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Ultrastructure of *Lampropedia hyalina*

JACK PANGBORN AND MORTIMER P. STARR

Electron Microscope Laboratory and Department of Bacteriology, University of California, Davis, California

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ABSTRACT

PANGBORN, JACK (University of California, Davis), AND MORTIMER P. STARR. Ultrastructure of *Lampropedia hyalina*. J. Bacteriol. **91**:2025–2030. 1966.—In an effort to learn more about the structural bases for the sheeting format of *Lampropedia hyalina*, ultrathin sections were cut which were precisely oriented either parallel to or perpendicular to the plane of growth; these were examined by electron microscopy. *Lampropedia* cells show cytological features typical of gram-negative bacteria. In addition, three uniquely structured layers are found exterior to the cell walls. Details are presented regarding the fine structures and geometric relationships of these layers; their probable origins and involvements in the characteristic cellular juxtaposition are discussed.

Many bacteria are arranged in arrays which are so distinctive and regular in occurrence that these arrangements are common criteria for classification and identification. We have been attracted for some time by the problem of finding the structural bases for the various bacterial juxtapositions. It is clear from the recent reviews by Hoffman (2) and Starr and Skerman (7) that such juxtapositions have a number of different determinants.

Lampropedia hyalina is a bacterial organism in which spheroid cells are arranged in rectangular sheets one cell thick (5). *Lampropedia* has been the subject of a genealogical study by Kuhn and Starr (3) which has an important bearing on juxtaposition. Here, one of the investigative methods was continuous observation of clones in microslide cultures under the phase-contrast microscope. The sequence of cell divisions and the development of microcolonies, as well as the formation and depletion of the prominent poly-β-hydroxybutyrate (PHB) granules, could thus be followed.

In a preliminary communication, it had been noted by Kuhn, Starr, and Pangborn (Bacteriol. Proc., p. 44, 1963) that an "extracellular matrix" was somehow responsible for the aggregation of the cells. Murray (4) reported that there are two structures important to the sheeting format; namely, an "intercalated" layer which holds together the cells of the sheet, and an outer sheathlike "punctate" layer. Both of these structures may be important to tablet formation, but Murray (4) considers that the intercalated layer is the more significant. Evidence was presented that occasional cells from a nonsheeting strain retained these layers in the appropriate anatomical regions, but there was no evidence that the layers can regenerate fully, and Murray (4) was not able to isolate sheeting *Lampropedia* from the defective strain. An excellent and detailed study of three extracellular layers found in *Lampropedia* has been presented by Chapman, Murray, and Salton (1). Their descriptions of these layers and of their relationships were based primarily upon shadowed and negatively stained preparations of isolated fragments of the cell envelopes.

The present communication reports our electron microscopic observations of thin sections prepared from precisely oriented intact sheets of *L. hyalina*, with particular reference to the ultrastructure of the components responsible for the sheeting format.

MATERIALS AND METHODS

The usual methods of cultivation, fixation, and embedding were modified to minimize disturbance of the delicate surface structures of the organism. Stock cultures of Pringsheim's (5) strain of *L. hyalina* were maintained according to the mat method described by Kuhn and Starr (3). Petri dishes containing 1.0% Difco yeast extract broth were inoculated from a 24-hr-old stock culture and were incubated at room temperature (25 C) for 24 hr, at which time the surface growth had covered approximately one-half the area of the petri dish. At this time, with minimal disturbance of the culture in the bottom of the dish, the dish cover was replaced with another cover containing filter paper moistened with 2.0% osmic acid. After 30 min of vapor fixation, the broth was pipetted from

the dish and replaced with molten 2.0% Difco agar in fixative buffer (without osmic acid) at 42 C. The molten agar was allowed to flow beneath the culture with only minor disturbance to the mat. After solidifying, the agar was cut into 0.25-inch (0.63-cm) squares by use of those areas of the culture which contained an unbroken mat. These agar squares, with the culture mat adhering to the surface, were then fixed and dehydrated according to the method of Ryter and Kellenberger (6). For purposes of spatial orientation, embedding in epoxy resins was carried out in shallow circular chambers. The plastic-embedded agar squares were cut from the circular blocks, oriented according to the desired sectioning plane by using the agar layer as the point of reference, and glued onto blank plastic rods. Sectioning was done on a Porter-Blum MT-2 microtome with a diamond knife. The sections were poststained with uranyl acetate in methanol and were examined in an RCA EMU-3 microscope. The three-dimensional drawing (Fig. 1) is based on stereomicrographs of serial sections taken at angles of $\pm 5°$.

RESULTS

Thin sections of *Lampropedia* show cytological features typical of gram-negative bacteria (Fig. 2). The PHB granules within the cell are bounded by single limiting membranes, which feature is presently under study in collaboration with D. A. Kuhn. In transverse section (horizontal to the plane of the sheet), the cells have a squarish morphology (Fig. 2); in longitudinal section (vertical to the plane of the sheet), the cells are oval in shape (Fig. 3) with the long axis perpendicular to the liquid surface of the medium.

Murray (4) described an amorphous intercalated zone between the cells, which he suggested was a cementing substance responsible for the characteristic tablet formation in *Lampropedia*. This zone was not present in a nonsheeting strain which had lost its ability to form packets of cells. Chapman et al. (1) described this intercalated layer as an electron-transparent zone with occasional "faint fibrillar interconnections." In our thin sections, this zone was heavily traversed with electron-dense fibers (Fig. 4) which, in stereo-mounted micrographs, appeared as thin extensions of the cell wall. The stereomicrographs gave the impression that adjacent cell walls had a "tacky" surface, which had been pulled apart. Our preparative technique, which minimized disturbance of the cells, improved the structural preservation of this zone and allowed the fibers to be demonstrated. The existence of these fibers within the intercalated zone supports Murray's (4) contention that this zone is the layer most implicated in the coherence of *Lampropedia* cells.

Two unique structures in *Lampropedia* are

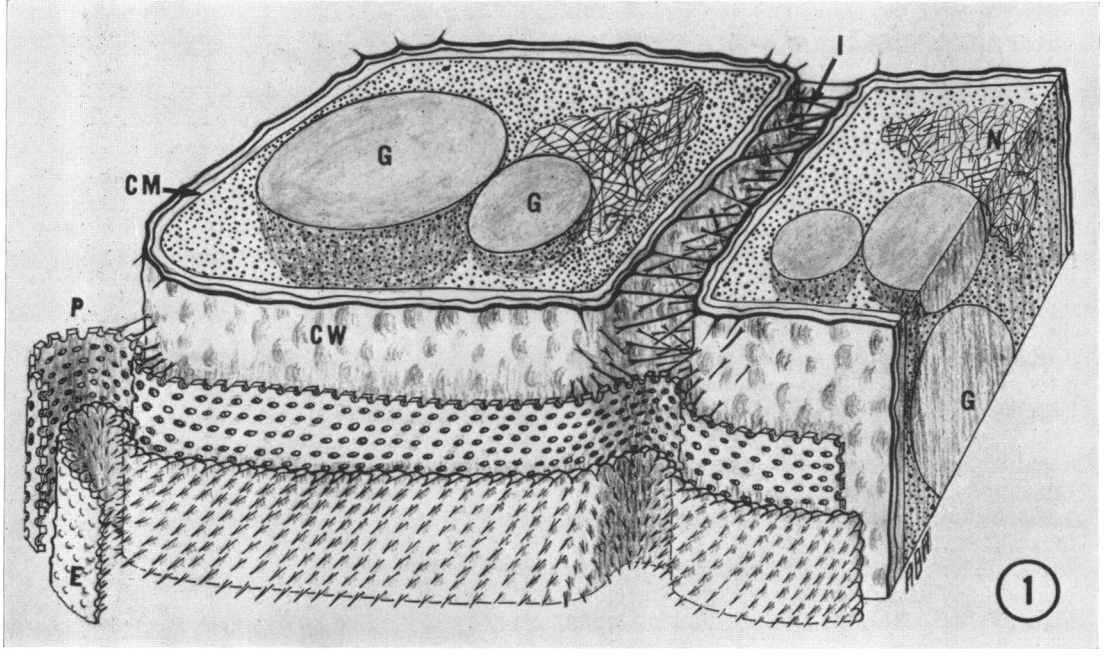


FIG. 1. *Artist's reconstruction of a cell of Lampropedia hyalina, based on serial stereomicrographs, showing the cytological features and unique surface layers. Abbreviations: CW = cell wall; CM = cytoplasmic membrane; E = echinulate layer; G = PHB granule; I = intercalated zone; N = nucleus; P = perforate layer.*

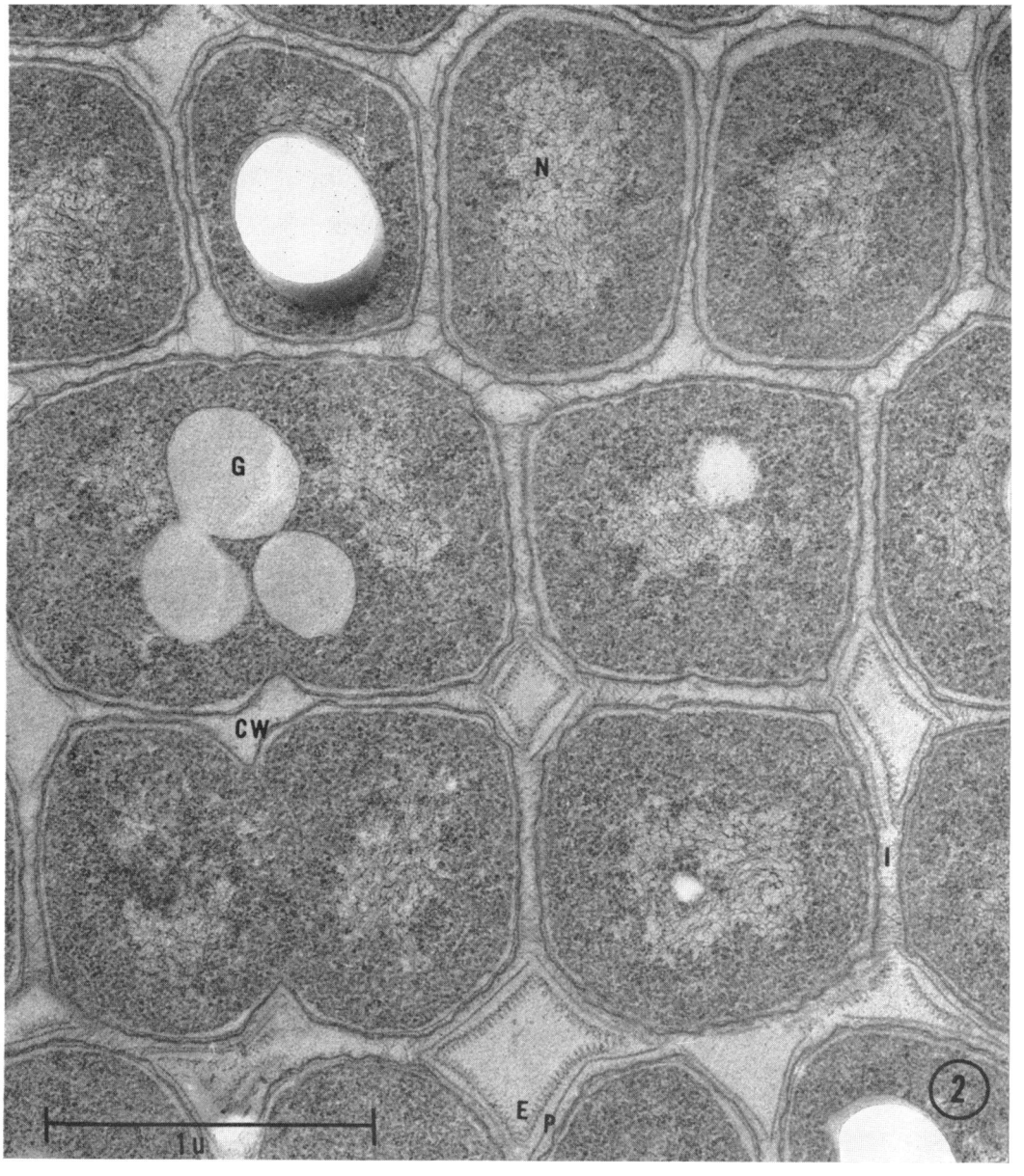
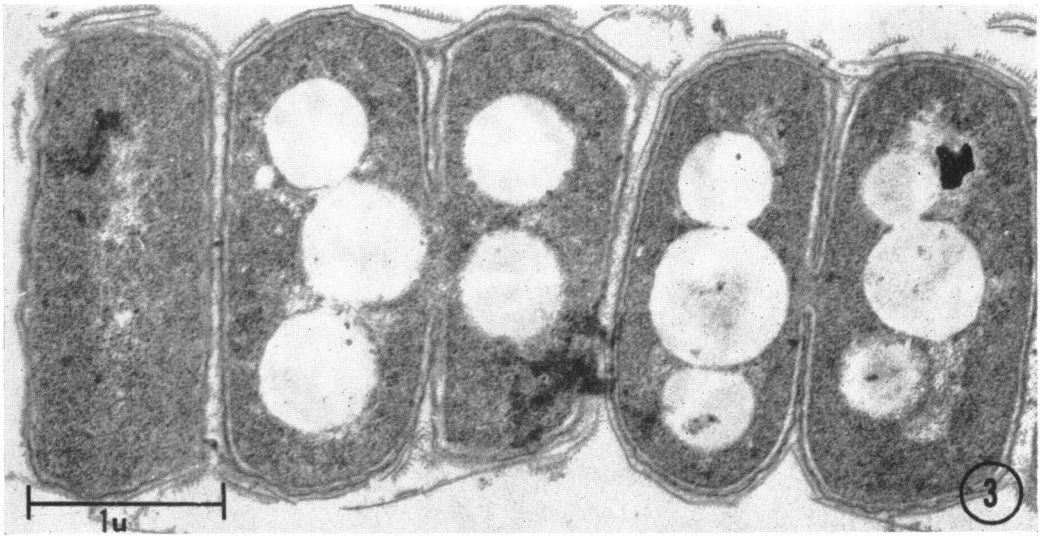


FIG. 2. *Transverse section of Lampropedia; i.e., cut parallel to the plane of the sheet. The cells have the cytology typical of gram-negative bacteria, and a squarish shape in this orientation. The intercalated, perforate, and echinulate layers can be seen between the cells. See Fig. 1 for abbreviations.*

found exterior to the intercalated zone. These are the perforate layer and the outermost punctate layer described by Murray (4) and, in more detail, by Chapman et al. (1) by use primarily of negative staining techniques on isolated cell envelope fragments. Our studies of these two surface layers with thin sections of relatively undisturbed sheets confirm the previous descriptions by the above authors and, in addition, serve to elucidate further structural details and relationships between the surface layers and the cell. Because of its spiny appearance, we prefer to designate the outermost layer as "echinulate" rather than "punctate."

Chapman et al. (1) considered the possibility that the honeycombed perforate layer consists of



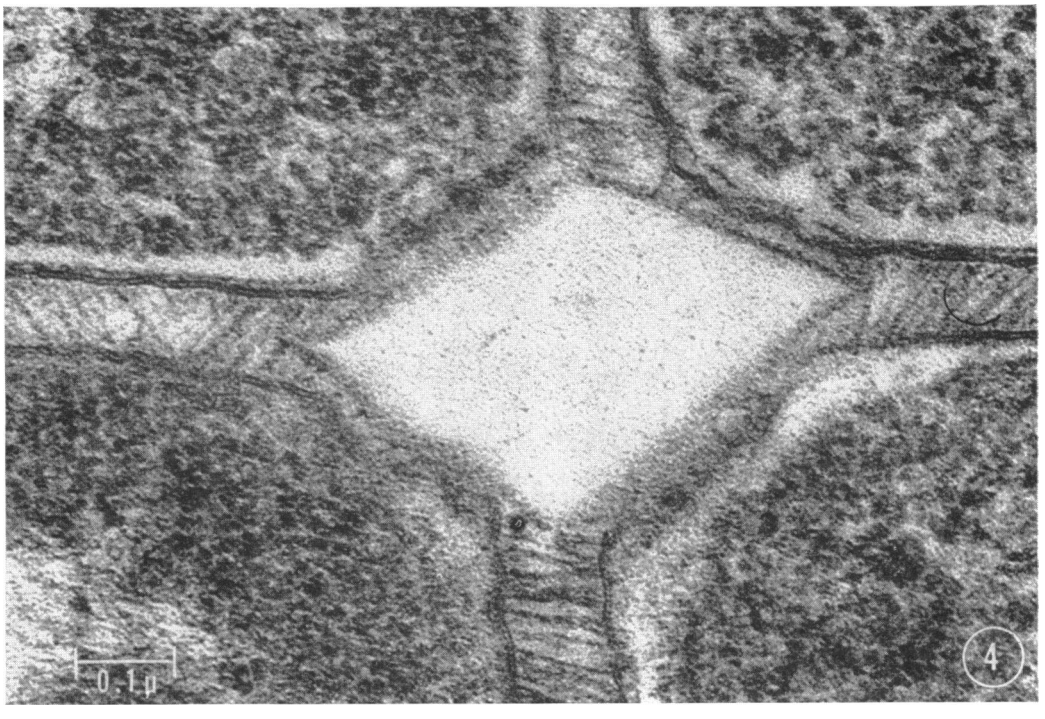


FIG. 3. *Longitudinal section (perpendicular to the plane of the sheet) showing the elongate shape of Lampropedia cells in this orientation. The growth medium had been at the bottom, and the air at the top, of the sheet shown in the micrograph. The perforate and echinulate layers are seen on both surfaces surrounding the cell packet.*

FIG. 4. *Higher magnification micrograph of the intercalated zone in Lampropedia. This zone is formed of fibrous extensions of the cell wall, and these fibers function in preserving the integrity of cell packets.*

interwoven fibers, set at angles of 120°, which leave hexagonal openings of 75 A in the "woven membrane." The spacing of these holes was reported to be 145 A by these workers, and is 135 A in our measurements. In areas where the perforate layer has not been disturbed, the fibrous intercalated zone, though much narrowed, continues immediately beneath the perforate

layer and appears to be connected to it (Fig. 5). This association of the perforate layer with the fibers of the intercalated layer supports the suggestion of Chapman et al. (1) regarding the fibrillar nature of the perforate layer. This association, also, would explain the greater durability of the perforate layer as opposed to the outermost echinulate layer, which is easily lost during preparation of the cells for electron microscopy.

Descriptions of the echinulate layer by the

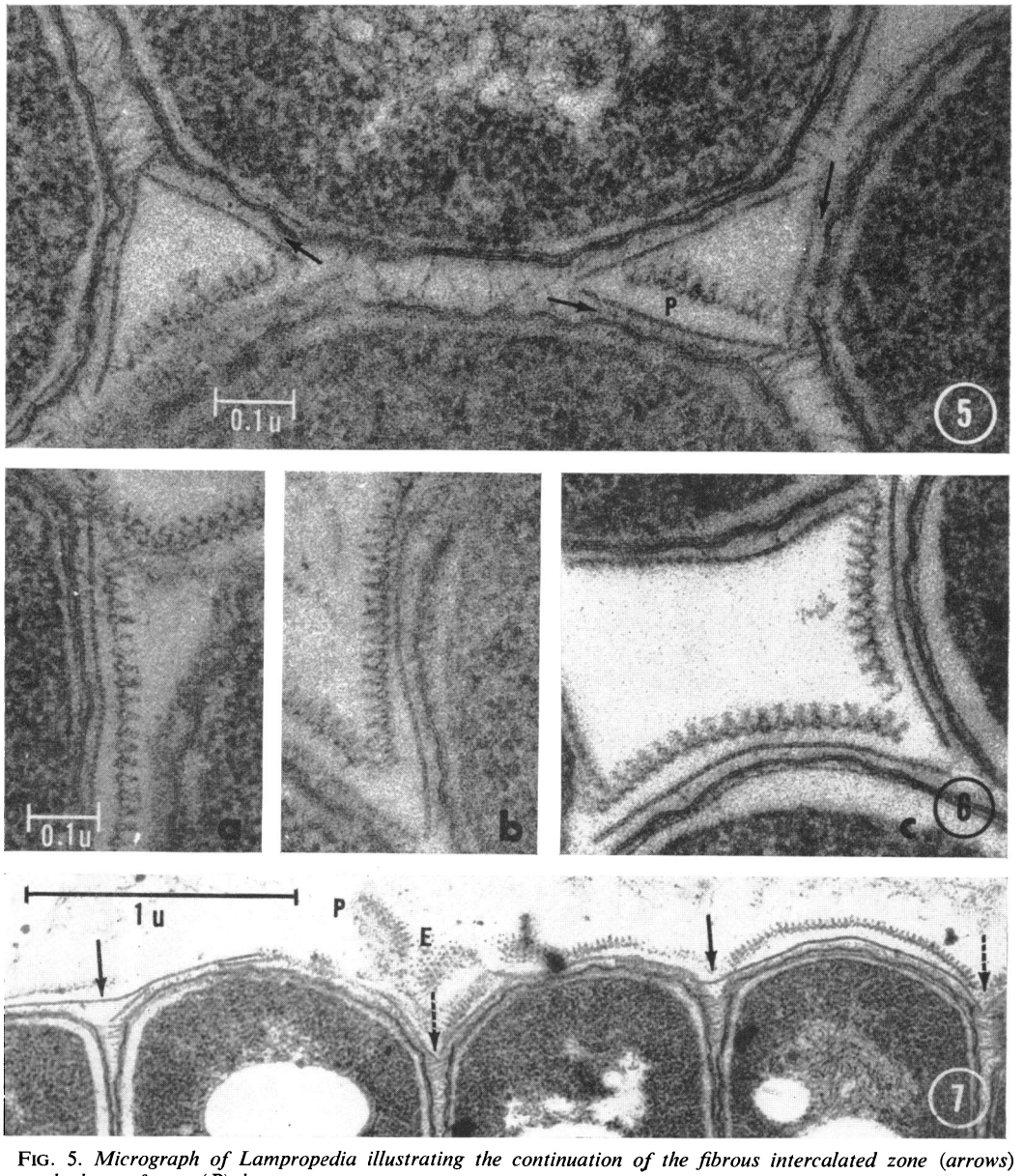


FIG. 5. *Micrograph of Lampropedia illustrating the continuation of the fibrous intercalated zone (arrows) beneath the perforate (P) layer.*

FIG. 6. *Higher magnification micrographs of the echinulate layer of Lampropedia, sectioned perpendicularly to the surface of the cell, showing the unique structure of the spines. (C) illustrates the split of the perforate and echinulate layers within the division cleft.*

FIG. 7. *Longitudinal section of Lampropedia showing the surface layers (arrows) common to adjacent cells, and early stages of splitting (dashed arrows) within the division cleft. An oblique cut through the perforate (P) and echinulate (E) layers can be seen.*

previous investigators were quite brief, because of the difficulty in preserving the delicate layer with the preparative procedures they used. Minimal disturbance of the cells by our procedure gave better, though not complete, preservation of this outermost layer. Our measurements of 230 A periodicity of the spines may be compared with the previously published measurements of 260 A (1). The echinulate layer has a unique morphology (Fig. 6). Each spine appears to be a hollow extension of a hollow bulb, round to oval in section. The bulbs are connected at their extremities to form a chain or mat and, in cross section, appear as a structured membrane with rounded projections on the inner surface and echinulate projections on the outer surface. The total width of the echinulate layer is approximately 290 A. Our studies neither confirm nor preclude the possible ribbed structure suggested by Chapman et al. (1). We can, however, confirm their idea that the spines are hollow.

Because *Lampropedia* grows on a liquid surface with one surface exposed to the relatively dry atmosphere, we considered that the sheathlike outer structure might possibly be an adaptive device which covers only the upper surface of the cells and thereby protects the cells from dehydration. However, longitudinal thin sections (Fig. 3) reveal that the two outer layers are found on both surfaces of the cell.

The pattern of division in *Lampropedia*, with the subsequent involvement of the surface layers, gives some indication of the mode of synthesis of the two outer layers. As described by Kuhn and Starr (3), a PHB-free cell from an old culture, placed on fresh medium in a microslide culture, first forms a distinctive single intracellular PHB granule. Before cell division takes place, a second granule starts to form. Subsequent divisions of the granules and the cells occur alternately at 90° from the previous division, synchronized in a manner such that the first granule of the clone is located in a corner cell of the clone. In the present study, it was noted that the first peripheral sign of division is an indentation of the cell wall in a plane perpendicular to the liquid surface. The outer two layers dip inward angularly, and closely follow the initial division cleft (Fig. 7). As division proceeds, the adjacent cell walls become parallel to one another and remain closely spaced. As the newly formed cell walls separate, and the intercalated zone widens, a split develops in the perforate layer and the separated ends of that layer penetrate the division plane (Fig. 6c). The echinulate layer also splits a discernible distance behind the perforate layer. The possibility exists that the separation of the outer layers within the division cleft is artifactual; however, it can be assumed that the division cleft would be a region protected from mechanical disturbance, and the possibility of damage to these layers during preparation of the sections would thus be minimal. In addition, although oblique sections through the echinulate layer are easily recognizable (Fig. 7), the outer two layers were never seen within the division cleft in such oblique cuts as would be expected if all the layers were continuous. We suggest, therefore, that the perforate layer is synthesized at the site of separation within the division cleft, and is followed by the synthesis of the echinulate layer. The perforate layer appears to "unzip" the fibers of the intercalated layer, supporting the suggestion that the perforate layer is formed directly from the fibers of the intercalated zone. There is general agreement that the two outer layers surround each tablet of cells to form a sheathlike structure. However, in view of our findings, this concept must be modified to include the discontinuity of this sheath at its point of synthesis within the division cleft.

ACKNOWLEDGMENTS

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