt/OsO₄; UA/PbC) ($\times 44,300$; bar, 0.25 μm). (From Chong, 1981.) Fig. 5. Part of a young haustorium mother cell (HMC) of *Puccinia graminis* f. sp. *tritici* after protease treatment. The hyphal wall (arrow) is almost completely extracted, but the HMC wall (arrowhead) is less affected. The HMC wall lacks its usual layered appearance (Glt-protease-OsO₄; UA/PbC) ($\times 30,400$; bar, 0.5 μm). (From R. Rohringer and J. Chong, unpublished.)

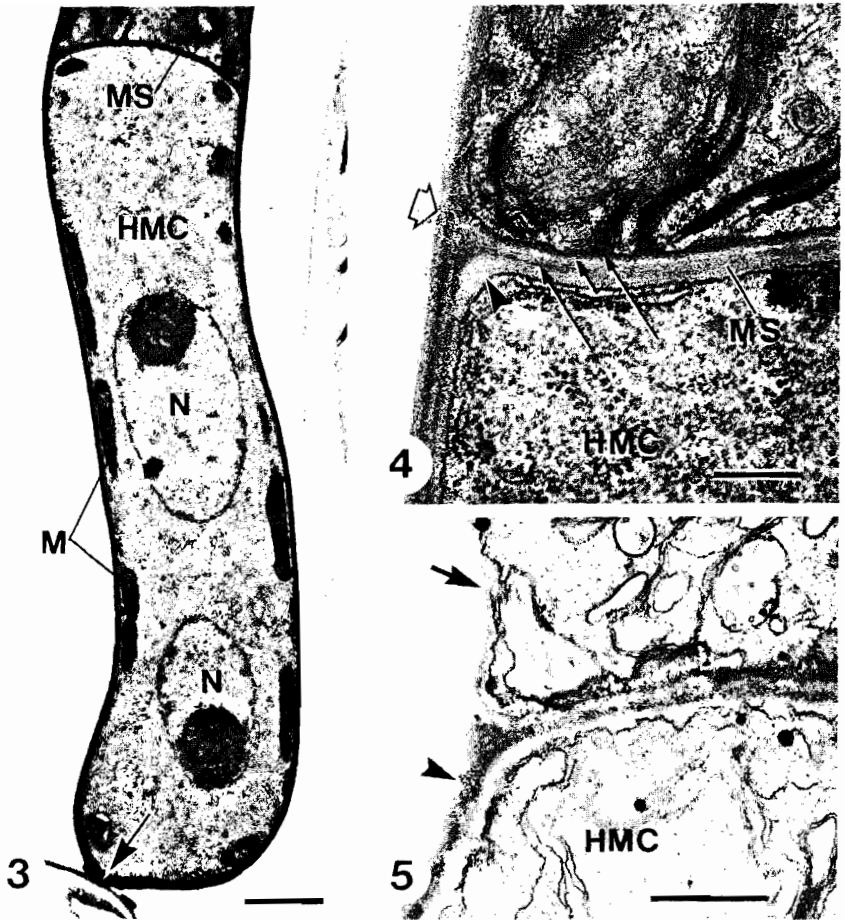


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contain an electron-dense material (Fig. 6, inset). The bounding membranes of the elaborations are continuous with the plasmalemma across the septum (Fig. 7). From these views and from serial sections we have deduced that each "protrusion" is an elongated flattened cisterna (a modification of the tubular protrusion as described by Heath and Heath, 1975), closed at the end distal to the HMC septum, containing a somewhat electron-dense matrix and a more electron-dense core. The elaborations are often interconnected (Chong, 1981), thus forming a large labyrinth-like complex. See Fig. 9 for an interpretation of this complex.

The complex is reminiscent of the wall-membrane elaborations of transfer cells, which facilitate the intercellular movement of substances. These basically involve "surface area amplification" (Gunning, 1977). Heath and Heath (1975) suggested that the occurrence of the septal elaborations during early haustorium formation provided additional membrane area to facilitate energy-requiring rapid transport of materials across the HMC septum.

Although there is no direct evidence for the function of this apparatus, the available information (see also Section IV,H,4) strongly suggests that transfer is a major function, hence we propose the term *septal transfer apparatus*. The term *elaboration* will be retained to designate each of the elongated membranous components of the apparatus.

The growth and retraction of the septal transfer apparatus in both *P. graminis* f. sp. *tritici* and *P. coronata* was closely correlated with stages of haustorium formation (Chong *et al.*, 1981), as summarized in Fig. 2. At first, the elaborations occurred on incompletely formed HMC septa, and varied in length on different septa. At this stage or slightly later, other membrane formations in the form of small whorls of very short duration appeared on the HMC side of the septum (Fig. 8). These stained similarly to fungal plasmalemma after PACP treatment, and may be derivatives of plasmalemma-type membrane.

The septal transfer apparatus attains its maximum size during host wall penetration, then decreases in length during subsequent growth of the haustorial neck. During retraction, the elaborations become angular in outline and later remain as an irregular ridge across the HMC septum. The angular appearance during retraction has been interpreted (Chong *et al.*, 1981) as due to a rapid loss of their contents. The septal transfer apparatus is thus a transient structure, persisting only until the time of haustorial neck formation.

Cytochemical tests of the septal transfer apparatus have indicated that the matrix of the elaborations contains some polysaccharide, un-

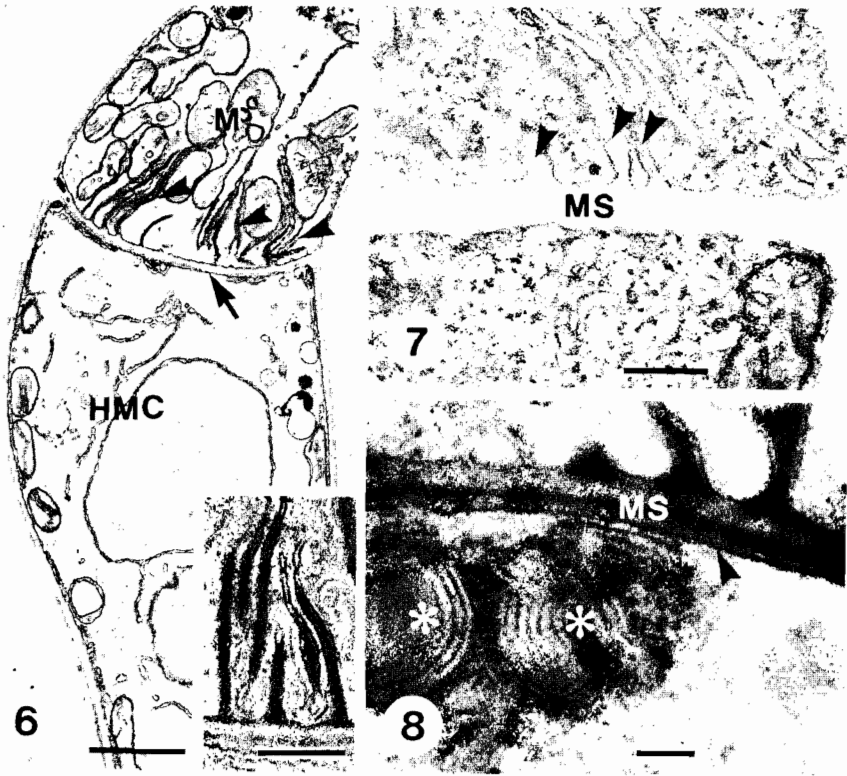


Fig. 6. A haustorium mother cell (HMC) of *Puccinia coronata* sectioned longitudinally to show the fungal plasmalemma elaborations (arrowheads) and associated mitochondria [M] on the hyphal side of the HMC septum [arrow] [Glt/OsO₄]. This section was partially oxidized with periodic acid, then stained with TCH-SP ($\times 12,600$; bar, 1 μm). (J. Chong, unpublished.) Inset: Each elaboration is bound by a membrane and contains an electron-dense matrix and a more electron-dense core [Glt/OsO₄; UA/PbC] ($\times 45,000$; bar, 0.25 μm). (From Chong *et al.*, 1981.) **Fig. 7.** Continuity of the bounding membranes of the elaborations (arrowheads) with the plasmalemma across the haustorium mother cell septum (MS) in *Puccinia coronata*. This is clearly demonstrated after treatment with acetone to extract the septal wall material [Glt-acetone-OsO₄; UA/PbC] ($\times 44,300$; bar, 0.25 μm). (From Chong, 1981.) **Fig. 8.** Whorls of membrane (asterisks) stained in a manner characteristic of the plasmalemma (arrowhead), found on the haustorial mother cell side of the septum (MS) in *Puccinia coronata* [Glt/OsO₄; PACP] ($\times 74,300$; bar, 0.1 μm). (From Chong, 1981.)

saturated lipids, and a large amount of protein (Chong *et al.*, 1981). By using selective extraction and specific staining it was concluded that the polysaccharide, lipid, and protein components may exist as a complex glycolipoprotein (Chong *et al.*, 1981).

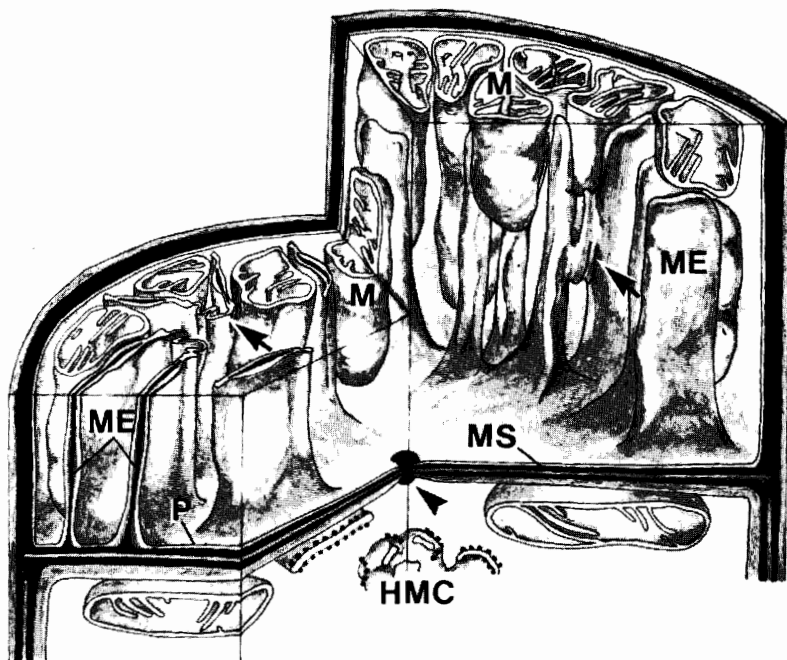


Fig. 9. Diagram of the haustorium mother cell (HMC) septal region cut open to give a three-dimensional interpretation of the principal components: membrane elaborations (ME) (= septal transfer apparatus), plasmalemma (P), HMC septum (MS), and mitochondria (M). For clarity they are not drawn to scale, and some are illustrated by only a few examples (e.g., membrane elaborations and mitochondria). Note the interconnection (arrows) among the membrane elaborations to form a large labyrinth-like complex. The HMC septal pore (arrowhead) is plugged at this stage.

C. HOST CELL PENETRATION

The host cell penetration phase begins with the formation of a penetration peg within an area of contact of the HMC with a host cell. At the site of host cell penetration the HMC wall thickens to assume a convex lens-like shape. This thickening of the HMC wall appears to be universal in all rusts so far examined, and appears to result from the deposition of new wall material (Chong, 1981; Littlefield and Heath, 1979). The penetration peg develops at the center of this thickened region. In the penetration zone the cytoplasm of the HMC is characterized by the presence of electron-dense granules, membranous

whorls, and microtubules (Fig. 10), indicating intense cytoplasmic activity.

The ultrastructural evidence indicates that host wall penetration is mainly a wall dissolution process (Littlefield and Heath, 1979). The act of host wall penetration is difficult to determine by electron microscopy because the process occurs rapidly, and a set of serial sections are required to determine unambiguously the level of penetration. One such set of serial sections obtained for *P. coronata* (Chong *et al.*, 1981) demonstrated that a halo occurred in the host wall in advance of the penetration peg after PA-TCH-SP staining (Fig. 11). This indicates that wall polysaccharides are being dissolved or modified in advance of the penetration peg.

One of the problem areas in the literature has been the evaluation of the occurrence of fungal wall material through the penetration zone. In conventionally processed material there is a very thin layer of fungal wall material in the penetration zone with *P. coronata* and a thicker layer with *P. graminis* f. sp. *tritici* (Chong, 1981). However, better resolution has been obtained by varying the preparative procedures. With *P. graminis* f. sp. *tritici*, material from both the middle and inner wall layers of the HMC wall could be seen in the penetration zone, respectively, after PACP (Chong *et al.*, 1981) and PA-TCH-SP (Fig. 12) staining. Treatment with gold-conjugated Con A, which differentiates host wall and fungal wall material, also showed fungal wall material in the penetration zone (R. Rohringer and J. Chong, unpublished). Our observations have indicated that material from the middle layer of the HMC wall intermingles with host wall material in the penetration zone.

Invasion by *P. coronata* or *P. graminis* f. sp. *tritici* results in the host wall immediately around the penetration site becoming resistant to cell wall-macerating enzymes (*P. graminis* f. sp. *tritici*, Fig. 13). The nature of this host wall modification is not known. The pore in the HMC septum apparently closes and opens during penetration and subsequent haustorial growth. Heath and Heath (1975) first observed that during penetration by *U. phaseoli* var. *vignae*, the HMC septal pore was plugged with a dense material, which persisted until the haustorial body had grown to just beyond its globose form. The pore then lost the dense "plug" and remained open during the mature haustorial phase. A similar sequence of "plugged" and "unplugged" states was found for *P. coronata* and *P. graminis* f. sp. *tritici* (Chong, 1981). This correlation is illustrated for *P. coronata* in Fig. 2. This sequence would appear to restrict the flow of materials out of the HMC during

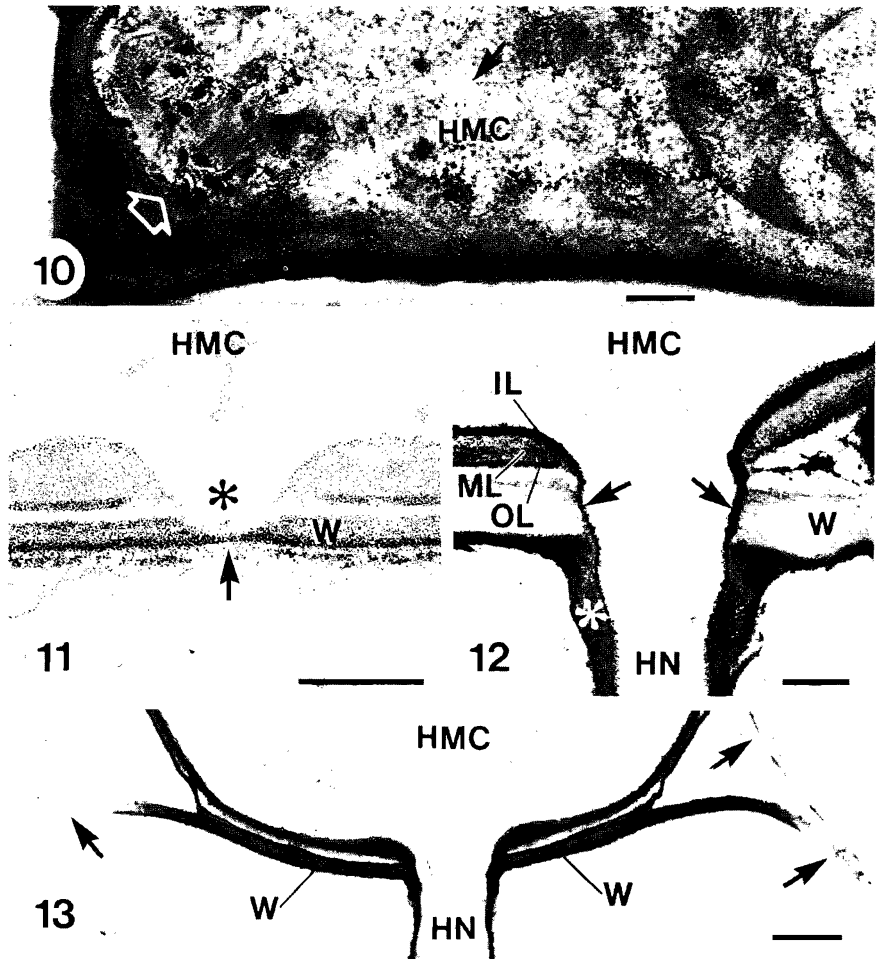


Fig. 10. A nonmedian section of a young haustorium mother cell (HMC) of *Puccinia coronata* to show a microtubule [arrow], membranous materials, and electron-dense granules in the HMC cytoplasm at the site of host penetration [open arrow] (Glt/OsO₄; UA/PbC) ($\times 35,700$; bar, 0.25 μm). [From Chong, 1981.] **Fig. 11.** A median section from a series of closely adjacent sections to show a penetration peg [asterisk] formed from a young haustorium mother cell (HMC) of *Puccinia coronata*. There is a halo [arrow] in the host wall (W) in advance of the penetration peg (Glt/OsO₄; PA-TCH-SP) ($\times 65,000$; bar, 0.25 μm). [From Chong *et al.*, 1981.] **Fig. 12.** A near-median section through the penetration region in *Puccinia graminis* f. sp. *tritici*. The PA-TCH-SP staining shows a distinct fungal wall layer [arrows] through the penetration region, which is continuous with the inner layer (IL) of the haustorium mother cell (HMC) wall, and with the haustorial neck (HN) wall (asterisk). ML and OL, Middle and outer layers of HMC wall, respectively; W, host cell wall (Glt; PA-TCH-SP) ($\times 35,700$; bar, 0.25 μm). [From R. Rohringer and J. Chong, unpublished.] **Fig. 13.** A median section through the penetra-

haustorium formation, then allow the reverse passage of materials after the haustorium has begun to mature.

D. POSTPENETRATION GROWTH OF THE HAUSTORIUM

1. *The Haustorial Neck*

The early postpenetration growth of the haustorium occurs as a tubular finger-like projection into the host cell (Figs. 14 and 15). This projection is referred to as the haustorium initial and later becomes the haustorial neck. Electron-dense granules, some of which are membrane-bound, or amorphous materials occur in the cytoplasm, but no other organelles are present. The cytoplasm is continuous with that of the HMC, which still contains all of the organelles. The electron-dense granules are probably similar to those that aggregate at the penetration site (see Fig. 10). Littlefield and Heath (1979) noted that similar granules in *Melampsora lini* may be involved in the secretion of host wall-degrading enzymes, and that they did not resemble the apical vesicles that are typical of hyphal tip cells. The latter interpretation may be valid, but the occurrence of similar bodies in the cytoplasm of the haustorium initial suggests another role. In histochemical tests the matrix of the transfer apparatus reacted similarly to the haustorial neck wall in *P. coronata* [Chong, 1981; Chong *et al.*, 1981], indicating a similar composition (see Section IV,E on the histochemistry of the neck wall). It is possible that the matrix of the transfer apparatus is used directly in the synthesis of the neck wall, and the electron-dense granules may represent a unique type of "apical vesicle" that is involved in the transfer of this material.

2. *Haustorium Expansion Phase*

After the haustorium initial has grown to a length of about 4 μm , the haustorial body begins to form (Fig. 16). Haustorial bodies at this stage are packed with mitochondria, which apparently migrate from the HMC. At this stage, the neck wall of *P. coronata* is seen to be composed of two moderately stained layers separated by a middle more

tion region in *Puccinia graminis* f. sp. *tritici*. The portion of the host cell wall (W) around the penetration region is resistant to macerating enzymes, whereas the rest (arrows) of the host cell wall is largely extracted. HMC, Haustorial mother cell; HN, haustorial neck [macerating enzymes—Glt/OsO₄; UA/PbC] ($\times 17,900$; bar, 0.5 μm). [From R. Rohringer and J. Chong, unpublished.]

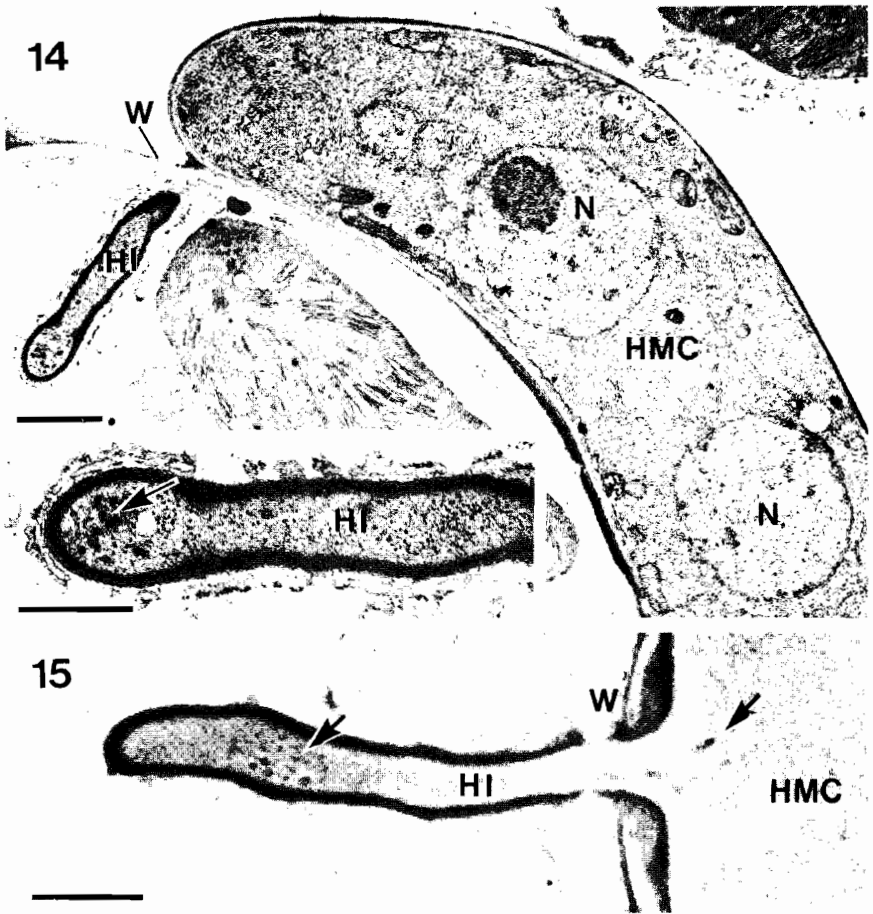


Fig. 14. A haustorium initial (HI) of *Puccinia coronata* consisting of a tubular finger-like projection about $2.6\ \mu\text{m}$ long, extending into the host cell. Adjacent sections did not reveal the presence of a haustorial body. HMC, Haustorial mother cell; N, nucleus; W, host cell wall. Inset: Higher magnification of the haustorium initial to show the presence of electron-dense granules (arrow) in the cytoplasm. The wall of the haustorium initial is seen as one densely staining layer (Glt/OsO₄; UA/PbC) (Fig. 14, $\times 11,100$; bar, $1\ \mu\text{m}$. Inset, $\times 30,400$; bar, $0.5\ \mu\text{m}$). (From Chong, 1981.) **Fig. 15.** A haustorium initial (HI) of *Puccinia graminis* f. sp. *tritici* consisting of a tubular finger-like projection. Note the electron-dense granules (arrows) in the fungal cytoplasm. HMC, Haustorial mother cell; W, host cell wall (Glt/OsO₄; PACP) ($\times 28,600$; bar, $0.5\ \mu\text{m}$). (From Chong, 1981.)

electron-opaque layer (Fig. 17). These layers appear to merge at the base of the haustorial body to form a single-layered body wall (Fig. 18). In contrast, the neck wall of *P. graminis* f. sp. *tritici* was seen to consist of only one densely staining layer in similarly processed material.

The EH membrane lies closely against the wall along the length of the neck, then becomes separated from the wall near the base of the haustorial body of either *P. coronata* or *P. graminis* f. sp. *tritici*. Near

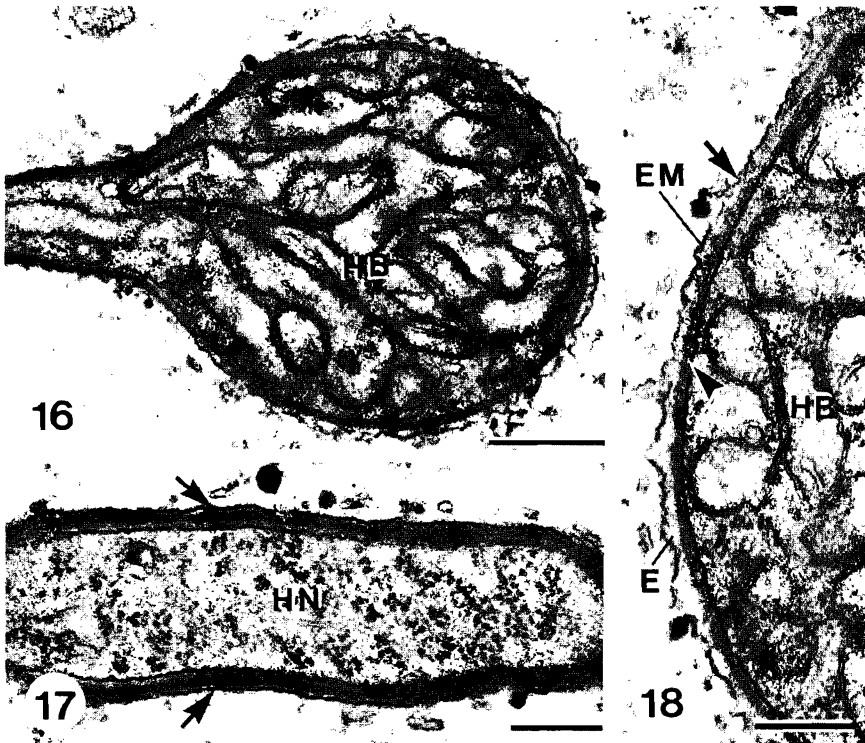


Fig. 16. A young haustorium of *Puccinia coronata* in the early expansion phase. The young haustorial body (HB) is packed with mitochondria (Glt/OsO₄; UA/PbC) ($\times 30,400$; bar, 0.5 μm). (From Chong, 1981.) **Fig. 17.** Part of the haustorial neck (HN) from the same haustorium shown in Fig. 16. Two moderately stained layers separated by a middle electron-dense layer can be seen in the entire neck wall. The extrahaustorial membrane (arrows) adheres tightly to the entire length of the neck. A neck ring is not present at this stage (Glt/OsO₄; UA/PbC) ($\times 47,100$; bar, 0.25 μm). (From Chong, 1981.) **Fig. 18.** Part of the haustorial body (HB) of the same haustorium shown in Fig. 16. The body wall (arrowhead) is composed of only one layer. The extrahaustorial membrane (EM) is separated from the wall near the base (arrow) of the body to form the extrahaustorial matrix (E) (Glt/OsO₄; UA/PbC) ($\times 51,400$; bar, 0.25 μm). (From Chong, 1981.)

this point a variable somewhat electron-lucent (after UA/PbC staining) area, the EH matrix (see Section IV,H,2), intervenes between the EH membrane and the body wall.

When the haustoria of *P. coronata* or *P. graminis* f. sp. *tritici* attain a size of about 5 μm in diameter, an electron-dense band appears in the neck wall and forms a ring around the neck. The neck ring forms approximately midway along the neck of *P. graminis* f. sp. *tritici* and about one-third of the way from the base of the body in *P. coronata* (Chong and Harder, 1980). The neck ring has been found in the D-haustoria of all rusts so far examined. The neck ring in the rusts has been consistently interpreted as a single, intensely osmiophilic band. However, a major variation in neck ring structure has been observed for *P. coronata* (Chong and Harder, 1980). When the neck ring in this fungus is first formed, it appears as a single broad band (Fig. 19), but in mature haustoria a second narrower band is clearly evident (Fig. 20). These two bands have been respectively designated as the α and β bands (Chong and Harder, 1980). The significance of the PA-TCH-SP "staining" of the α band in Fig. 19 is discussed later in Section IV,E. In contrast, only a single band has been resolved in the haustorial necks of *P. graminis* f. sp. *tritici* (Chong, 1981).

A possible explanation for the function of the neck ring was provided by Heath (1976) when it was shown that uranyl acetate crystals occurred between the host and fungal cell membranes up to but not beyond (proximal end of the neck) the neck ring of *P. sorghi*. This indicates that there is an apoplastic flow of materials along the neck wall that is stopped by the neck ring. This observation strengthened an earlier suggestion that the tight association of host and fungal membranes with the neck ring is reminiscent of the Casparian strip of endodermal cells in roots of higher plants (Littlefield and Bracker, 1972). The tight association of the extrahaustorial membrane and the neck ring of *P. coronata* is clearly demonstrated after protease treatment where the neck wall is extracted, but the neck ring remains intact, and the extrahaustorial membrane remains bound to the neck ring (see Fig. 24). The neck ring is arguably a structure that has been evolved by the rust fungi to force a symplastic route (through the haustorium) for the movement of solutes from host to parasite.

Despite the importance of the view just advanced, little is known about the chemical composition of the neck ring. In *P. coronata* the α band is resistant to periodic acid digestion (Fig. 19), but the β band is extracted. Similarly, the entire single bands of mature haustoria of *P. graminis* f. sp. *tritici* (Fig. 21) or *Melampsora lini* (Littlefield and Bracker, 1972) are extracted by periodic acid. It was shown (Chong and

Harder, 1980) that both bands of *P. coronata* are inherently electron-dense, and using energy-dispersive X-ray (EDX) analysis, the α band was found to have a high silicon content, while the β band had iron and phosphorus, probably in the form of ferric pyrophosphate (Chong and Harder, 1980). Although the single bands of *P. graminis* f. sp. *tritici* or *M. lini* have not been subjected to EDX analysis, the fact that they are periodic acid-extractable, similar to the β band of *P. coronata*, suggests that they may be similar in composition.

A haustorium is considered to be mature when nearly all of the cytoplasm of the HMC has migrated into the haustorium, leaving the HMC largely vacuolate. In these haustoria the mitochondria tend to retain the peripheral distribution as in the HMC (Chong, 1981), but they again assume the more irregular, less compact form similar to that in the intercellular hyphae (see Harder, Chapter 11, this volume). The peripheral distribution of the haustorial mitochondria appears to offer an advantage in the active transport of materials into the haustorium.

The nuclei in the mature haustoria become more irregular in shape as compared to their more compact ovoid form in the HMCs. The nucleoli in haustoria of either *P. coronata* or *P. graminis* f. sp. *tritici*

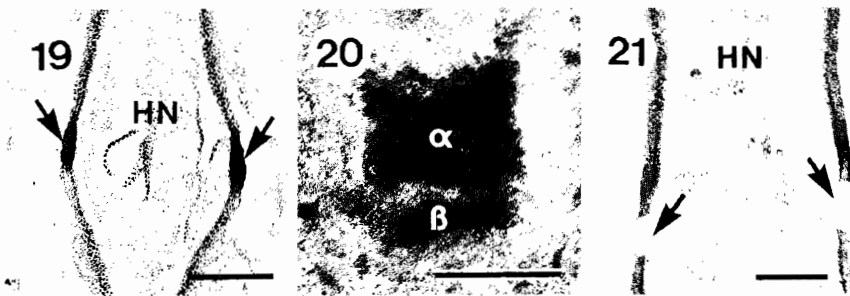


Fig. 19. A near-longitudinal section of the neck (HN) of a young haustorium of *Puccinia coronata* with only a single band present [Glt/OsO₄; PA-TCH-SP] ($\times 44,600$; bar, 0.25 μm). [From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.] **Fig. 20.** An oblique tangential section of a mature haustorial neck of *Puccinia coronata*. Note the presence of two bands. The larger band closer to the haustorial mother cell is designated as the α band, the smaller one closer to the haustorial body, the β band [Glt/OsO₄; UA/PbC] ($\times 66,400$; bar, 0.25 μm). [From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.] **Fig. 21.** A near-longitudinal section of a haustorial neck (HN) of a mature haustorium of *Puccinia graminis* f. sp. *tritici* after PACP staining. The entire neck ring (arrows) is electron-lucent [Glt; PACP] ($\times 36,400$; bar, 0.25 μm). [From R. Rohringer and J. Chong, unpublished.]

(Harder *et al.*, 1978, and unpublished) are smaller and without intranucleolar lacunae, and are less granular than those in active intercellular hyphal cells. Nucleolar granules are considered to be precursors of cytoplasmic ribosomes (Smetana and Busch, 1974) and thus are associated with synthetic activity. The reduced granular component of nucleoli in haustoria suggests that the haustorium is not actively involved in the synthesis of new materials.

By light microscopy, the mature haustoria of *P. coronata* (Ruttle and Fraser, 1927) and *P. recondita* (Allen, 1926) were found to contain only one nucleus. We have examined numerous haustoria of the former fungus by electron microscopy and have never seen more than one nucleus, although two nuclei are always found in the young HMCs. A similar observation was made for *P. poarum* by Al-Khesraji and Lösel (1981). It has not been particularly difficult to find two nuclei in haustoria of other rusts by electron microscopy. The significance of the observation of a single nucleus in haustoria of *P. coronata* or *P. poarum* awaits further investigation.

E. ORGANIZATION AND CYTOCHEMISTRY OF THE HAUSTORIAL WALLS

The organization and cytochemistry of the haustorial walls is of considerable interest because these form part of the host-pathogen interface (Bracker and Littlefield, 1973), and they may be involved in plant and fungus recognition (Rohringer *et al.*, 1982). A number of histochemical tests have been performed to identify components of walls of both immature and mature haustoria of *P. coronata* (Chong, 1981; Chong *et al.*, 1981) and *P. graminis* f. sp. *tritici* (Chong, 1981; Rohringer *et al.*, 1984). The results of these tests for *P. graminis* f. sp. *tritici* are summarized in Fig. 22. For a description of the tests used, see Section II. The diagram in Fig. 22 is included to facilitate identification of different portions of the neck or body walls and to indicate that the inner layer (IL) of the neck wall becomes thicker to comprise the bulk of the body wall, while the thick outer layer (OL) remains as a narrow band around the haustorium. The haustorial walls of *P. coronata* responded similarly to the tests in Fig. 22 except for the response to protease. In *P. coronata* the entire outer layer of the neck wall between the penetration peg and haustorial body is digested by protease (Fig. 24), whereas in *P. graminis* f. sp. *tritici* the portion of the neck wall between the neck ring and penetration peg is resistant to this enzyme (Figs. 22 and 23). These results indicate two types of neck wall organi-

Immature				Mature					
PA-TCH-SP	WGL	Con A		PA-TCH-SP	WGL	Con A	Protease	Con A after protease	
OL	+	-	+	OL	+	-	+	-	-
IL	++	-	+	IL	++	-	+	-	+
OL	+	-	+	OL	+	-	+	+	-
IL	++	-	+	IL	++	-	+	-	-
OL	+	-	+	OL	+	?	?	+	?
IL	++	-	+	IL	++	+	-	+	-

Fig. 22. Positive (+) or negative (-) reactions of the outer [OL] or inner [IL] wall layers of immature or mature haustoria of *Puccinia graminis* f. sp. *tritici* after treatment with PA-TCH-SP, WGL, Con A, or protease. Note that the OL and IL of the neck wall become reversed in relative thickness around the haustorial body. The treatments [also see Section II] are for detection of substances as follows: PA-TCH-SP, Polysaccharides with vicinal hydroxyl groups; WGL, chitin; Con A, α -linked glucose or mannose; protease, protein; Con A after protease, α -linked carbohydrates that are not bound to proteins.

zation: One in which the neck ring marks an abrupt transition in the properties of the neck wall (*P. graminis* f. sp. *tritici*), and the other in which the entire neck wall appears to be uniform in composition (*P. coronata*). Similar conclusions regarding two different types of neck wall organization in the rust fungi were made by Littlefield and Heath (1979).

The conclusions drawn from the application of the cytochemical tests in various combinations were that the major components of the walls (i.e., protein, carbohydrate, and lipid) exist in complex forms, probably as glycoproteins, lipoproteins, or glycolipoproteins (Chong, 1981; Chong *et al.*, 1981). Also, the properties of the walls change as the haustoria mature; the haustorial body walls become more resistant to protease and acquire a chitin component as indicated by the increased wheat germ lectin binding. Probably the most significant finding is that the neck wall is unique in composition relative to the walls of any other part of the rust fungal thallus, particularly in its apparent lack of chitin. It has been suggested (Rohringer *et al.*, 1982) that the neck wall may carry host-rust recognition factors in the determination of compatibility or incompatibility in the interaction between wheat and *P. graminis* and f. sp. *tritici* containing the *P6* gene for avirulence.

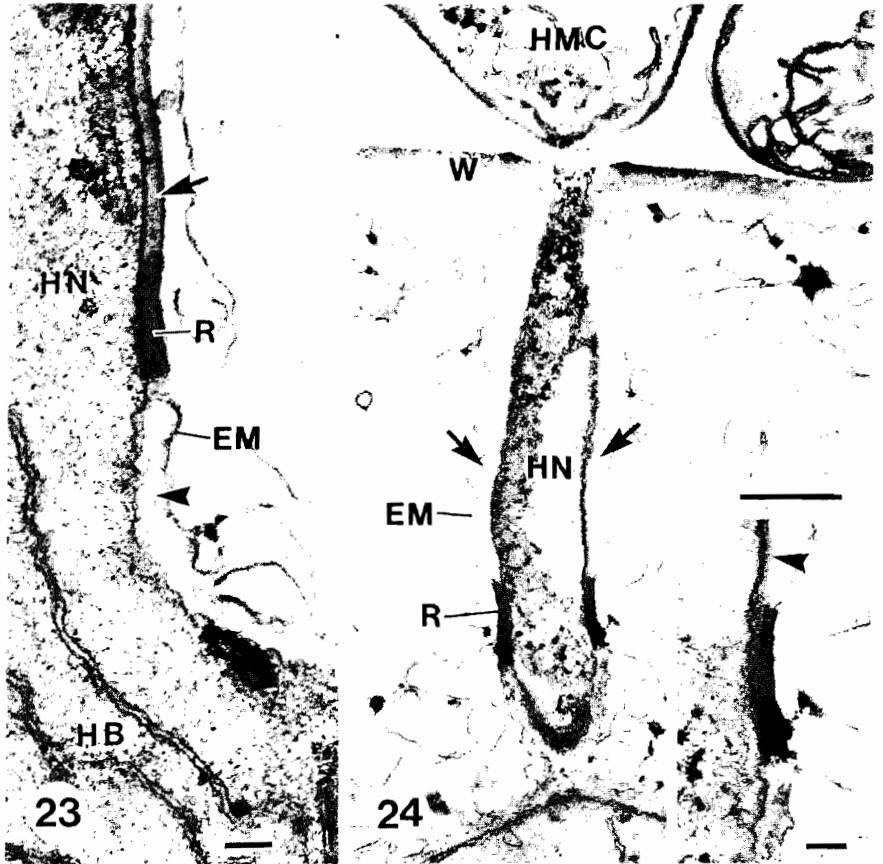


Fig. 23. Differential extraction of the haustorial walls of a mature haustorium of *Puccinia graminis* f. sp. *tritici* after protease treatment. The part of the neck (HN) wall (arrows) between the neck ring (R) and the penetration peg is resistant to protease, but the portion (arrowhead) above the neck ring and the body (HB) wall have been largely digested. The extrahaustorial membrane (EM) adheres tightly to the neck ring and to the part of the neck wall that is not affected by protease, but is convoluted where the wall has been digested (Glt-protease-OsO₄; UA/PbC) ($\times 62,500$; bar, 0.1 μm). [From R. Rohringer and J. Chong, unpublished.] Fig. 24. The haustorial neck (HN) wall (arrows) of a mature haustorium of *Puccinia coronata* is largely electron-lucent after protease treatment. The extrahaustorial membrane (EM) has been freed from the neck except at the neck ring (R), where it remains tightly bound. Note separation of the haustorium mother cell (HMC) from its haustorium. W, Host cell wall. Inset shows the remaining thin layer (arrowhead) of fungal wall material along the neck (Glt-protease-OsO₄; UA/PbC) (Fig. 24: $\times 26,300$; bar, 0.5 μm . Inset: $\times 53,000$; bar, 0.1 μm). [Fig. 24: From Chong *et al.*, 1981. Inset: From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.]

F. POLYPHOSPHATES

In older haustoria and in young HMCs, small vacuoles that contain electron-dense granules are frequently observed. In glutaraldehyde-fixed, unstained sections of haustoria of *P. coronata*, these granules are electron-dense (Fig. 25), suggesting that they have a mineral composition. With EDX analysis these granules were found to be rich in phosphorus and to contain some iron and sulfur (Chong and Harder, 1982a), indicating that they may contain polyphosphate. Polyphosphates commonly occur in the fungi, and in *P. graminis* f. sp. *tritici* much of the polyphosphate occurs in the urediospores (Bennett and Scott, 1971).

G. HAUSTORIA IN THE CENTERS OF AGED COLONIES

Ruttle and Fraser (1927) noted by light microscopy that the haustoria and HMCs near the center of older, well-developed colonies of *P. coronata* appeared to be aberrant. In particular, the HMCs were distorted

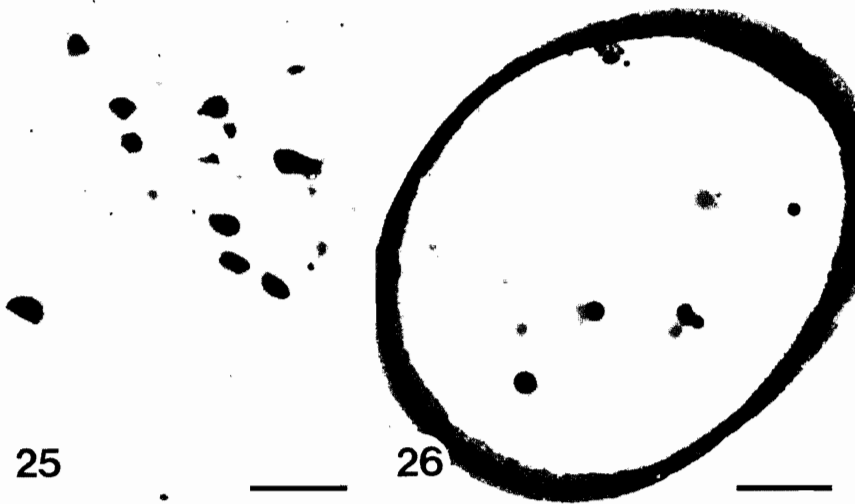


Fig. 25. A semithin unstained section of part of an old haustorium of *Puccinia coronata* showing the presence of large electron-dense granules in the protoplast. This section had been subjected to EDX analysis (Glt; unstained) ($\times 12,600$; bar, $1 \mu\text{m}$). (From Chong, 1981.) **Fig. 26.** A semithin unstained section of an old haustorium of *Puccinia coronata* showing the heavy accumulation of electron-dense deposits in the wall. This section had been subjected to EDX analysis (Glt; unstained) ($\times 25,700$; bar, $0.5 \mu\text{m}$). (From Chong, 1981.)

and the walls were glassy in appearance, and in extreme cases the lumen of these cells was almost completely obliterated by the swollen wall. Electron microscopy showed that similarly located haustoria were distorted and densely staining (Chong, 1981), similar to necrotic haustoria in incompatible interactions. When the tissue was fixed only in glutaraldehyde and the sections left unstained, electron-dense deposits were found in the walls of the HMCs (Fig. 26) as well as the HMC septa. These modified walls were subsequently shown by EDX analysis to be heavily silicified (Chong, 1981). This finding explains the "glassy" appearance noted by Ruttle and Fraser (1927). This also provides a clue to a possible unique mechanism evolved by this fungus to protect itself from toxic by-products. During the late stages of rust infection many of the host cells become disorganized, presumably releasing products that are detrimental to the fungus. This results in a physiologically incompatible situation, in which the haustoria die (Chong, 1981). However, to prevent deleterious products from reaching the remainder of the fungal thallus, the HMC walls and the HMC septum become heavily silicified, likely making them resistant to the passage of these products. Similar electron-dense deposits were also found in the walls and septa of many of the HMCs of *P. graminis* f. sp. *tritici* located at or near the center of the colonies.

H. THE HOST-HAUSTORIAL INTERFACE

1. *The Extrahaustorial (EH) Membrane*

During the growth of rust haustoria in their host cells, the host plasmalemma becomes invaginated and surrounds the entire haustorium. The invaginated plasmalemma consists of newly synthesized membrane. The part of the invaginated host plasmalemma that surrounds the haustorium, beginning at the penetration site, is referred to as the extrahaustorial (EH) membrane. The EH membrane is closely associated with the haustorial neck wall (Harder *et al.*, 1978), but around the haustorial body a matrix of material intervenes between the body wall and EH membrane.

In most conventionally processed tissue the EH membrane is undulated (Fig. 27), but preliminary results from freeze-substitution indicate that this membrane is in fact smooth (D. E. Harder and K. Mendgen, unpublished). The fixation with glutaraldehyde, as performed for conventional electron microscopy, may result in alteration of membrane conformation (Willison and Brown, 1979). The conformation of the membrane may also be affected by the age of the haustorium. In

freeze-etched preparations, EH membranes range from smooth to very rough under similar conditions of preparation, apparently varying with age (D. E. Harder and K. Mendgen, unpublished). The EH membrane in Fig. 28 represents a view of a moderately rough EH membrane.

In most profiles seen by electron microscopy the EH membrane is thicker than the other host membranes, and continues to thicken and may attain a more diffuse outline as the haustorium ages (Harder *et al.*, 1978). The EH membrane is continuous with the host plasmalemma and presumably would share some of its properties. The PACP stain, which is specific for plant plasma membrane, also intensely stains the EH membrane in *P. coronata* (Fig. 27) as well as in *P. graminis* f. sp. *tritici* infections (Harder *et al.*, 1978). In this respect the EH membrane is similar to the plasmalemma. However, Harder and Mendgen (1982) showed by freeze-etch electron microscopy after filipin treatment that the EH membrane contains considerably less sterol than does the host plasmalemma. Also, Spencer-Phillips and Gay (1981) demonstrated a lack of ATPase activity at the extrahaustorial membrane as compared to the noninvaginated host plasmalemma in *U. appendiculatus* infections. It was suggested by the latter workers that an enzyme-deficient host plasma membrane is developed around the haustoria.

A specific role, if any, of the EH membrane in rust–host interactions is still speculative. One possible role is the control of the flow of

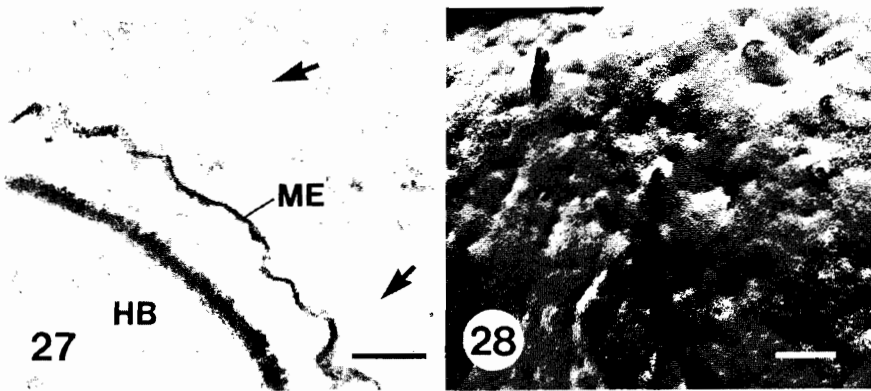


Fig. 27. The extrahaustorial membrane (ME) around a haustorial body (HB) of *Puccinia coronata* is undulated and stains more densely than tubule membranes (arrows) and other membranes of the host (Glt/OsO₄; PACP) [$\times 40,000$; bar, 0.5 μm]. (From Chong *et al.*, 1981.) **Fig. 28.** A freeze-etch replica of the extrahaustorial membrane around a haustorium of *Puccinia coronata*. The extrahaustorial membrane has a moderately rough profile (Glt; freeze-etch) [$\times 30,900$; bar, 0.5 μm]. (D. E. Harder and K. Mendgen, unpublished.)

metabolites into or out of the haustorium through alterations in permeability. Membrane sterol is known to play a role in membrane permeability, thus the change in sterol content may reflect such a role. A more intriguing possibility involves the association of the EH membrane with other host membranes. As will be seen (Section IV,H,3) there is an extensive association of the EH membrane with host endoplasmic reticulum. Such direct associations between the plasmalemma and endoplasmic reticulum are rare, and the change in sterol content of the EH membrane may result in greater compatibility between these two types of membranes. An alternate view to the control of metabolite flow directly through the EH membrane will be presented in Section IV,H,4.

2. The Extrahaustorial (EH) Matrix

No part of the haustorial apparatus has led to more speculation than the EH matrix. In the rust fungi, the EH matrix is of universal occurrence around the body of the haustorium but is highly variable in appearance. The matrix ranges from a narrow, nearly electron-lucent band to an apparently broader zone containing various amounts of fibrillar or granular electron-dense substances. The variability in appearance has frequently been related to the age of the haustorium or degree of compatibility with the host. The matrix has variously been considered to be derived from the fungal wall, to be of host origin, or to be an artifact resulting from histological preparation procedures. Despite the attention paid to the EH matrix, there is little definitive information in the literature on its composition.

Recent work on *P. coronata* (Chong, 1981; Chong *et al.*, 1981) and *P. graminis* f. sp. *tritici* (Rohringer *et al.*, 1984) has provided some information on the composition of the matrix. The EH matrices of *P. coronata* and *P. graminis* f. sp. *tritici* were shown to contain mixtures of lipid, larger amounts of polysaccharide, and protein. At least two types of polysaccharides were apparent: cellulose, which may be a response of the host to build a wall at this interface, and protein-bound polysaccharide (glycoprotein). The variability in electron density that is normally encountered in the matrix is probably due to the level of solubilization, or accumulation and polymerization of its components. The latter appears to increase with increasing age of the haustorium. In any case, the contents of the matrix are clearly not an artifact of preparation procedures. Some preliminary work on freeze-substitution of haustoria of *P. coronata* or *U. appendiculatus* var. *appendiculatus* (D.

E. Harder and K. Mendgen, unpublished) has indicated that the matrix is structurally a uniform and easily recognizable entity.

The differentiation of the matrix from the haustorial wall is frequently unclear. Many micrographs show a diffuse, somewhat frayed zone at the juncture of the matrix and the body wall. This led Littlefield and Heath (1979) to suggest that although the wall and matrix appeared distinct, perhaps matrix material impinged into the outer surface of the haustorial wall (or vice versa). Histochemical tests (Chong, 1981; Chong *et al.*, 1981) showed that the wall and matrix are clearly distinguished in mature haustoria of the two rusts studied: There were no WGL receptor sites (i.e., chitin) in the matrix, but they were common in the wall. In mature haustoria of *P. graminis* f. sp. *tritici*, there were no Con A receptor sites in the body wall, but they were common in the matrix. However, the outer surface of the wall is probably less smooth than the inner surface; whether this is an introduced artifact is not certain, but it may represent a larger wall surface area for solute transfer.

3. Association of Host Endoplasmic Reticulum (ER)

Invasion of the host cell results in marked alteration of the distribution and configuration of host ER. This appears to be a generalized phenomenon throughout the rusts (see Littlefield and Heath, 1979). Endoplasmic reticulum occurs around the bodies of young haustoria of *P. graminis* f. sp. *tritici*, but the greatest association of ER is in the neck region of young developing haustoria (Ehrlich and Ehrlich, 1971; Harder *et al.*, 1978). The extent of ER association in *P. graminis* f. sp. *tritici* is seen in Fig. 29, where much of the ER radiates from the haustorial neck region into the surrounding host cytoplasm. The association of ER with young haustoria is the most striking in *P. graminis* f. sp. *tritici* infections, and has not been observed to such an extent in other host-rust interactions. In *P. coronata* infections the ER cisternae tend to be parallel to the EH membrane (Harder, 1978) (Fig. 30). In *P. graminis* f. sp. *tritici* the extensive association of ER with the haustoria in the neck region tends to diminish as the haustoria mature. Although the host ER has been shown to contact the EH membrane extensively in the neck region, direct-line continuity between these membranes has not been established. However, there appears to be direct-line continuity between ER and the EH membrane around the haustorial body where the EH matrix is apparent (Harder *et al.*, 1978). Convincing

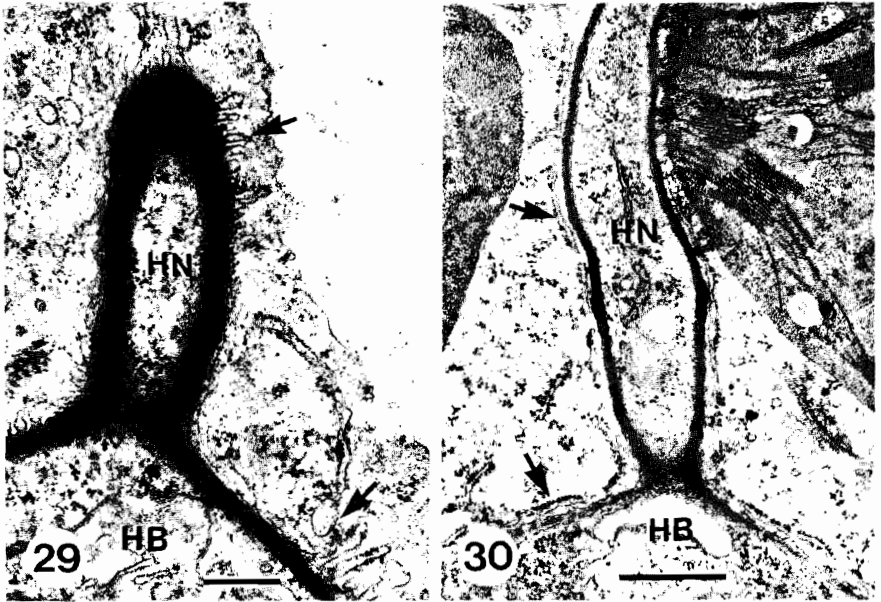


Fig. 29. A nonmedian section of a young haustorium of *Puccinia graminis* f. sp. *tritici* showing the extensive association of host endoplasmic reticulum [arrows] with the neck (HN) and body (HB) [Glt/OsO₄; UA/PbC] ($\times 19,300$; bar, 0.5 μm). (From D. E. Harder and R. Rohringer, unpublished.) Fig. 30. The host endoplasmic reticulum cisternae (arrows) tend to lie parallel to the extrahaustorial membrane in *Puccinia coronata*. HN, Haustorial neck; HB, haustorial body [Glt/OsO₄; UA/PbC] ($\times 27,900$; bar, 0.5 μm). (From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.)

evidence for such continuity is difficult to find, and only in a few cases is it readily apparent.

4. Association of Host Membrane Complexes

A number of reports describe vesicles or tubular membranous structures in the host cytoplasm near the haustoria (Chong *et al.*, 1981; Ehrlich and Ehrlich, 1963, 1971; Harder, 1978; Harder *et al.*, 1978; Rijkenberg, 1975; Van Dyke and Hooker, 1969; Yudkin and Reiter, 1979). The vesicular configurations as noted in several of those articles are probably parts of tubules, as indicated in work on *P. graminis* f. sp. *tritici* (Harder *et al.*, 1978) and *P. coronata* (Harder, 1978; Chong *et al.*, 1981). (See 2 in Note Added in Proof.) In *P. coronata* the tubules develop as an irregular network (Fig. 31), whereas in *P. graminis* f. sp. *tritici* there is a more highly organized complex of small and large tubules

(Figs. 33 and 34). The latter complexes were shown to be derived from the host ER (Harder *et al.*, 1978), and the same appears to be true for *P. coronata* (J. Chong and D. E. Harder, unpublished). The small and large tubules in *P. graminis* f. sp. *tritici* infections are interconnected, and the entire complex may surround part of the haustorium. A three-dimensional interpretation of this complex is shown in Fig. 36. The membranes of these complexes, both in *P. coronata* and *P. graminis* f. sp. *tritici* infections, have frequently been observed to be continuous with the EH membrane (Fig. 32, inset). In *P. coronata* infections the tubular complexes were most commonly found in the host cytoplasm between the haustorium and adjacent host nucleus (Fig. 32) (Chong, 1981). This has not been observed for *P. graminis* f. sp. *tritici* infections. For the latter, individual complexes were observed to be interconnected by ER, ramifying extensively around the haustorium (Harder *et al.*, 1978).

Membrane configurations similar to the complexes in the *P. graminis* f. sp. *tritici* or *P. coronata* infections have never been seen in the absence of infection, thus the complexes are probably specifically induced by the invading fungus. The type of complex induced in oats by *P. graminis* f. sp. *avenae* (Fig. 35) is similar to those induced in wheat by *P. graminis* f. sp. *tritici*, as distinct from the type induced in oats by *P. coronata*. This demonstrates alteration of host processes that are specific to the species of the invading fungus; that is, the fungus is able to pass a message(s) into the cell to alter specifically the metabolic processes in that cell.

The structure of the components of the membranous-tubular complexes are reminiscent of the transfer apparatus associated with the HMC septum (Section IV,B). The main feature involves an electron-dense core bound by a membrane. This was consistent for the two rusts studied regardless of the organization of the complexes. As mentioned earlier, membrane structures of somewhat similar shape have been regarded as functional sites where intensive secretion or absorption may take place (Berridge and Oschman, 1972; Gunning, 1977). We interpret the haustorium-associated membranous complexes to be synthetic or secretory bodies related directly to the requirements of the fungus. In regard to the host-pathogen interface, the concept that the EH membrane forms the most immediate interface between the host and pathogen protoplasts requires revision in view of the large ramification of the complexes around the haustorium. These complexes are open directly to the EH matrix, and are themselves interconnected via the host ER system. This network most likely includes the nucleus, as the ER is also continuous with the outer membrane of the nuclear

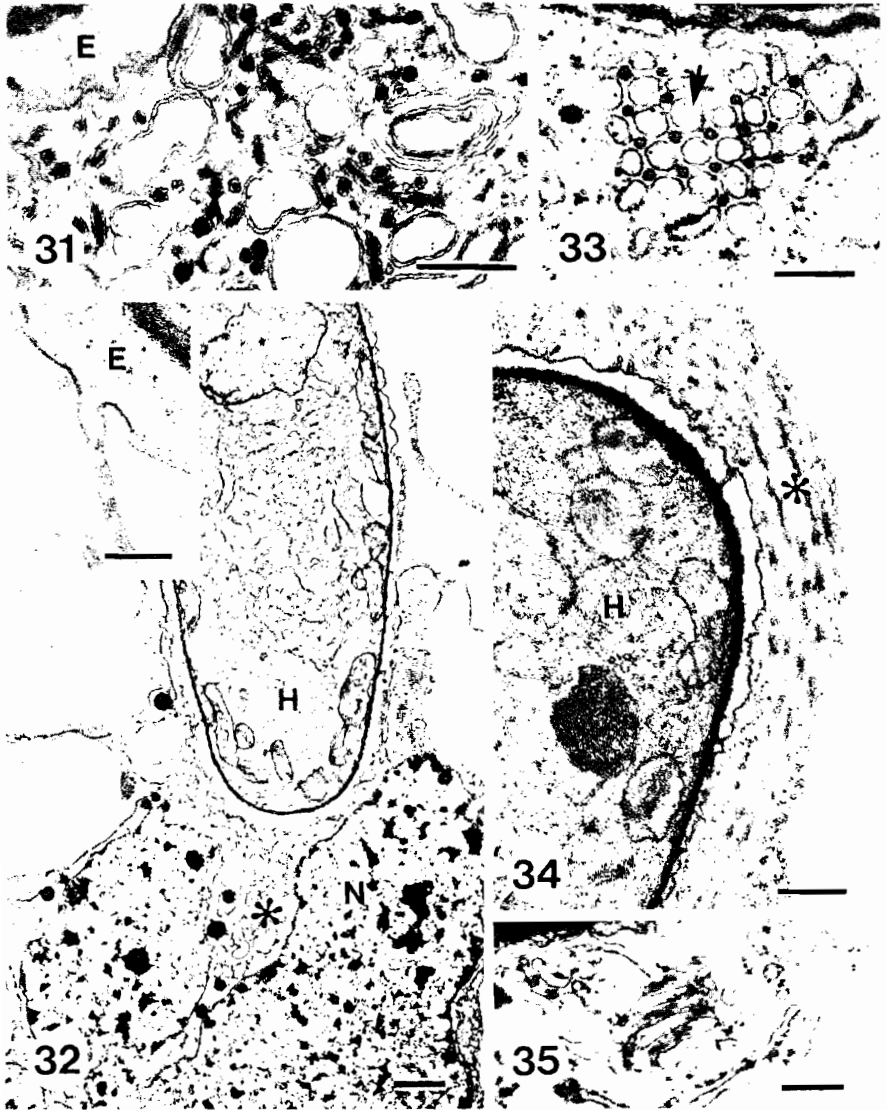


Fig. 31. Development of an irregular network of tubules in the host cytoplasm near the haustorium of *Puccinia coronata*. The tubules contained an electron-dense core. E, Extrahaustorial matrix (Glt/OsO₄; UA/PbC) ($\times 25,700$; bar, 0.5 μm). (From Chong, 1981.) Fig. 32. Close association between a haustorium (H) of *Puccinia coronata* and the host nucleus [N]. Cytoplasmic tubules are found in the region (asterisk) between the haustorium and the host nucleus. The portion of the host nucleus surrounding the tubule complex is lobed (Glt/OsO₄-K₃Fe(CN)₆) ($\times 6900$; bar, 1.0 μm). Inset shows the continuity of a tubule with the extrahaustorial matrix (E) in *Puccinia gaminis* f. sp.

envelope (Morré and Mollenhauer, 1974). The "functional" interface in effect extends throughout the host cell. Gunning (1977) suggested that the undulated nature of the EH membrane around fungal haustoria was to increase the surface area to facilitate transfer of substances. However, this may be relatively less important, for as noted earlier, the undulation of the EH membrane may be largely artifactual (D. E. Harder and K. Mendgen, unpublished). In view of the extensive access to the host cell's metabolic machinery through the EH membrane-associated tubular complexes, emphasis should also be placed on the complexes to provide and facilitate the flow of metabolites.

V. Monokaryotic Haustoria

Observations by light microscopy on the basidiospore-derived monokaryotic infections of several cereal rusts (Allen, 1930, 1932a,b) showed that their intracellular structures were more filamentous than the haustoria in the dikaryotic infections. Electron microscopy has supplemented these findings and has clearly shown that the monokaryotic (M) intracellular structures of a number of rusts have little of the structural specialization of the D-haustorial apparatus. The type of intracellular structure formed is considered to be dependent on the karyotic state of the thallus and not on the host species infected (Gold *et al.*, 1979). Within the cereal rusts, the M-intracellular structure has variously been designated as haustorium (Allen, 1930, 1932a,b), P-haustorium (Harder, 1978), or intracellular hypha (Rijkenberg and Truter, 1973; Al-Khesraji *et al.*, 1980; Al-Khesraji and Lösel, 1980, 1981; Gold *et al.*, 1979). Littlefield and Heath (1979) introduced the term *M-haustorium* to cover

tritici (Glt/OsO₄; PA-TCH-SP) [$\times 34,300$; bar, 0.25 μm]. (From Chong, 1981.) **Fig. 33.** Cross section of a membrane complex near a haustorium of *Puccinia graminis* f. sp. *tritici*. Note the orderly arrangement of two types of tubules: smaller ones containing an electron-dense core and larger ones (arrow) with electron-lucent contents (Glt/OsO₄; UA/PbC) [$\times 41,400$; bar, 0.25 μm]. (From Harder *et al.*, 1978. Reproduced by permission from the National Research Council of Canada.) **Fig. 34.** A near-longitudinal section of a large membrane complex (asterisk) similar to that shown in Fig. 33, in the host cytoplasm near a mature haustorium (H) of *Puccinia graminis* f. sp. *tritici* (Glt/OsO₄; UA/PbC) [$\times 18,000$; bar, 0.5 μm]. (From D. E. Harder and R. Rohringer, unpublished.) **Fig. 35.** An oblique section of a membrane complex induced in an oat mesophyll cell by *Puccinia graminis* f. sp. *avenae*. This complex is characteristic of those induced in wheat cells by *Puccinia graminis* f. sp. *tritici* (Glt/OsO₄; UA/PbC) [$\times 33,200$; bar, 0.25 μm]. (From D. E. Harder, unpublished.)

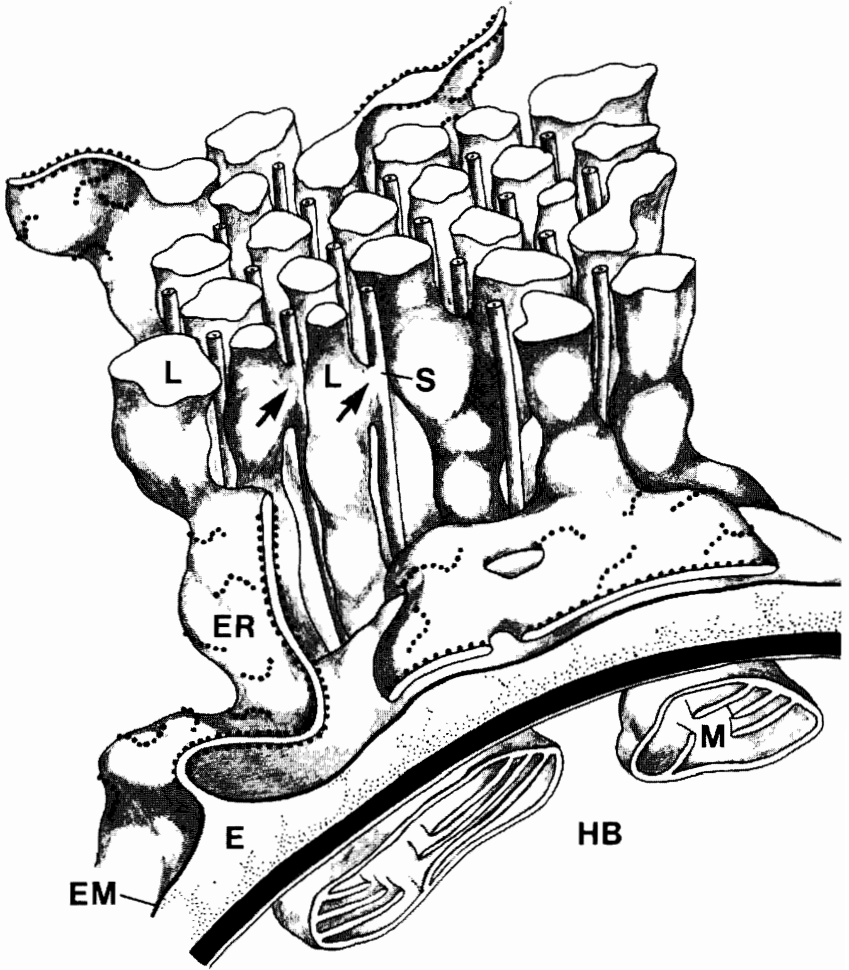


Fig. 36. Diagram of a haustorium (*Puccinia graminis* f. sp. *tritici*)-associated organized membrane complex (reconstructed from a series of serial sections) cut open to show the principal components and their interrelationships. Note the connections (arrows) between the large (L) and small (S) tubules. The large tubules are also connected to the surrounding host endoplasmic reticulum (ER), which in turn is continuous with the extrahaustorial matrix [E]. EM, Extrahaustorial membrane; HB, haustorial body; M, mitochondria.

the M-intracellular structures of all the rusts, and this terminology will be retained here. (See 3 in Note Added in Proof.)

The features that typify the development and structure of a M-haustorium of *P. coronata* are illustrated in the drawing in Fig. 37. Although this drawing is summarized from observations involving *P. coronata*, the structural features, except as noted later, are generally representative of the M-haustoria of the cereal rusts known so far from ultrastructural studies (Chong, 1981; Chong *et al.*, 1981; Al-Khesraji and Lösel, 1980, 1981; Al-Khesraji *et al.*, 1980; Gold *et al.*, 1979; Hard-

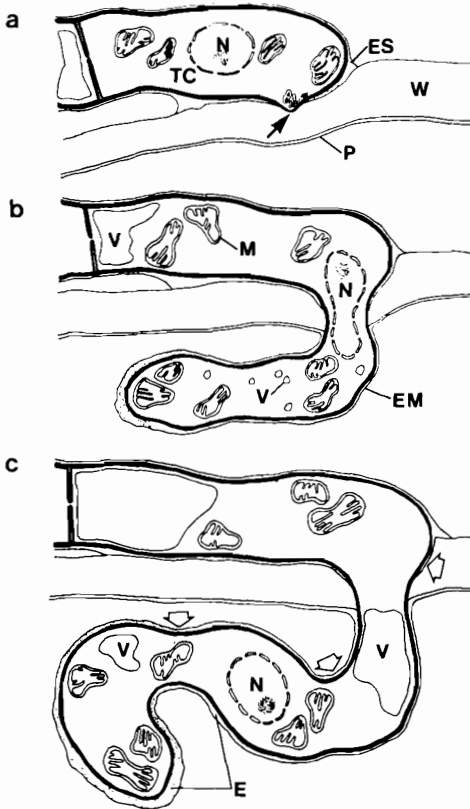


Fig. 37. A diagrammatic, chronological representation of M-haustorium development in *Puccinia coronata*. (a) M-haustorium formation is initiated by a protuberance (arrow) from a terminal cell (TC). (b) After penetration, subsequent growth of the protuberance forms the haustorium. (c) Old M-haustoria are often septate, and possible location of septation is indicated (open arrows). E, Extrahaustorial matrix; EM, extrahaustorial membrane; ES, extracellular coating substance; M, mitochondrion; N, nucleus; P, plasmalemma; V, vesicle; W, host cell wall.

er, 1978; Rijkenberg and Truter, 1973]. The following description of M-haustoria is based on the morphological features of D-haustoria to illustrate how these two intracellular structures compare and where they differ.

1. The M-haustorium arises from a terminal intercellular hyphal cell.
2. There is no differentiation of a specialized HMC nor development of a specialized HMC septum with its transfer apparatus.
3. Penetration is likely accomplished by enzymatic digestion of the host wall.
4. There is only a slight constriction of the penetration peg.
5. The wall of the terminal intercellular hyphal cell is continuous with that of the M-haustorium and remains unmodified.
6. The growth of the M-haustorium is filamentous, and no neck ring is formed.
7. Centripetal septum formation may occur at any point intracellularly or at various points outside the penetration region.
8. There is no redistribution of mitochondria.
9. The host plasmalemma becomes invaginated to form an EH membrane, and an EH matrix is evident. In *P. coronata* the EH matrix is most pronounced around the distal end of the M-haustorium.
10. A collar may or may not form around the "neck."
11. There is less extensive association of host ER with the M-haustorium, and the development of membranous transfer-type complexes in the host cytoplasm has not been observed.
12. Growth is usually terminal in the invaded host cell, although it has been reported (Gold *et al.*, 1979) that the M-haustoria of *P. recondita* may exit from their host cells.

In cytochemical tests the EH matrix of the M-haustoria of *P. coronata* exhibits some unique characteristics (Chong *et al.*, 1981). It is more pronounced around the distal end, and in this respect it resembles the EH matrix around the D-haustoria of the same fungus. However, in the M-haustoria the EH matrix stains more intensely and more uniformly with UA/PbC or PA-TCH-SP, indicating a higher concentration of substances, particularly polysaccharides. Interestingly, the EH matrix stained differentially and oppositely with UA/PbC and PACP at the proximal or distal ends, indicating regional specialization of composition of the matrix (Chong, 1981; Chong *et al.*, 1981).

The designation of the M-haustoria as haustoria or intracellular hyphae has been a problem since Allen's light microscopic descrip-

tions (1932a,b, 1933). Electron microscopy has shown the M-haustoria to be essentially unaltered hyphae that invade and grow inside a host cell. One of the criteria outlined by Bushnell (1972) to define haustoria was that they are terminal in their host cells. In this sense the M-haustoria of *P. coronata* (Allen, 1932b; Harder, 1978) or *P. poarum* (Al-Khesraji and Lösel, 1980) fit the definition of a haustorium. However, the morphologically similar M-haustoria of *P. recondita* (Gold *et al.*, 1979) or others (see Littlefield and Heath, 1979) have been observed to exit from invaded host cells. The latter are more suitably defined as intracellular hyphae. A functional definition is also not without difficulties, as there is little direct information concerning the functions of either D- or M-haustoria. Thus far, association with the host cytoplasm and apparent nutrient uptake are the most obvious features shared by D- and M-haustoria, and this provides the basis for the designation of both as haustoria (Littlefield and Heath, 1979). However, the latter authors also pointed out that either a haustorial or intracellular hyphal designation could be used if the respective structures are known.

VI. Collars

A common response of the host cell to the invasion of the rust fungi is the deposition of a collar of material around the fungus in the region of host cell penetration. Collars are not an integral part of the haustorial apparatus; thus they are considered separately here.

A. DIKARYOTIC INFECTIONS

Collars are not formed in every invaded cell, and their formation is frequently linked to the degree of host-rust fungal compatibility (Heath, 1974; Heath and Heath, 1971). Collar formation is seen as a nonspecific response by the host to wall off the fungus, and in some cases of incompatibility, the entire invading haustorium may be encased by the collar (Heath, 1971; Heath and Heath, 1971).

In genotypically compatible interactions a collar may form at the point of penetration, but it rarely extends beyond the haustorial neck. Collars are more frequently observed in older infections in a variety of host-rust interactions (Chong, 1981; Coffey *et al.*, 1972; Ehrlich *et al.*, 1968; Heath and Heath, 1971), which is probably related to a general decrease in the degree of host-rust fungal compatibility in older infec-

tions (Chong, 1981; Littlefield and Heath, 1979). In our observations, collars have been more frequently observed in compatible interactions involving *P. coronata* than those of *P. graminis* f. sp. *tritici* (D. E. Harder and J. Chong, unpublished).

The following description of collars and their formation in infections of *P. coronata* or *P. graminis* f. sp. *tritici* is summarized from Chong and Harder (1982b) and Harder (1978). The mode of collar formation most frequently observed is interpreted in the drawing in Fig. 38. These collars are formed after fungal penetration and correspond to the type I collar designated by Littlefield and Heath (1979). Although these collars surround the haustorial neck, a zone of host material normally intervenes between the collar and the neck.

Where collars occur in *P. coronata* or *P. graminis* f. sp. *tritici* infections, they are initiated by the deposition of material against the host wall in the region where the fungus enters the host cell. In one version of collar formation, small membrane-bound vesicles, some containing electron-opaque material, aggregate near the base of the neck. These vesicles appear to be derived from Golgi bodies that have aggregated at this site (see also Littlefield and Bracker, 1972). The vesicles then apparently coalesce to form the bulk of the collar. Mature collars frequently contain trapped membranes, which probably reflects this mode of formation. However, some collars do not contain the trapped membranes, and there may be variations in their mode of formation (see Littlefield and Heath, 1979). The more homogeneous type of collar may be the result of fusion of vesicular contents (a reverse pinocytotic process) rather than direct fusion of the entire vesicles.

In *P. coronata* infections in particular, the collars are often variable in shape, which may reflect stages in their formation. Collars frequently have long projections radiating into the host cytoplasm. Host ER and Golgi bodies are associated with these projections (Fig. 41). The involvement of ER and vesicles in collar formation is not unexpected, because the collars are essentially a wall apposition, and secretory vesicles have been implicated in wall thickening induced either artificially (Wheeler, 1974; Wheeler *et al.*, 1972) or pathogenically (Tu and Hiruki, 1971). For further discussion of wall appositions in plant pathogenesis see Aist (1976) and Bracker and Littlefield (1973).

The collars in most rust infections are of variable electron density after conventional processing. Callose-like compounds have been suggested to be a major component of collars, although other carbohydrate substances may also be present (Littlefield and Heath, 1979). Collars in *P. coronata* (Fig. 39) or *P. graminis* f. sp. *tritici* (Fig. 40) show intense staining with PA-TCH-SP, particularly for *P. coronata*. The PA-

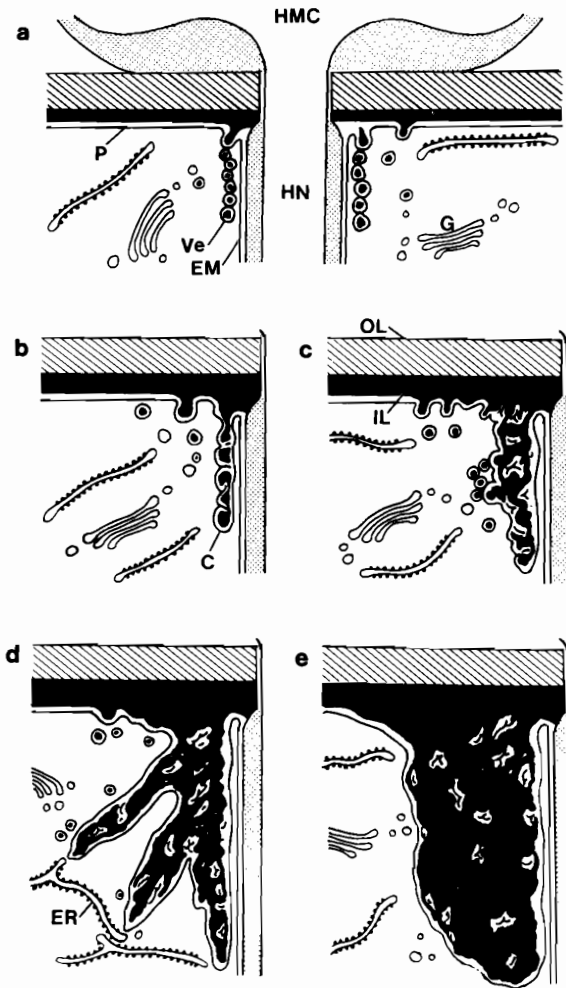


Fig. 38. A diagrammatic chronological representation of one possible mode of collar formation in the D-infection of *Puccinia coronata*. (a) Vesicles [Ve] containing collar material are found adjacent to the extrahaustorial membrane (EM). (b) Vesicles coalesce to form a small collar (C). (c) The collar grows as more material is being deposited into the collar and to the inner layers (IL) of the host wall. (d) The growth of the collar is enhanced by the presence of large projections that are interconnected by host endoplasmic reticulum (ER). (e) Subsequently, a large collar is formed around the haustorial neck, and is integral with the inner layer of the host wall. G, Golgi body; HMC, haustorial mother cell; HN, haustorial neck; OL, outer layer of host cell wall; P, plasmalemma.

TCH-SP procedure does not stain callose [which is a $\beta(1\rightarrow3)$ -glucan], thus the heavy PA-TCH-SP staining is due to polysaccharides other than callose. Similarly, Heath and Heath (1971) indicated that collars in an immune bean rust interaction were rich in polysaccharides as shown by periodic acid-silver hexamine staining. In other cytochemical tests (Chong, 1981), treatments with protease, cellulase, lipid solvents, or for glycogen were negative. From Fig. 39 it is clear that the collar is integral with the inner layer of the host wall. In *P. graminis* f. sp. *tritici* infections the collar is more distinct from the inner wall layer, although this may represent a difference in concentration of PA-TCH-SP stainable polysaccharides. The conclusions from these tests are that the collars are mainly composed of carbohydrate, and particularly in *P. coronata* infections, much of this carbohydrate is in a form other than callose. The amount of callose in the latter infection remains to be determined.

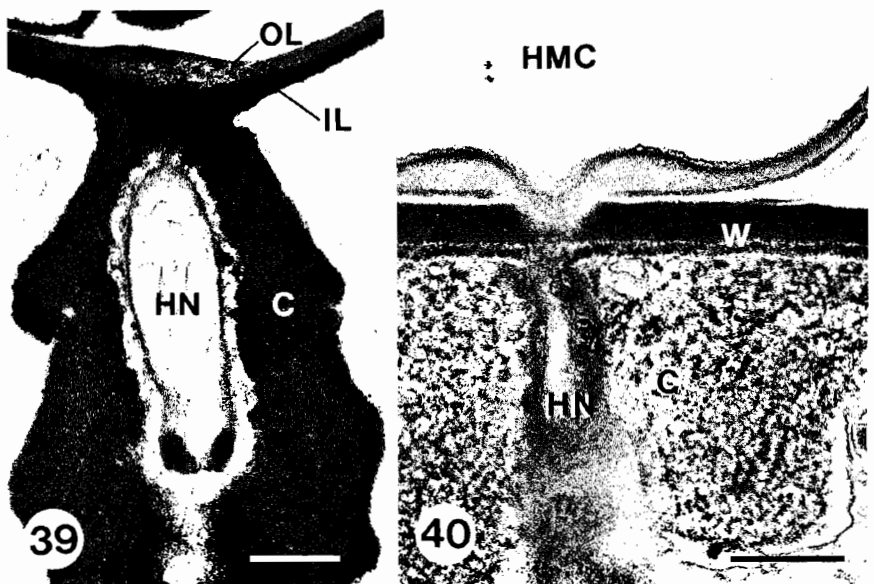


Fig. 39. A large collar (C) around a haustorial (HN) neck of *Puccinia coronata*. Material making up most of the collar is intensely stained except for the small area immediately adjacent to the neck. The collar material is integral with the inner layer (IL) of the host cell wall. OL, Outer layer of host cell wall [Glt/OsO₄; PA-TCH-SP] ($\times 24,300$; bar, 0.5 μm). (From Chong, 1981.) Fig. 40. A well-developed collar (C) around a haustorial (HN) neck of *Puccinia graminis* f. sp. *tritici*. Collar material is stained, but it has a diffuse and granular appearance and is more lightly staining than the host wall (W). HMC, Haustorium mother cell [Glt/OsO₄; PA-TCH-SP] ($\times 30,000$; bar, 0.5 μm). (From Chong, 1981.)

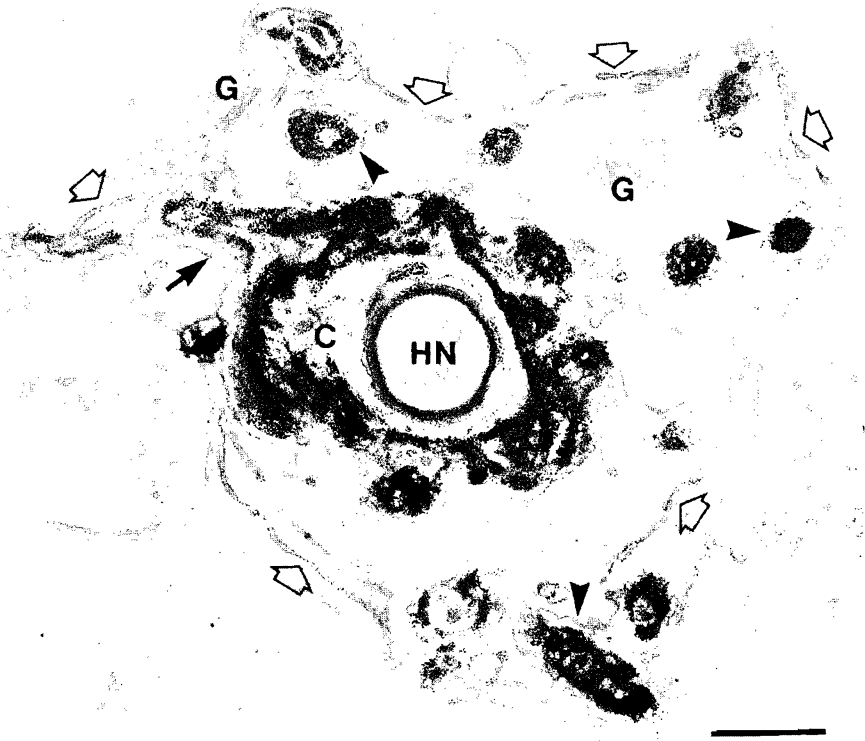


Fig. 41. A developing collar (C) around a haustorial neck (HN) of *Puccinia coronata* with a projection (arrow). Serial sections showed that the nearby large vesicles (arrowheads) containing densely staining material were cross sections of projections radiating out from the collar. Host endoplasmic reticulum (open arrows) and Golgi bodies (G) are associated with these projections [Glt/OsO₄; PA-TCH-SP] ($\times 31,400$; bar, 0.5 μm). (From Chong, 1981.)

B. MONOKARYOTIC INFECTIONS

Similar to D-infections, collars have been observed to occur to a greater or lesser extent around the M-haustoria of *P. poarum* (Al-Khesraji and Lösel, 1981), *P. coronata* (Harder, 1978), and *P. sorghi* (Rijkenberg and Truter, 1973). In all these cases the collar appears continuous with and is morphologically poorly distinguished from the host wall. The collar also fuses with the wall of the M-haustoria to a greater or lesser distance from the penetration site. Thus these collars are quite distinct from those of D-infections, and correspond to the type II collars of Littlefield and Heath (1979). Rijkenberg and Truter

(1973) considered the collars in M-infections of *P. sorghi* to be indistinguishable from the EH matrix, although it is evident from *P. coronata* infections (Harder, 1978) that the collars are discrete and of limited extent. The morphology of the collars may be a reflection of the growth habit of the M-thallus. Al-Khesraji and Lösel (1981) show the intercellular hyphae of *P. poarum* to be embedded in the host walls and to grow through the middle lamellar layer between host cells. A similar growth habit is exhibited by M-hyphae of *P. coronata* (D. E. Harder, unpublished). The collars then may result from the fungus growing between or within the walls, and where the hyphae turn into the host cell, the host wall extends around the penetration site.

VII. Haustorial Function

In this section we will discuss the possible function(s) of rust haustoria on the basis of what is currently known of their structural features and their development from early formation to maturity. The fact that rust haustoria are intracellular organs generally leads to the assumption that their primary function is nutrient absorption from the invaded host cells. However, there is as yet no direct evidence for this role. Although autoradiographic studies have shown transfer of substances between the host and some rust fungi (Ehrlich and Ehrlich, 1970; Favali and Marte, 1973; Manocha, 1975; Mendgen and Heitefuss, 1975; Mendgen, 1977, 1979; Onoe *et al.*, 1973; see Littlefield and Heath, 1979), it has not been verified that the route of transfer has occurred directly through the D-haustoria. There are claims that intercellular mycelial growth can occur to some extent in the absence of haustoria (Onoe *et al.*, 1973; Pady, 1935). This implies that the intercellular rust mycelium is able to obtain at least some nutrients directly from the host without passage through the haustorium. Also, the axenic culturability of some of the rusts (see Williams, Chapter 13, this volume) indicates that nutrient uptake via the hyphae is sufficient for a certain amount of growth. The D-haustorium may have a more specific role than that of extracting basic nutrients from the invaded host cell. As shown by Onoe *et al.* (1973), the D-haustoria of *P. coronata* can take up more complicated substances than can the intercellular mycelium. The specific types of nutrients and perhaps the efficiencies of their uptake are the more important factors to consider when dealing with the nutritional role of D-haustoria.

Evidence from ultrastructural studies supports an absorptive role for

haustoria at least in D-infections. As described earlier, haustorium-associated host tubular complexes are found in *P. graminis* f. sp. *tritici*, *P. coronata*, and perhaps in other uredial infections (Rijkenberg, 1975; Van Dyke and Hooker, 1969; Yudkin and Reiter, 1979). In *P. graminis* f. sp. *tritici* in particular, the buildup of the organized tubular complexes is extensive. These complexes are interconnected via the host ER system, and the tubules in turn are open to the extrahaustorial matrix. In effect, the host-pathogen interface extends throughout the entire host cytoplasm. The net result is a large amplification of the "functional" interfacial area, thereby effecting a more efficient transport of materials, presumably from the host cell to the haustorium.

Further evidence, though indirect, that supports a nutritional role for haustoria is the unique structure of the D-haustorium. Although the D-haustoria are intracellular, they do not in fact penetrate the host plasmalemma. After host wall penetration, the D-haustorium invaginates the host plasmalemma as it grows into the host cell. Thus except for blockage by the neck ring, the region between the extrahaustorial membrane and the haustorial wall is open to the host cell wall. This would allow materials that are transported from the host cytoplasm to the extrahaustorial matrix to flow along the haustorial wall into the host wall region (apoplastic flow), thus to be lost to the fungus. The neck ring appears to be a unique structure evolved by the rust fungi to prohibit this apoplastic "escape" of host solutes (Heath, 1976). The mineral composition of the neck ring suggests its ability to act as a barrier. This, combined with the tight adherence of the extrahaustorial membrane to the ring, argues for a forced route of metabolites from the extrahaustorial matrix through the haustorium (the symplast route), thus increasing the efficiency of metabolite transfer. Further, the peripheral distribution of the mitochondria in the haustoria would appear to offer an advantage in the active transport of materials.

Comparison of growth and reproduction between the M- and D-life cycle phases is instructive relative to haustorial physiology. Intercellular growth in the M-phase of *P. poarum* (Al-Khesraji and Lösel, 1980) and *P. sorghi* (Rijkenberg and Truter, 1973) was much more profuse, but with a relative paucity of intracellular structures as compared to the D-phase of either fungus. Rijkenberg and Truter (1973) concluded that the M-phase could subsist largely on substances diffusing from host cells. Further, the M-haustoria are able to invade vascular tissue (Al-Khesraji and Lösel, 1980; Harder, 1978) and thereby have direct access to the host's nutritional resources. In the macrocyclic rusts the uredial stage is the main reproductive phase, whereas the pycnial-acial stage is more short-lived. Thus the M-haustoria may be

less important for nutrition of the fungus. This is reflected in the low level of specialization of the M-haustoria. The D-haustoria by contrast are highly differentiated structurally, and their differentiation appears to be adapted for efficiency of metabolite uptake and transfer. The relatively greater amount of intracellular growth and sporulation in the D-phase indicates that the thallus is more dependent on the haustoria, implicating a nutritional role for them.

The D-haustorium may also have a role in altering the metabolism of the host to suit its own requirements. During the early stages of haustorium formation there is extensive association of host ER with the young haustorium, and formation of the haustorium-associated host membranous complexes is initiated. The configurations of the host membrane alterations appear to be related to the rust fungal species rather than the host species (see Section IV,H,4). This demonstrates that during the initial stages of haustorium development, information is passed into the host cell that results in alterations of the host endomembrane system. These alterations could have two effects: One may be to alter metabolism and to synthesize metabolites peculiar to the requirements of the fungus; the other is to provide for an efficient and controlled means of transport of metabolites into the haustorium. The latter possibility would provide a means for the fungus to draw on the resources of the host with a minimum of physical disruption to the host cell. In effect, the host alterations are accomplished in a subtle way so as to favor the fungus but to keep the host cell functioning. These observations are consistent with the suggestion by Spencer-Phillips and Gay (1981) that the host cooperates in passing solutes to the fungus (which could be via the membranous complexes), but the pathogen actually controls the efflux from the host. The latter authors indicated that the control activity may occur at the level of the haustorial plasma membrane.

The D-haustorial apparatus is thus a remarkably specialized adaptation of the rusts. Not only does it appear to be structurally specialized to conduct functions required for a compatible host-fungus interaction, but when the infection of a cell has run its course and moribundity sets in, the haustorial apparatus becomes encased in silicon, presumably to limit now deleterious metabolites from spreading through the thallus.

Note Added in Proof

1. Haustoria are the only known intracellular structures in the dikaryotic life cycle stage of most rusts. However, *Physopella zaeae*,

an example of a direct-penetrating (uredial stage) tropical rust, has been shown to grow extensively as intracellular hyphae and to form typical D-haustoria from the same thallus (Heath and Bonde, 1983).

2. Heath and Bonde (1983) demonstrated that vesicles with electron-dense contents, along with tubules, formed near haustoria of the maize rust fungus *Physopella zaeae*.
3. Recently Gold (1983) introduced the term *haploid (H)-haustorium* as a possibly more appropriate term than monokaryotic-haustorium. This was to emphasize more strongly the haploid stage of the life cycle during which they occur rather than their nuclear complement.

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