A NEW CYTOPLASMIC COMPONENT OF PLANT CELLS

MARY A. BONNEVILLE and BRUCE R. VOELLER. From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, and The Rockefeller Institute, New York. Dr. Bonneville's present address is Department of Dermatology, Tufts University School of Medicine, Boston

During an investigation of the relationship between the parasitic flowering plant dodder (*Cuscuta campestris* Yuncker) and its hosts, an unusual cytoplasmic component was observed in certain of the dodder cells. Both in electron and in light micrographs our attention was drawn to the cytoplasmically rich cells at the tip of the newly initiated haustorium, or sucker. These long cells appeared to contain a previously undescribed cytoplasmic component in addition to the structures commonly observed in plant cells. Because of the unusual nature of this component and its prominence in the cells most actively involved in penetration of the host, we have described its appearance and considered its possible role in the physiology of the host-parasite relationship.

MATERIALS AND METHODS

Developing haustoria of dodder, *Cuscuta campestris* Yuncker,[1] together with the surrounding host tissues from the stem of Paris daisy (*Chrysanthemum frutescens* L.), were placed for 22 hours in a fixative containing equal parts of 2 per cent aqueous osmium tetroxide and 0.2 M Sorensen's phosphate buffer, pH 6.8. This procedure was adopted at the suggestion of Dr. Myron Ledbetter, of Harvard University. Dehydration in alcohol and propylene oxide was carried out at 4°C. The tissues were embedded in Epon in the manner described by Luft (4). Sections were made with a diamond knife (Du Pont) on a Porter-Blum microtome. For electron microscopy, thin sections, supported on grids coated with collodion, were stained with lead citrate solution (9) and covered with a thin carbon film. All observations were made with an RCA microscope, model 3C, at magnifications of 3000 to 11,000 times. For light microscopy, sections 1.5 μ thick were placed on glass slides and mounted in paraffin oil.

[1] Identification was kindly confirmed by Dr. Howard Irwin, of the New York Botanical Garden.

OBSERVATIONS AND DISCUSSION

Early descriptions of haustorial development in dodder (7, 10) indicate that haustorial initial cells arise deep in the cortex at the periphery of the vascular region of the dodder stem, push through the parenchymatous and epidermal tissues en-

closing them, and enter the host tissue. Penetration appears to be facilitated by the action of secretions derived from the apical rows of cells which form the spearhead of the haustorium (7, 8, 10). These cells then establish permanent functional contact with the host vascular system.

Within longitudinal sections of the young haustorium, shortly before its penetration of the host tissues, a group of large, elongated hyphal cells, strikingly different from those in other regions of the dodder, is easily identified (Fig. 1, arrows). In these cells, large nuclei, containing prominent nucleoli, are surrounded by dense granular cytoplasm. The cell group forms a blunt spearhead oriented toward the host. At this stage the haustorium is separated from the host by several layers of dodder cortical cells (*Co*) that later will be pushed aside by the elongating haustorium. Behind the spearhead are several rows of cuboidal cells (*Cu*), which also have dense, granular cytoplasm. The appearance of both the hyphal and the cuboidal cells contrasts with that of the vacuolated cytoplasm found in the cells around them.

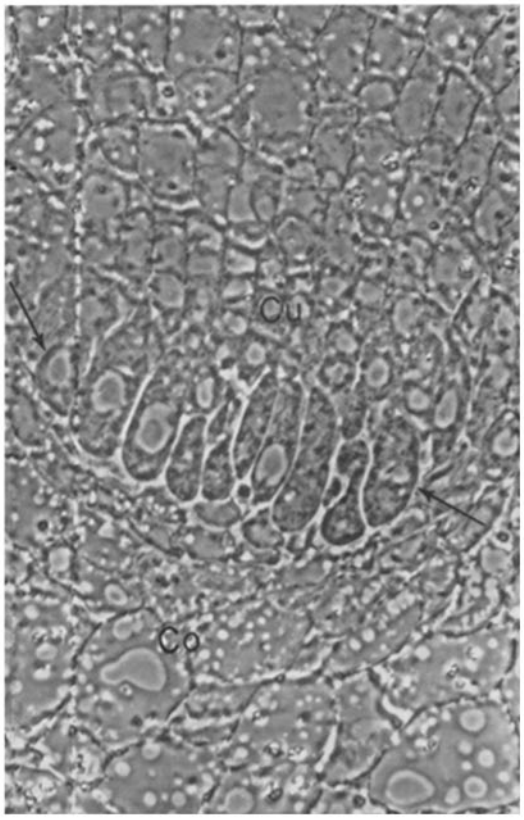


FIGURE 1 Photomicrograph of a young developing haustorium of dodder; haustorium shown in longitudinal section. A blunt spearhead of elongated hyphal cells (arrows) stands out clearly by virtue of its dense granular cytoplasm. The large nuclei and prominent nucleoli of these cells are easily identified. Cuboidal cells (*Cu*), which also have a densely granular cytoplasm, lie within the haustorium behind the hyphal cells. The haustorium is covered by several layers of cortical cells (*Co*), which are characterized by their vacuolated cytoplasm. At this stage the haustorium has neither erupted through the cortex of the dodder stem nor penetrated the host tissue. × 425.

The cytoplasmic fine structure of the young hyphal cell is striking in several respects. Compared with those in other osmium tetroxide-fixed plant cells (*cf.* 11), rough-surfaced cisternae of the endoplasmic reticulum (Fig. 2, *ER*) are surprisingly common. Free ribosomes are densely crowded within the cytoplasmic matrix. Mitochondria (*M*) with well developed cristae are abundant, and dictyosomes (*D*) are particularly numerous. Most remarkable, however, is the presence in the young hyphal cells of a large membranous system which, to the knowledge of the authors, has not been described previously. In some sections this system appears as a bowl-shaped stack of flattened sacs (Fig. 3, *S*), which are dilated at their peripheries (*X*). Near by lie rounded vesicles (*Vs*), some of which are probably parts of adjacent sacs. Within the "bowl" are spherical vesicles, some of which are of considerable size (*Vs'*). Such a profile of the membrane system in question would result whenever stacks of membranes are cut transversely. However, if a section were cut roughly parallel to the piles of membranes (see black line, Fig. 3), the system would then appear as a large central vacuole (*Vs''*) surrounded by smaller vesicles and portions of flattened membranous sacs cut tangentially.

Usually, however, this membrane system does not seem to possess a highly regular form. Often its vesicles (Fig. 5, *Vs*) and sacs (*S*) are associated in irregular array. Irregularity may be due in part to the tangential orientation of sections, but it probably indicates, in addition, that the system is continually changing its configuration and lacks a rigid structure. Furthermore, the membrane system is apparently an extensive one, since several portions of it may appear in a single section of one hyphal cell (Figs. 3 and 5).

All the components of this cytoplasmic appara-

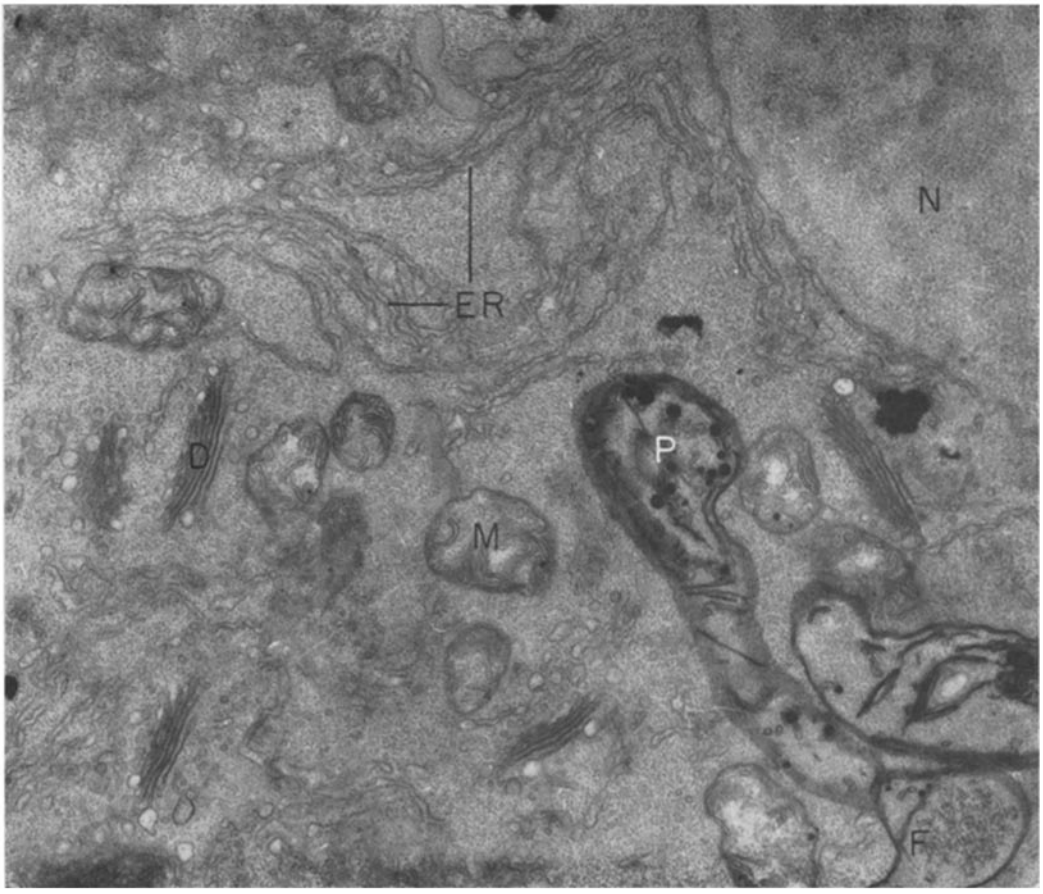
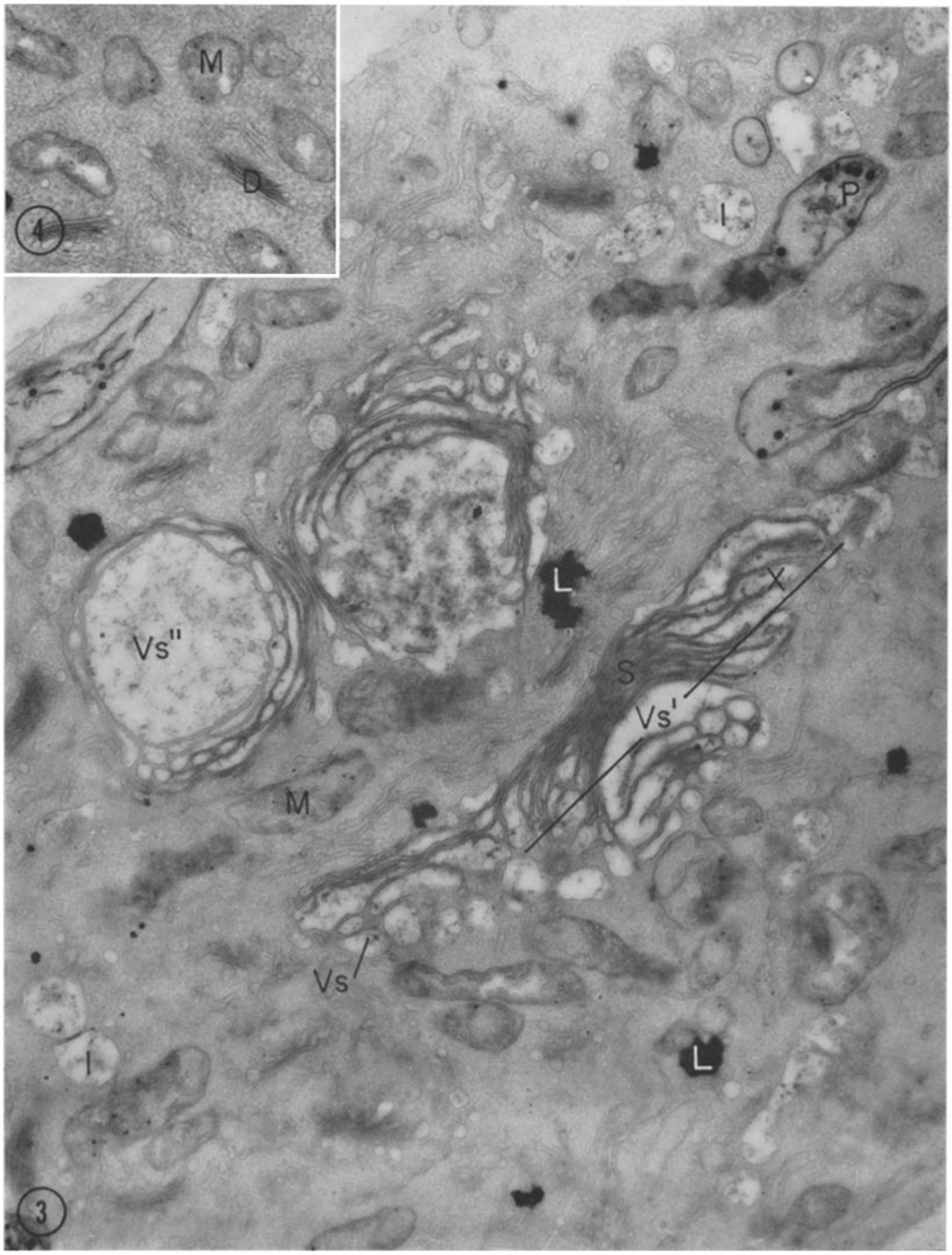


FIGURE 2 This electron micrograph illustrates the fine structure characteristic of hyphal cells in the young haustorium. At upper right, a portion of the nucleus (*N*) is present. In the surrounding cytoplasm, rough-surfaced cisternal elements of the endoplasmic reticulum (*ER*) are shown. Mitochondria (*M*), dictyosomes (*D*), and proplastids (*P*) are also visible. The extreme density of the two outer membranes of the proplastids, as well as certain of the inner membranes, is always striking in these osmium tetroxide–fixed preparations. The presence within the proplastids of lipidlike droplets, as well as fine dense granules (*F*) which are perhaps phytoferritin (3), is also characteristic. The fine dense granules always appear at one end of a proplastid section. × 17,500.

tus have within them irregular clumps of material of unknown nature. Moreover, many isolated vesicles (Figs. 3 and 5, *I*) appear to have within them material similar to that observed within the compact groups of membranes. Such vesicles may be related to the membrane systems, perhaps even produced by them.

Dictyosomes, which are ubiquitous in the various plant taxa (*cf.* 11), are generally considered at present the counterpart of the animal Golgi complex. The studies of Buvat (2), Mollenhauer *et al.* (6), Bouck (1), Mollenhauer and Whaley (5), and Whaley and Mollenhauer (12) suggest that, like the Golgi complex in animals, dictyosomes in plants play a role in cellular secretion. It is thus of particular interest to find in an achlorophyllous plant tissue, known to have secretory activity (*cf.* 7, 8, 10), an additional membrane system resembling the Golgi complex. Although in the young hyphal cells the two types of membrane system occur side by side, they are quite different in size, number, and apparent plasticity of form. When examined at the same magnification, as shown in Fig. 3, a typical dictyosome (Fig. 4, *D*) is much smaller than this new membrane system. On the other hand, dictyosomes are much more



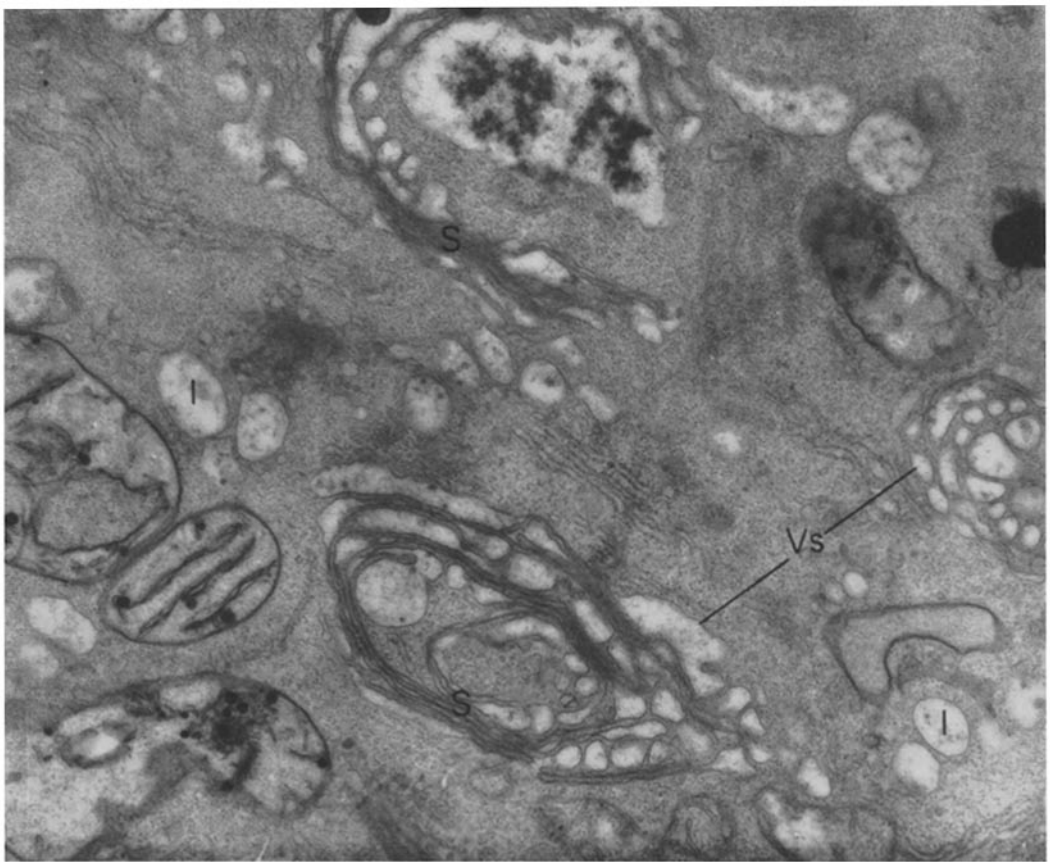


FIGURE 5 Portions of the extensive membranous system often appear as an irregular array of vesicles (*Vs*) and flattened sacs (*S*). This irregularity probably results both from the tangential angle of the section and from lack of an intrinsically rigid structure. Isolated membrane-bound vesicles (*I*) have within them material resembling that found within the membranous system itself and may therefore be related to, perhaps produced by, that system. × 19,000.

numerous. Scores of dictyosomes have been counted in individual hyphal cells, but rarely more than three or four of the new systems per cell have been observed. The two types of membrane system also differ in their distribution in the haustorium; dictyosomes are universally distributed, but the system here described is apparently restricted to the hyphal cells. However, unlike the Golgi com-

FIGURES 3 AND 4 The large membranous system present in hyphal cells of the young haustorium is illustrated in this electron micrograph. Piles of flattened sacs (*S*), dilated at their peripheries (*X*), form a bowl- or cup-shaped structure within which are rounded vesicles (*Vs'*), some of considerable size. Similar vesicles (*Vs*) lie near the lateral margin of the sacs. The images (*Vs''*) such as those at upper left are believed to result from sections cut parallel to the pile of sacs (black line). Irregularly clumped material is found in the intramembranous spaces. Material of similar appearance is also found in vesicles (*I*) lying scattered in the other regions of the cytoplasm. This membrane system is extremely large as compared with mitochondria (*M*), proplastids (*P*), and dictyosomes (*D*, Fig. 4) examined at the same magnification. Opaque bodies (*L*) are probably lipid droplets. × 14,000.

plex of animal secretory cells, neither membrane system appears to show any typical localization, in plant cells, with respect to the nucleus or cell apex.

It seems quite plausible that this system which resembles the Golgi complex is the source of the enzymatic secretions that bring about the breakdown and absorption of the host cell wall and cell contents. Further studies to elucidate this possibility, and to distinguish between the roles of the two smooth-surfaced membrane systems, are in progress.

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