ELECTRON-OPAQUE FIBRILS AND GRANULES IN
AND BETWEEN THE CELL WALLS OF HIGHER PLANTS

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ABSTRACT

The components of higher-plant cell walls which become electron-opaque after staining with ruthenium-osmium were studied by electron microscopy. A fibrillar material which absorbs this stain is a major wall constituent in the root epidermal cells of carrot and morning glory. In both form and size, these fibrils resemble those found on the surface of suspension-cultured cells of the same species. Some cells of woody species show an irregular distribution of electron-opaque material in the cell wall matrix and middle lamella. This material, which has an amorphous appearance with many electron stains, is shown by ruthenium-osmium staining to be an aggregate of discrete granules, 150-220 A in diameter. These observations are not consistent with the concept of the cell wall matrix and middle lamella as an amorphous, uniform gel.

INTRODUCTION

With usual methods of fixation and staining (glutaraldehyde and/or osmium tetroxide followed by uranium and/or lead salts), sectioned plant cell walls and the material on their surfaces often lack contrast. They may appear to be more electron-transparent than the organelles within the cell. However, a method of staining with ruthenium and osmium has been developed recently which shows electron-opaque fibrils associated with the cell wall of cultured plant cells (Leppard et al., 1971, Leppard and Colvin, 1971). Since cultured plant cells are grown under special conditions which may change cell wall physiology (Halperin and Jensen, 1967), it was of interest to extend the studies to native plant tissues to see if they also contain electron-opaque fibrils associated with their cell walls.

The extension of studies was divided into two parts. First, roots of the three species which are known to have electron-opaque fibrillar material in suspension culture (carrot, bean, morning glory) were examined for the presence of similar fibrils. Second, sections of aerial portions of specimens of commercial wood species (pine, spruce, poplar, and fir) were examined for the same substance(s), with special emphasis placed on the middle lamella and spaces between the cells.

MATERIALS AND METHODS

Seeds of Daucus carota, Phaseolus vulgaris var. red kidney bean, and Psoralea sp. were germinated in the dark at room temperature in Petri dishes on filter paper moistened with distilled water. After 1 wk, the young roots were placed in the glutaraldehyde fixative solution. While immersed, the root tips were gently excised and the rest of the root was discarded. The fixation in glutaraldehyde, followed by ruthenium-osmium staining, and the subsequent processing for electron microscopy were done as for the suspension-cultured cells of the same species (Leppard et al., 1971).

The woody species examined were pine (Pinus resina-
A word is necessary on the use of the term "fibrils" in the subsequent sections. In this paper, "fibril" means any thin, longitudinally extended, threadlike structure but it does not imply necessarily any internal order or crystallinity.

RESULTS

Electron-Opaque Fibrils

Initially, sections of all tissues were examined at low magnification for electron-opaque patches or electron-opaque fibrils in, on, or between cell walls. Only these areas were considered for subsequent, high-resolution examination.

Electron-opaque, fibrillar material forms an extensive, extracellular, tenuous coating on the outside surface of the wall of epidermal cells of carrot root tips (Fig. 1). This coating corresponds in position to the swollen wall layer which is seen by light microscopy (Esau, 1967, p. 121). Fibrillar material also forms a system of sheetlike layers between the cuticle and the cell wall of epidermal cells of root tips of morning glory (Fig. 2). Epidermal cells of morning glory sometimes show electron-opaque fibrils in the wall itself or external to the cuticle. Cuticle layers and the fibril layers are sometimes fused (Fig. 2). The largest fibrils of these two species resemble those of the largest fibrils seen in tissue culture (Leppard et al., 1971). The smallest fibrils have diameters approaching the resolution limit for sectioned material, as in suspension-cultured cells. The cell walls of root tips of carrot and morning glory had no discretely stained regions, but these walls occasionally showed electron-opaque fibrils with the minimum resolvable diameter.

Indistinct, electron-opaque, wall fibrils and a fibrillar middle lamella are found rarely on some pine branch cell surfaces (Fig. 3). A different fibrillar material sometimes covers the secondary layer in old tracheids of fir (Fig. 4). These structures are found most readily in association with tears in the

The dimension markers on the electron micrographs represent 0.5 μ. Those on the fluorescence micrographs (Figs. 7 A and 7 B) represent 10 μ.

Abbreviations

W, wall
E, exterior
C, cuticle
S, space
L, lumen of tracheid
C, chloroplast
M, cell membrane
F, fibril aggregate

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Figure 1 External surface of the wall of a carrot root epidermal cell. Note the form of the electron-opaque fibrils. × 180,000.
Figure 2  Cell wall with cuticle of a morning glory root epidermal cell. Note the complex layering of the fibrils between the cuticle proper and the cell wall. × 80,000.
wall where it is least compressed. Whether the failure to observe electron-opaque fibrils in significant numbers in woody species is a result of the compression of cell surfaces or their rarity cannot be determined at present.

No distinct fibrils were found in bean root, pine leaf, spruce branch, spruce leaf, or poplar branch. There was some indication that the bean-root cell walls do not take up the stain well and that, as a consequence, patches of fibrils may have been missed. A comparison of well stained and weakly stained patches of carrot and morning glory fibrils showed that weakly stained fibrils of fine dimensions had little contrast relative to the Epon. This problem of contrast is particularly well illustrated by a comparison of the large and fine fibrils of Fig. 1 with similar examples from the intensely stained, tissue culture fibrils described before (Leppard et al., 1971).

**Electron-Opaque Granules**

Patches and bands of electron-opaque granules are observed in the cell walls and middle lamellae of many branch and leaf tissues treated with the ruthenium-osmium stain (Fig. 5). These aggregations are prominent and are not associated with electron-opaque fibrils. In general, granules were particularly numerous in the middle lamellae and in a thin band along the inner face of the cell wall (Fig. 5). In some sections, the granules appear to be the bulk of middle lamella substance. Individual granules are rounded in form (but not circular) and are not all of the same size (Fig. 6). Many are similar in size to cytoplasmic ribosomes seen in the same sections. Because the patches frequently have a very irregular shape and are sometimes very large in relation to section thickness, no attempt was made to describe in detail the irregular mosaic pattern of granule aggregates at the cell surface. The patches have little relation to cell wall banding and frequently interrupt such banding haphazardly.

**Discussion**

The foregoing observations show that fibrillar material, which can be stained with ruthenium-osmium, may be sometimes detected on the outside of or between the cells of native tissues of the same species as the suspension-cultured cells (Leppard et al., 1971). They verify the previous suggestion that such fibrillar material may be a component of ordinary plant tissues and is not just an artifact of the methods of culture. The electron-opaque fibrillar material found on the outside of suspension-cultured cells differs in form sometimes from that in some of the native tissues studied, and it may be too soon to assume that all the fibrillar material(s) observed has a similar function or composition outside the cell. However, it is also quite possible that this difference in form of the electron-opaque fibrils may be only a reflection of differences in mode of deposition (or compression) of a material with the same composition and use.

If we assume, in the absence of any present evidence to the contrary, that the electron-opaque fibrils outside the cells of native tissues have the same general composition as the electron-opaque fibrils of cells of the same species in suspension culture, then we may use present knowledge of the composition of the suspension-culture fibrils to interpret observations on the native tissues. It has been established by isolation and analysis of the fibrils from suspension culture that these fibrils are composed predominantly of very long chains of polygalacturonic acid to which are attached, either covalently or by irreversible adsorption, ultraviolet-absorbing groups or branches (Leppard et al., 1971; Leppard and Colvin, 1972, Perry et al., 1972). In brief, they are fibrillar pectin with ultraviolet-absorbing groups attached. This composition explains the strong adsorption of ruthenium...
by the suspension-culture fibrils, their complete lack of neutral sugars (i.e., not cellulose or hemicellulose), their resistance to attack by any enzyme yet tried including pectinase, their weak positive response to the Wiesner reagent, deposition of silver granules from the silver nitrate-hexamine reagent, and partial extraction by aqueous dioxane. There seems no reason at present to doubt that the electron-opaque fibrils outside the cells from native tissues of the same species may have some of the same properties. It is also reasonable to suppose that the electron-opaque fibrils outside the cells of native tissues of other species may have a similar composition. Whether the granules have the same general composition is more doubtful. At the present time, the relation of the genesis and function of this form of fibrillar polygalacturonic acid to ordinary forms of pectin is wholly speculative.

In spite of some reservations, it is useful to compare the present morphological observations with others. Scott et al. (1958) studied the mucilage layer on onion root tips but were unable to resolve any fibrillar material in this layer other than cellulose microfibrils from the wall. Other workers have used ruthenium red to illustrate layers identified as principally pectin which were associated with the cuticle of epidermal cells (Roelofsen, 1959; Martin and Juniper, 1970). The fine structure of these layers, however, was not resolved. Scott (1963) studied root-surface structures and emphasized that an increased knowledge of the root surface would be required to understand better the solute absorption, root exudation, and the invasion of pathogens. Several groups have described extracellular, fibrillar material at the outer wall surface of cultured plant cells. Israel and Steward (1966) described a fibrillar material on carrot explants which they interpreted to be 30 Å electron-transparent microfibrils coated with electron-opaque granules. Halperin and Jensen (1967) described electron-opaque fibrils, presumed to be cellulose, which grew profusely on suspension-cultured carrot cells. Sutton-Jones and Street (1968) observed electron-opaque, fibrillar aggregates on suspension-cultured cells of Acer pseudoplatanus, L. and interpreted these aggregates as teased-out microfibrils from torn wall surfaces. We believe that the fibrils described by the latter three groups of investigators are the noncellulosic surface fibrils described by us (Leppard et al., 1971; Leppard and Colvin, 1972).

A word of caution is in order about interpretation of photographs of the cell wall. Nearly all recent reviews (Roelofsen, 1965; Mühlethaler, 1967; Kreger, 1969) consider cellulose as the sole substance with a microfibrillar form in or on the higher plant cell wall, with the exception of endospore mannan. The observations presented here show that this view is too simple. Microfibrillar material other than cellulose exists on or in native plant cell walls and must not be ignored when considering their structure. In fact, this point of view was presented clearly by Roelofsen and his colleagues 20 years ago but has tended to be forgotten (Roelofsen and Kreger, 1951, 1954; Preston, 1952).

The location and staining properties of the aggregates of granules in the middle lamellae suggest that they may be a structural variant of the fibrils, but no other evidence exists yet. In addition, their haphazard distribution within the wall is further evidence for the nonuniform distribution of wall components (Colvin and Leppard, 1971). The ruthenium-osmium staining suggests local concentrations of a form of wall substance which has not been recognized previously. A mosaic-like concept of the plant cell wall may be more consistent with its properties than that of a nearly uniform, reinforced gel.

Figure 3  Space between four cells of a pine branch tissue. The middle lamella has a fibrillar aspect as does the torn wall-matrix material projecting into the space. The section from which this photograph was taken (and that of Fig. 4) was cut thicker than normal in order to enhance contrast. × 38,000.

Figure 4  A fibrillar material coating the surface of the lumen of the secondary wall of an old tracheid from a branch of fir. × 50,000.

Figure 5  Cell walls of pine leaf showing an irregular distribution of electron-opaque components. × 65,000.
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Figure 6. Cell wall of a spruce leaf cell showing individual granules. Note the granules attached to the cell membrane, the indistinct inner wall boundary, and the increased concentration of granules towards the middle lamella. × 96,000.

Figure 7. Surfaces of carrot cells photographed by the fluorescence microscope at 2800 Å. Fig. 7A: Longitudinal section of embedded carrot root showing the epidermal layer and external material. Note that the fibril aggregates absorb more strongly than the nuclei. Fig. 7B: clump of suspension-cultured carrot cells in aqueous milieu. These cells were not dehydrated before photography. × 850.