Ultrastructure of Haustorium Development in *Puccinia coronata avenae*: Some Host Responses

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Based on a thesis submitted by the senior author for partial fulfillment of the requirements for a Ph.D. degree. The technical assistance of Ken Shewchuk is gratefully acknowledged.
Contribution 1021 from Agriculture Canada, Research Station, Winnipeg, Manitoba, Canada.
This manuscript is the third in a series begun in another journal (see reference 5).
Accepted for publication 5 May 1982.

ABSTRACT

Chong, J., and Harder, D. E. 1982. Ultrastructure of haustorium development in *Puccinia coronata avenae*: Some host responses. Phytopathology 72:1527-1533.

The initial response of host cells to the presence of haustoria in a compatible interaction between *Avena sativa* and *Puccinia coronata avenae* was the association of host endoplasmic reticulum with the young haustoria. Subsequently, host Golgi bodies accumulated around the developing haustoria, but no direct association of Golgi bodies with haustoria could be ascertained. There was a close association between the host nuclei and haustoria. The portions of the nuclei that were adjacent to the haustoria were lobed and were often indented by lobes of the haustoria. Tubular complexes occurred in the host cytoplasm between the haustoria and nuclei, but not elsewhere in the invaded host cells. Possible implications of this arrangement of tubules are discussed. Collars were frequently found around the necks of older haustoria. Collar formation was initiated by the deposition of material against the host cell wall at the penetration region and along the haustorial neck. Developing collars were variable in shape; many had long projections radiating into the host cytoplasm. Host endoplasmic reticulum extended between these projections. All collars were continuous with the inner layer of the host cell wall. The collars were largely unaffected by protease, cellulase, or lipid-solvent treatments, but stained heavily with periodic acid-thiocarbohydrazide-silver proteinate. The collars were most likely composed of several types of polysaccharides.

Light microscopy investigations (2,29,30) have shown a definite attraction between host nuclei and haustoria in many dikaryotic rust infections. With corn rust, the association was so marked that deformation of the host nucleus was described as an "amoeboid-like" enwrapment of the haustorium (29). Recent studies (1,25) conducted with the electron microscope confirm the close association of the host nucleus and the haustorium in many rust infections. Associations between host mitochondria, chloroplasts, and haustoria also have been reported, but only the host nucleus-haustorial association seems convincingly to be a characteristic response. Al-Khesraji and Lösel (1) indicated that there is an interaction between the host nucleus and the developing haustorium of *Puccinia poarum*. There is, however, little definitive information on the structural relations between host nuclei and haustoria.

Deposition of a "collar" of material at the point where the fungus enters the host cell is another common response of the host to the presence of haustoria. Calloselike compounds have been demonstrated by light microscopy in some collars (17,18), and involvement of host endoplasmic reticulum (ER) has been implicated in the development of the more extensive, but otherwise similar, collars and encasements in some incompatible rust-host interactions (20). Apart from such information, little is known of the composition of the collars and their relationship to the host cell wall.

In this paper, observations on the structural aspects of the host responses in a compatible interaction between oat and *Puccinia coronata* Cda. f. sp. *avenae* Eriks. are presented. An attempt was also made to study the composition of the collars and their development in this host-parasite system.

MATERIALS AND METHODS

Plant material and inoculation. Seedlings of *Avena sativa* L. 'Pendek' were grown and inoculated with urediospores of a compatible race (race 326) of *P. coronata avenae* as outlined previously (5).

Conventional fixing and staining for electron microscopy. Developing rust colonies were sampled at various times beginning 5 days after inoculation. The excised infected tissue was fixed with glutaraldehyde, postfixed with osmium tetroxide (OsO_4), and embedded in Epon-Araldite (5). Ultrathin sections were taken from near the edge and center of the infection colonies, mounted on formvar- and carbon-coated 149-μm (100-mesh) or single-hole copper grids, stained with uranyl acetate and lead citrate (UA/PbC), and examined with a Hitachi HU-12 electron microscope.

Cytochemical methods. The Thiéry (31) periodic acid-thiocarbohydrazide-silver proteinate staining method was used. Periodate oxidation (27) and control treatments (10,11) for the Thiéry method have been described (5).

The $K_3Fe(CN)_6$ fixing method of de Bruijn (4) was used to visualize the localization of glycogen. Sections were either examined directly or stained as described before with UA/PbC.

Solvent extractions and enzyme treatments. Infected tissue fixed with glutaraldehyde was subjected to organic solvent extraction (ether/ethanol, acetone, or chloroform/methanol) or enzyme treatment before postfixation with OsO_4 as described previously (6). The enzymes were protease (Sigma, type V, purified, 1–5 mg/ml in 0.05 M tris-HCl buffer, pH 7.5), cellulase (Sigma, type 1, practical grade, 5 mg/ml in 0.05 M phosphate buffer, pH 5.5). Ultrathin sections of specimens treated with organic solvents or enzymes were poststained with UA/PbC, or by the Thiéry staining procedure as described above.

RESULTS AND DISCUSSION

Cytoplasmic changes. The immediate postpenetration growth of the fungus occurred as a tubular fingerlike projection into the host cell (Fig. 1). This projection later became the haustorial neck. Amorphous materials occurred in the cytoplasm of this fungal structure, but no other organelles were present. At this stage, host ER cisternae were seen lying parallel to the invaginated host plasmalemma along the nascent neck (Fig. 1). After the haustorial body was formed, host ER cisternae were also found associated with the haustorial body. Association of host ER with haustoria

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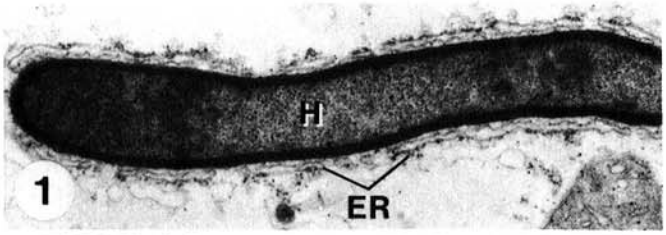
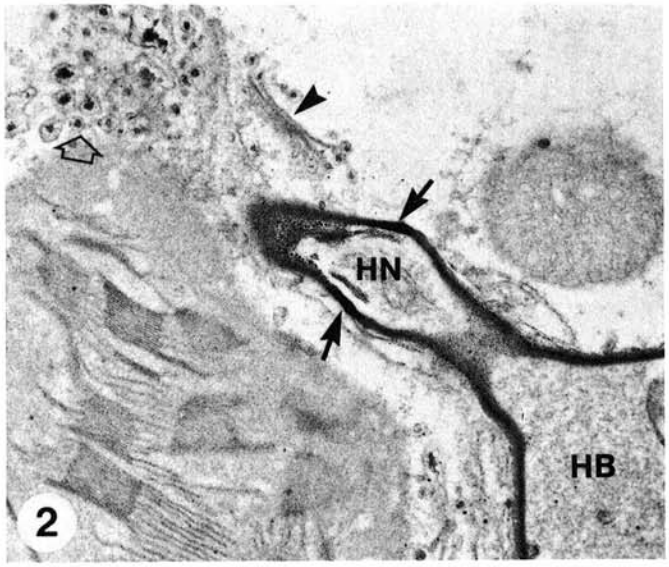
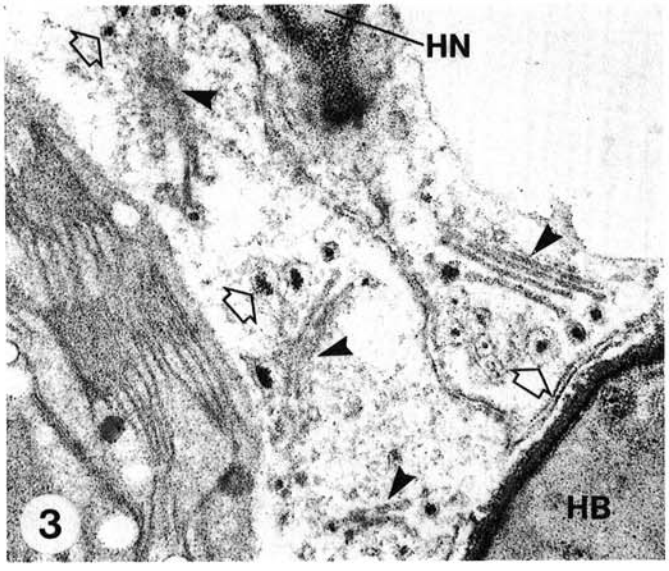
has been reported in other rust infections (see review [25]).

Host organelles (such as Golgi bodies) were often seen in the vicinity of the developing haustoria of *P. coronata avenae*. Figures 2 and 3 are micrographs of adjacent sections of a young haustorium stained by the Thiéry method. The relative age of this haustorium is indicated by the presence of only the α band in the neck ring (Fig. 2) (5). An aggregation of Golgi bodies and vesicles was clearly evident around the haustorial neck region. The contents of the vesicles were stained intensely by the Thiéry method and appear to have been derived from the Golgi bodies (Figs. 2 and 3). Golgi bodies were also abundant in the host cytoplasm around the haustorial body. An increase in the number of Golgi bodies in the host cytoplasm around haustoria has been reported in other rust infections (see review cited by Littlefield and Heath [25]). In *P. coronata avenae* infections, however, it was difficult to determine without quantitative data whether the apparent increase in number of host Golgi bodies around the haustoria resulted from a redistribution of such organelles in the host protoplast or from an increase in the total number of Golgi bodies in each infected cell. The Golgi bodies did not appear to be involved in any of the described associations of the ER and the invaginated host plasmalemma, despite their accumulation around haustoria. They also did not appear to be involved in the large tubular complexes (see later) associated with the haustoria. The Golgi bodies normally have an intermediary role in the transformation of ER-type to plasmalemma-type membranes (26). It is not clear whether the Golgi bodies have a similar role in the host membrane-haustorium association.







Figs. 1–3. Ultrathin sections of *Puccinia coronata avenae*. Abbreviations: ER, endoplasmic reticulum; Glt, glutaraldehyde; H, haustorium; HB, haustorial body; HN, haustorial neck; OsO_4, osmium tetroxide; PA-TCH-SP, periodic acid-thiocarbohydrazide-silver proteinate; UA/PbC, uranyl acetate and lead citrate. **1,** A tubular fingerlike projection, about 4.1 μm long, extending into the host cell. Note the presence of host endoplasmic reticulum lying parallel to the entire length of the fingerlike structure. Glt/OsO_4. UA/PbC (×23,100). **2** and **3,** Two adjacent sections of the same haustorium. Only the α band (arrows) is present in the neck ring. Golgi bodies (arrowheads) are around the haustorial neck region. Vesicles with densely staining contents (open arrow) appeared to have been derived from the Golgi bodies. Glt/OsO_4. PA-TCH-SP. **2,** ×25,700. **3,** ×38,600.

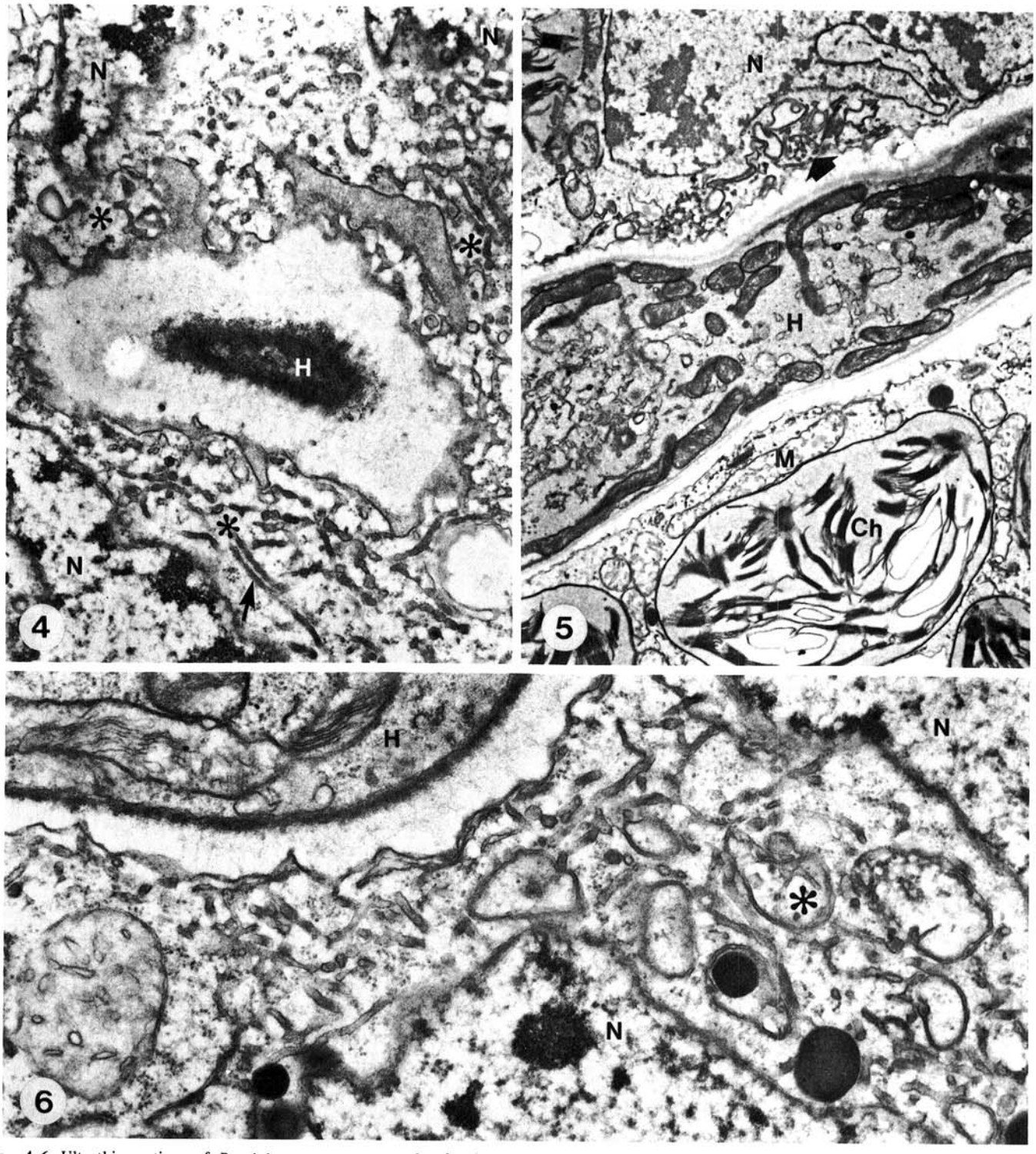
The development of haustoria of *P. coronata avenae* is accompanied by the proliferation of large tubular complexes in the host cytoplasm (6,15). Although cytochemical aspects were previously reported (6), no information was available on specificity of location in the host cell.

In the present study, the host nuclei were frequently seen appressed to one side of the haustorium and occasionally the nucleus was indented by a haustorial lobe (Fig. 4). The tubular complexes were found mainly in the cytoplasmic region between the host nuclei and the nearby haustoria (Figs. 5 and 6), but were seldom seen in areas of the cytoplasm away from the nucleus. Figure 5 shows a general view of the association of a mature haustorium with a portion of the host nucleus. The high frequency of this observation in the present study suggests that the association may be a characteristic response of the host. The proliferation of these tubules was more pronounced especially in the indented region where a haustorial lobe was located (Fig. 4). In this region, the portion of the nucleus adjacent to the cytoplasm containing these tubules was highly lobed (Figs. 5 and 6), more so than the rest of the nucleus. While direct continuity between the tubules and the invaginated host plasmalemma around the extrahaustorial matrix has been reported (6), continuity between the tubular membranes and the nuclear envelope was not found despite extensive efforts made to find these connections. The occasional association of these tubules with chloroplasts most likely was by chance and due to the nucleus being just out of the plane of sectioning.

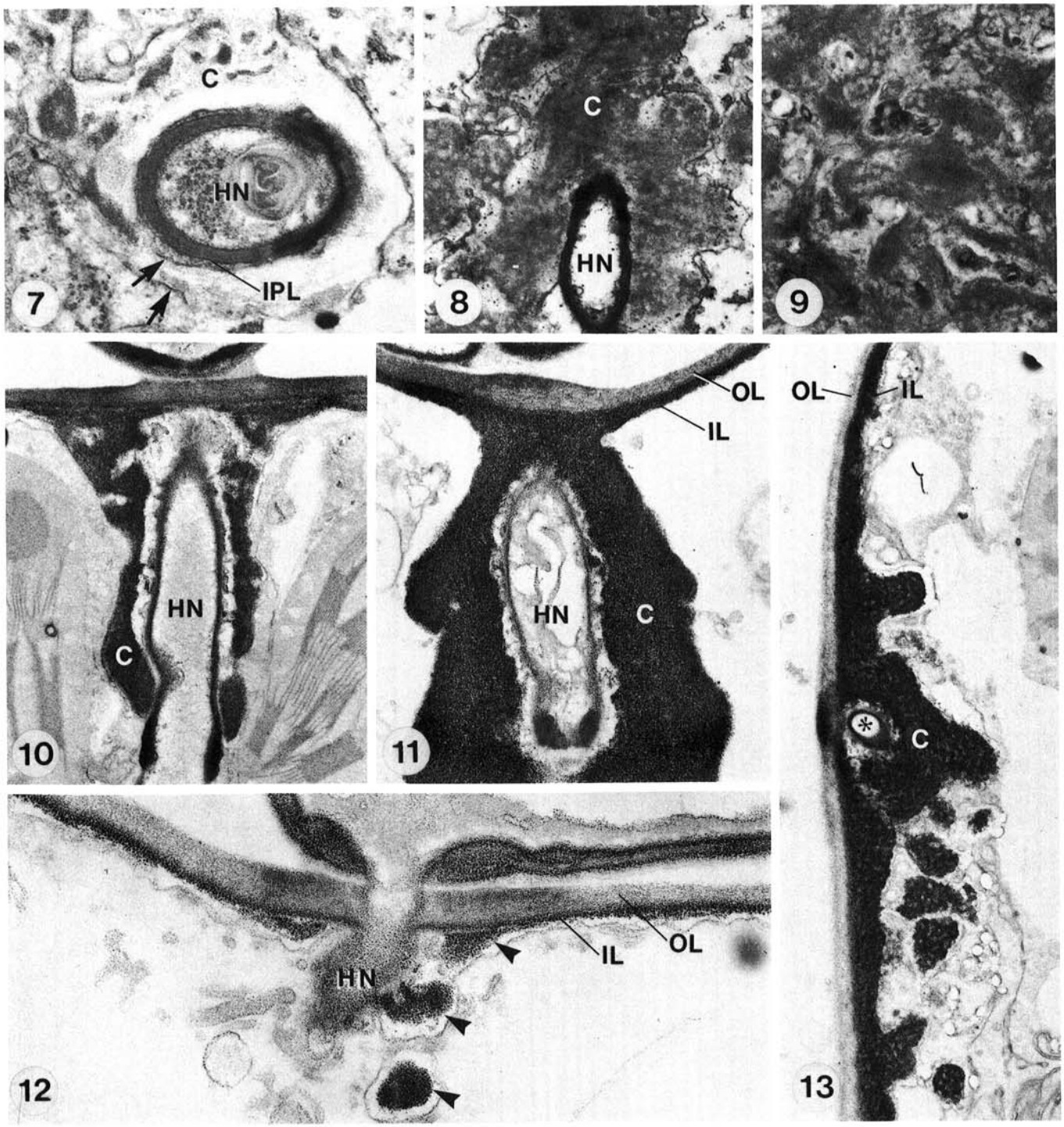
Close association of haustoria with host nuclei has been reported in other rust infections and appears to be a general phenomenon (25). The present study indicates a more extensive association between the haustoria of *P. coronata avenae* and host nuclei than has been previously indicated. Striking alterations in form and size of host nuclei associated with haustoria of *P. poarum* were reported in a recent light microscope study (1). These observations were interpreted as changes in nuclear content or in the properties of the nuclear membrane, and a direct involvement of nucleic acid metabolism of the host during host-parasite interactions was implicated (1). The behavior of the host nuclei and the occurrence of tubules, mainly between haustoria and host nuclei in *P. coronata avenae* infections, may be of significance in this connection. Similar to membrane protrusions associated with the haustorial mother cell septum during early haustorium formation (6), the occurrence of the tubular complexes is most likely to provide additional membrane area. Membrane structures of similar shape in plants and animals have been regarded as functional sites where intensive secretion or absorption may take place (3,14). This presents the intriguing possibility of a high level of exchange between host and fungus, including products of nuclear metabolism. Alternatively, the tubular complexes may play a synthetic-transport-intermediary role to meet the needs of the parasitic fungus. It was suggested previously (6) that the cellulose content of the tubules and the extrahaustorial matrix may be a response by the host to build a wall at the host-pathogen interface.

In principle, the tubular complexes in the oat-*P. coronata avenae* associations are similar, but they do not show the regular lattice-like organization found in wheat-*P. graminis tritici* interactions (16). In this respect, it is of interest that the tubular complexes found in oat-*P. graminis avenae* interactions (*unpublished*) are similar to those in the wheat-*P. graminis tritici* interactions. The form of the complexes thus appears to be specific to the pathogen, and not to the host. It is apparent that rust fungal pathogens are able to specifically induce membrane alterations in the host cytoplasm. This implicates specific alteration of host metabolic processes, which are likely to meet particular needs of the fungus.

Collars. Deposition of material against the host cell wall occasionally occurred at the point where the haustorium entered the cell. Such wall deposits usually resulted in the formation of a



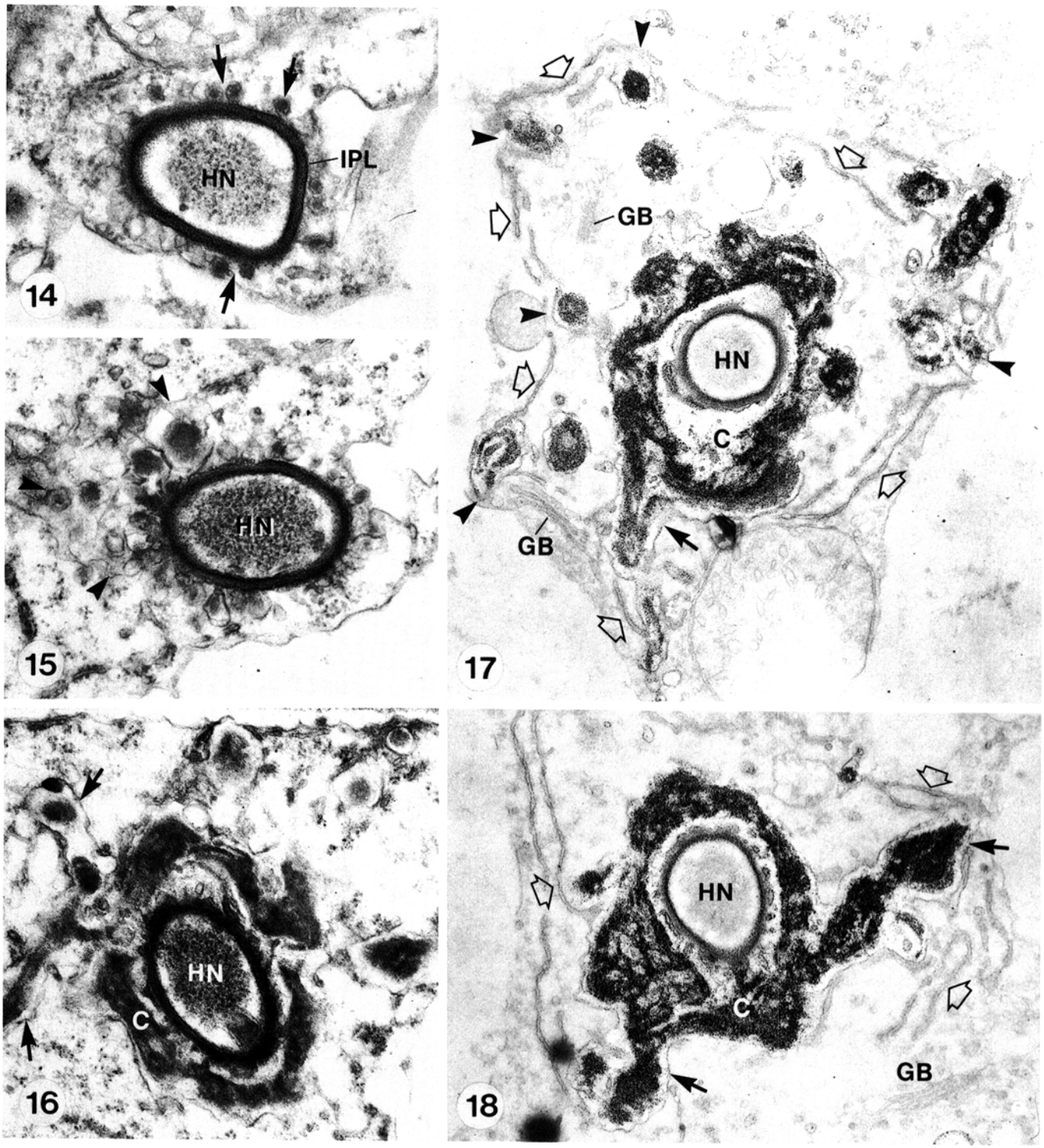
Figs. 4–6. Ultrathin sections of *Puccinia coronata avenae* showing host nucleus-haustorial association. Abbreviations: Ch, chloroplast; Glt/OsO_4-$K_3Fe(CN)_6$, de Bruijn fixation method; H, haustorium; M, mitochondrion; N, host nucleus; UA/PbC, uranyl acetate and lead citrate. **4,** A host nucleus indented by a haustorial lobe. Host tubules (arrow) occur in the cytoplasmic regions (asterisks) between the haustorium and the nucleus. Glt/OsO_4-$K_3Fe(CN)_6$. UA/PbC (×29,800). **5,** A general view of the association of a mature haustorium with the lobed portion of the host nucleus. Cytoplasmic tubules (arrow) are found in the region between the haustorium and the nucleus, and not in other areas around chloroplasts and mitochondria. Glt/OsO_4-$K_3Fe(CN)_6$. UA/PbC (×10,300). **6,** Host tubules found in the cytoplasmic region (asterisk) between the host nucleus and a haustorium. Two lobes of the nucleus are seen. Glt/OsO_4-$K_3Fe(CN)_6$. UA/PbC (×35,200).



Figs. 7–13. Ultrathin sections of collars around haustorial necks in infections of oats by *Puccinia coronata avenae*. Abbreviations: C, collar; Glt, glutaraldehyde; HN, haustorial neck; IL, inner layer; IPL, invaginated host plasmalemma; OL, outer layer; OsO_4, osmium tetroxide; PA-TCH-SP, periodic acid-thiocarbohydrazide-silver proteinate; UA/PbC, uranyl acetate and lead citrate. **7,** A small collar around the neck of a haustorium. There are some electron-opaque patches in the collar but it is mainly electron-lucent, especially in the area around the neck. Both the outer and inner peripheries of the collar are lined by the host plasma membrane (arrows). There is a thin layer of host cytoplasm between the host plasma membrane, which lines the inner periphery of the collar and the invaginated host plasma membrane around the neck. Glt/OsO_4. UA/PbC (×51,800). **8,** A large electron-opaque collar around a haustorial neck. Glt/OsO_4. UA/PbC (×24,000). **9,** Part of a large collar with membranous material and electron-opaque patches. Glt/OsO_4. UA/PbC (×25,700). **10,** A small collar around a haustorial neck. Except for the immediate area around the neck, the collar material is intensely stained. Glt/OsO_4. PA-TCH-SP (×30,000). **11,** A large collar around a haustorial neck. Material making up most of the collar is intensely stained except for the small area immediately adjacent to the neck. The collar is continuous with the inner layer of the host wall. Glt/OsO_4. PA-TCH-SP (×27,100). **12,** An oblique section through the penetration region. Dense staining material (arrowheads) has been deposited against the haustorial neck and inner layer of the host cell wall. The inner layer of the host wall is thinner than the outer layer. Glt/OsO_4. PA-TCH-SP (×44,600). **13,** An oblique cross section of a haustorial neck (asterisk) surrounded by a well-developed collar, the material of which is intensely stained. Wall appositions are observed at some distance from the penetration region, and the inner layer of the host wall is much thicker than the outer layer. Glt/OsO_4. PA-TCH-SP (×20,000).

collar around the haustorial neck, but it rarely extended beyond the neck. These collars were generally associated with older haustoria.

After UA/PbC staining, a collar in the early development stage was electron-lucent mainly in the area immediately adjacent to the neck (Fig. 7). Some electron-opaque patches occurred in the collar near the outer periphery. In Fig. 7, host plasma membrane was seen to line both the outer and inner peripheries of the collar. Occasionally a thin layer of host cytoplasm occurred in the region between the host plasma membrane, which lined the inner periphery of the collar, and the invaginated host plasma membrane



Figs. 14–18. Ultrathin sections of collars around haustorial necks in infection of oats by *Puccinia coronata avenae*. Abbreviations: C, collar; GB, Golgi bodies; Glt, glutaraldehyde; Glt/OsO_4-$K_3Fe(CN)_6$, de Bruijn fixation method; HN, haustorial neck; IPL, invaginated host plasmalemma; OsO_4, osmium tetroxide; PA-TCH-SP, periodic acid-thiocarbohydrazide-silver proteinate; UA/PbC, uranyl acetate and lead citrate. **14,** Small membrane-bound vesicles, some containing electron-opaque material (arrows) were found adjacent to the invaginated host plasmalemma along the haustorial neck. Glt/OsO_4-$K_3Fe(CN)_6$. UA/PbC (×45,700). **15,** An aggregation of vesicles (arrowheads) occurring along the haustorial neck. Glt/OsO_4-$K_3Fe(CN)_6$. UA/PbC (×38,900). **16,** A small collar with projections (arrows) radiating into the host cytoplasm. Glt/OsO_4. UA/PbC (×36,400). **17,** A developing collar 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 are associated with these projections. Glt/OsO_4. PA-TCH-SP (×31,400). **18,** An adjacent section to that in 17. Projections of the collar are indicated by arrows. Note the close association of host endoplasmic reticulum (open arrows) with the collar. Glt/OsO_4. PA-TCH-SP (×31,400).

around the haustorial neck (Fig. 7). Some mature collars were electron-opaque (Fig. 8) while others contained membranous material and extensive electron-opaque patches (Fig. 9). After Thiéry staining, the material making up most of the collar was intensely stained, although some regions, especially the portion of the collar immediately adjacent to the haustorial neck, remained unstained (Figs. 10 and 11). By using the same staining method, two host wall layers were revealed: a thicker, lightly staining outer layer and a thinner, more densely staining inner layer (Fig. 12). In all cases, the collar material was continuous with the inner layer of the host wall (Figs. 11 and 13). This inner layer of the host wall increased in thickness initially in regions around the penetration site as the collar developed. In some infections where well-developed collars were present, deposition of collar material into the inner host wall in these regions was so intense that this inner layer of the host wall became much thicker than the outer layer (compare Fig. 13 to Fig. 12). In well-developed collars, these wall appositions could be observed at some distance from the penetration region of the haustorium (Fig. 13).

The onset of wall apposition was initiated by deposition of material against the host cell wall at the penetration region of the haustorium (Fig. 12). In the neck region, small membrane-bound vesicles, some containing electron-opaque material, were found adjacent to the invaginated host plasmalemma (Fig. 14). At a later stage, more vesicles were found (Fig. 15), which probably coalesced to form the collar. A collar formed in this manner would contain trapped membranous materials, which are remnants of the vesicles after having released their contents during fusion. The absence of trapped membranes in some collars (Fig. 8) suggested that not all collars were formed in the manner described above. Furthermore, the collars were variable in shape. Many collars had long projections radiating out from the main collar into the host cytoplasm (Figs. 16–18). In some views these projections resembled large vesicles (Fig. 17), but this appearance was due to the plane of sectioning. Where these projections occurred, host ER and Golgi bodies were found associated with them (Figs. 17 and 18). The ER extended between these large projections, possibly linking them, although actual continuity between the ER and the projections was not observed. Figure 17 shows an extensive ER-collar projection profile occurring in the host cytoplasm around a collar.

Characteristic organization and distribution patterns of ER cisternae have been associated with cell wall formation in higher plants (7), including callose deposition (8,28). The characteristic association of the ER cisterne with the collar projections seen in the present study is probably representative of a stage in the growth of the collar. It has been implicated that ER plays a role in the development of the more extensive, but otherwise similar, collars and encasements found in some incompatible rust-host interactions (20), and ER involvement in polysaccharide synthesis has been suggested for other systems (7). However, collar deposits always stained more intensely than the contents of the nearby ER cisternae. As pointed out by Heath and Heath (20), it was possible that precursors of collar material were synthesized in the endoplasmic reticulum, then either the contents changed in composition or degree of polymerization after discharge, or possibly the substances of the collar were secreted in some other way.

Collars containing fibrillar and electron-lucent components have been observed in a number of infections (9,17,20,24). Since callose is typically electron-lucent (8,13,21), one would expect callose was at least present in those collars containing electron-lucent components. In compatible interactions with *Uromyces appendiculatus* (17) and in incompatible interactions with *U. phaseoli vignae* (18), histological studies with light microscopy suggested that a calloselike compound was present in these collars. It is likely that callose was present in the electron-lucent compartments of the collars found in *P. coronata avenae* infections. However, the collars were not wholly electron-lucent, and they probably contained other materials as well.

As mentioned above, the collars in *P. coronata avenae* infections were intensely stained with the Thiéry procedure, and they remained unstained in all the control treatments. The staining pattern of the collar with the Thiéry procedure was not affected after cellulase treatment, suggesting that cellulose was not a major constituent of the collar material. The de Bruijn fixation method did not reveal the presence of glycogen-like compounds. With protease treatment extensive extraction occurred in the haustorial neck walls (5,6), but the collar material that was stainable with UA/PbC and the Thiéry procedures remained unchanged. Solvent extraction did not affect the appearance of the collar or appear to affect its polysaccharide content.

The above cytochemical tests suggested that polysaccharides were the main constituents of the collar deposits in susceptible plants infected with *P. coronata avenae*. These polysaccharides reacted positively to the Thiéry procedure, indicating that they were polysaccharides containing vicinal hydroxyl groups. Callose, on the other hand, is known to be a β-1,3-glucan (12,23). This glucan is resistant to periodate oxidation (20), thus is negative to Thiéry staining. If it could be confirmed that callose was also present in the electron-lucent compartments of the collars found in *P. coronata avenae* infections, this would suggest that there were at least two types of polysaccharides constituting the collar deposits. In the incompatible interactions with *U. phaseoli vignae*, there is indication that the encasements also may contain other types of polysaccharides in addition to callose (20). These encasements were stained with the periodic acid-silver hexamine treatment, which is also a test for polysaccharides containing vicinal hydroxyl groups.

Results of a number of studies have shown that callose deposition could be induced, increased, or decreased by such factors as mechanical injury, temperature, application of various chemicals, or by changes brought about by senescence (see review cited in [22]). The formation of primarily callose-containing collars may similarly represent a nonspecific response to invasion of the host cell. However, it seems that other polysaccharides are often found in apparently large concentrations in the collars of *P. coronata avenae* infections and the development of such collars appears to be closely associated with host ER, thus representing a kind of host response that is perhaps more specific than that elicited by injury alone.

It is interesting that collars are not induced in every invaded cell. As pointed out by Heath and Heath (20) and Heath (19), this could indicate suppression of this response in compatible associations as long as the haustorium remained alive and unimpaired. Such a hypothesis would further explain why collars were more frequent in older infections seen in the present study and in other rust infections (9,20). Further, it has been indicated that the degree of compatibility between host and fungus, and the health of the latter, could decrease with time, particularly after the onset of sporulation (25).

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