INTERNATIONAL

Review of Cytology

EDITED BY

G. H. BOURNE J. F. DANIELLI

ASSISTANT EDITOR
K. W. JEON

VOLUME 53

INTERNATIONAL

Review of Cytology

EDITED BY

G. H. BOURNE
Yerkes Regional Primate Research Center
Emory University
Atlanta, Georgia

J. F. DANIELLI
Worcester Polytechnic Institute
Worcester, Massachusetts

ASSISTANT EDITOR
K. W. JEON
Department of Zoology
University of Tennessee
Knoxville, Tennessee

VOLUME 53

ACADEMIC PRESS New York San Francisco London 1978
A Subsidiary of Harcourt Brace Jovanovich, Publishers

COPYRIGHT © 1978, BY ACADEMIC PRESS, INC.
ALL RIGHTS RESERVED.
NO PART OF THIS PUBLICATION MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM OR BY ANY MEANS, ELECTRONIC OR MECHANICAL, INCLUDING PHOTOCOPY, RECORDING, OR ANY INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT PERMISSION IN WRITING FROM THE PUBLISHER.

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52-5203

ISBN 0-12-364353-8

PRINTED IN THE UNITED STATES OF AMERICA

List of Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

J. CHAYEN (333), *Division of Cellular Biology and World Health Organization Collaborating Laboratory for Cytochemical Bioassays, The Mathilda and Terence Kennedy Institute of Rheumatology, London, England*

FREDERICK GRINNELL (65), *Department of Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas*

D. C. R. HAUSER (145), *Haskins Laboratories at Pace University, New York, New York*

P. HEIZMANN (211), *Department of General and Applied Biology, Claude Bernard Lyon-I University, Villeurbanne, France*

R. N. KAPIL (291), *Department of Botany, University of Delhi, Delhi, India*

M. LEVANDOWSKY (145), *Haskins Laboratories at Pace University, New York, New York*

V. NIGON (211), *Department of General and Applied Biology, Claude Bernard Lyon-I University, Villeurbanne, France*

UWE B. SLEYTR (1), *University of Agriculture, Institute of Food Technology, Vienna, Austria*

S. C. TIWARI (291), *Department of Botany, University of Delhi, Delhi, India*

Contents

此为试读,需要完整PDF请访问: www.ertongbook.com

Morphology, Biochemistry, and Genetics of Plastid Development in *Euglena gracilis*

V. NIGON AND P. HEIZMANN

Plant Embryological Investigations and Fluorescence Microscopy: An Assessment of Integration

R. N. KAPIL AND B. C. TIWARI

The Cytochemical Approach to Hormone Assay

J. CHAYEN

Regular Arrays of Macromolecules on Bacterial Cell Walls: Structure, Chemistry, Assembly, and Function

UWE B. SLEYTR

University of Agriculture, Institute of Food Technology, Vienna, Austria

I. Introduction

One of the most remarkable features of a variety of bacteria is the presence of regular arrays of macromolecules on their outer surfaces.

The first surface pattern to be described was observed by Houwink (1953) in a *Spirillum* species, using shadowing techniques. Since then, regular macromolecular surface patterns have been demonstrated on the cell walls of an increasing number of both gram-positive and gram-negative bacteria, particularly since the introduction of negative-staining and freeze-etching techniques.

The outer surfaces of bacterial cells play an important biological role, since they are involved in constant interactions between the cell and its environment, and the highly ordered two-dimensional arrays of macromolecules found in some bacteria permit the study of a variety of questions related to cell surface properties and the assembly of biological structures. Information on regular cell wall structures is still fragmentary, but the data that have accumulated during the past few years of intensive research on a variety of organisms justify the presentation of a comprehensive review of the field.

It is hoped that the speculative parts of this article will stimulate and provoke further work, especially on the biological role of patterned layers.

II. Topography of the Bacterial Cell Envelope and the Location of Regular Patterns

Early studies on the topography of bacterial cell walls were summarized in an excellent review by Glauert and Thornley (1969). The techniques used included shadowing, negative staining, thin-sectioning, and freeze-etching. Since that time the use of freeze-etching has been extended, and the technique has proved to be particularly valuable in the detection and characterization of surface patterns in bacteria (Remsen and Watson, 1972; Thornley *et al.*, 1974; Sleytr and Glauert, 1975; Thornley, 1975; Glauert *et al.*, 1976).

Although there is considerable variation in the complexity and structure of bacterial cell envelopes, it is possible to classify most bacterial cell wall profiles into two main categories, corresponding to the division between gram-positive and gram-negative bacteria (Glauert and Thornley, 1969; Buckmire, 1970).

In the following discussion the term "cell envelope" is used for the complex of (layered) structures outside the cytoplasm including the cytoplasmic membrane. "Cell wall" is used for the same complex of structures excluding the cytoplasmic membrane.

A. Gram-Positive Cell Envelopes

In thin sections a typical gram-positive cell wall appears as a 15- to 80-nm thick, fairly homogeneous, electron-dense layer. Depending on the species examined and the fixation and staining conditions used, the appearance and the dimensions of the cell wall vary considerably, and indications of layering have been reported (Glauert and Thornley, 1969; Buckmire, 1970; Millward and Reaveley, 1974). For an individual species, modifications in growth conditions, and especially in the age of the culture, cause variations in the thickness of the cell wall (Boothby *et al.*, 1973; Neujahr and Weibull, 1975). Controlled enzymic digestion with lysozyme or other wall-degrading enzymes, followed by chemical analysis, has shown that the main constituent of the gram-positive cell wall is peptidoglycan. Teichoic acids, polysaccharides, and proteins are present in varying amounts as additional components (Rogers and Perkins, 1968; Salton, 1973).

The appearance of typical gram-positive cell envelopes, as seen in thin sections, is illustrated in Figs. 1 and 2. The additional external layer composed of regularly arranged subunits (the S layer) has been observed in numerous organisms (Fig. 2), although thin-sectioning techniques do not always reveal it. Underneath the cell wall is the plasma membrane, which is approximately 8 nm wide and has the typical structure of a unit membrane, consisting of two dense layers sepa-

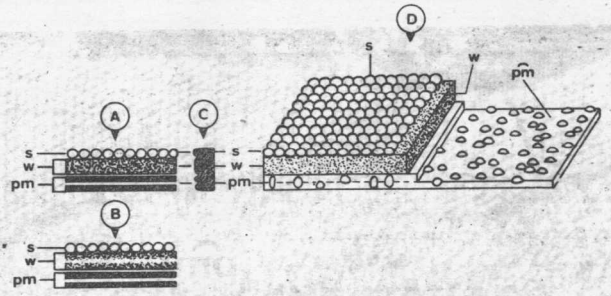

FIG. 1. Diagrams showing the relationships between the layers revealed in thin sections (A and B) and in freeze-etched preparations (C and D) of the envelopes of gram-positive bacteria with regularly arranged surface subunits. (A and B) Diagrams illustrating the structure of the envelopes as seen in thin sections. Depending on the organism, the fixation and staining method, or the growth conditions, the cell wall (w) may appear as a single layer of uniform density (A) or as two densely staining layers separated by a less dense layer (B). s, S layer composed of regularly arranged subunits. (C) The cell envelope as it appears after cross-fracture. The three main ridges represent the plasma membrane (pm), the cell wall (w), and the S layer (s). (D) An obliquely fractured cell envelope with a regular array of surface subunits. The pattern of the subunits is seen on the etched S layer (s) of the bacterium. The underlying cell wall (w) is seen as a ridge. p̂m, Internal fracture face of the plasma membrane.

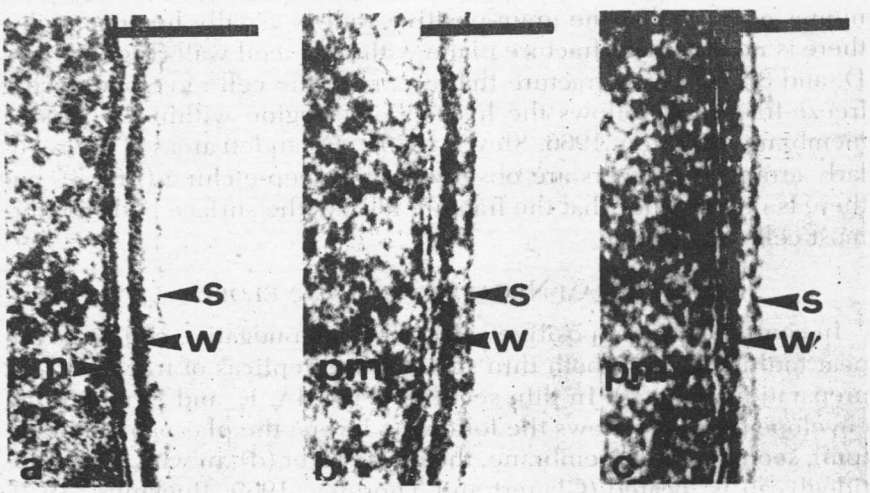

FIG. 2. Ultrathin sections of envelopes of gram-positive bacteria with regularly arranged S layers. (a) *Bacillus sphaericus*. (b) *Bacillus stearothermophilus*. (c) *Clostridium thermohydrosulfuricum*. pm, Plasma membrane; w, cell wall; s, S layer. Bar represents 0.1 μm.

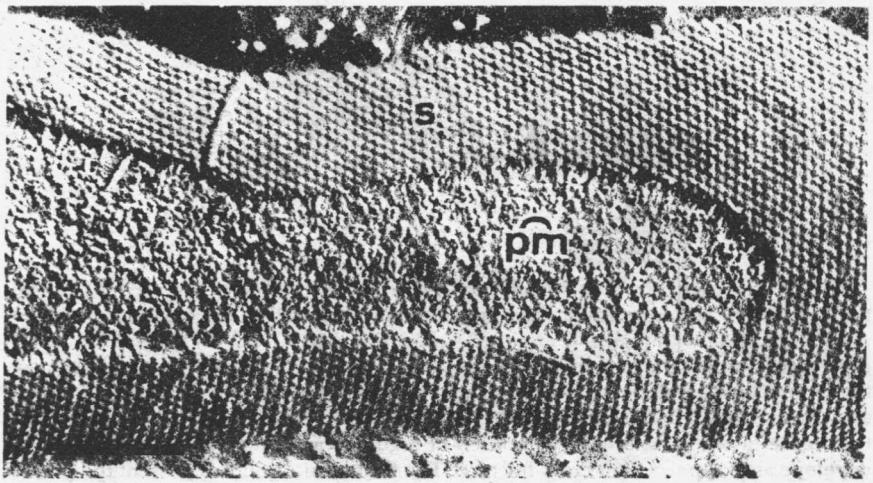

FIG. 3. Electron micrograph of a freeze-etched preparation of a gram-positive bacterium *C. thermohydrosulfuricum*. A large area covered with a regular hexagonal array of subunits (s) is visible on the etched outer surface of the cell. Fracture has taken place within the plasma membrane, revealing the convex face of the plasma membrane ($\widehat{pm}$) adjacent to the cytoplasm. Bar represents 0.2 μm.

rated by a less dense layer. Observations with freeze-etching techniques confirm that the gram-positive wall is usually homogeneous; there is no dominant fracture plane within the cell wall (Figs. 1C and D, and 3). The only fracture that occurs in the cell envelope during freeze-fracturing follows the hydrophobic region within the plasma membrane (Branton, 1966; Sleytr, 1970a). Extended areas of the regularly arranged S layers are observed after deep-etching (Fig. 3), but there is no evidence that the fracture follows the surface of this outermost cell wall layer.

B. GRAM-NEGATIVE CELL ENVELOPES

In contrast to gram-positive cell walls, gram-negative cell walls appear multilayered in both thin sections and replicas of freeze-etched preparations (Fig. 4). In thin sections (Figs. 4A, E, and F, and 5) the envelope typically shows the following layers: the plasma membrane (pm), seen as a unit membrane, the dense layer (d), in which the peptidoglycan is located (Glauert and Thornley, 1969; Buckmire, 1970; Murray *et al.*, 1965), the intermediate region, and the outer membrane (om), which has a unit membrane structure similar to that of the plasma membrane. The outer membrane contains lipopolysaccharide, protein, and phospholipids (Nikaido, 1973; Costerton *et al.*, 1974a;

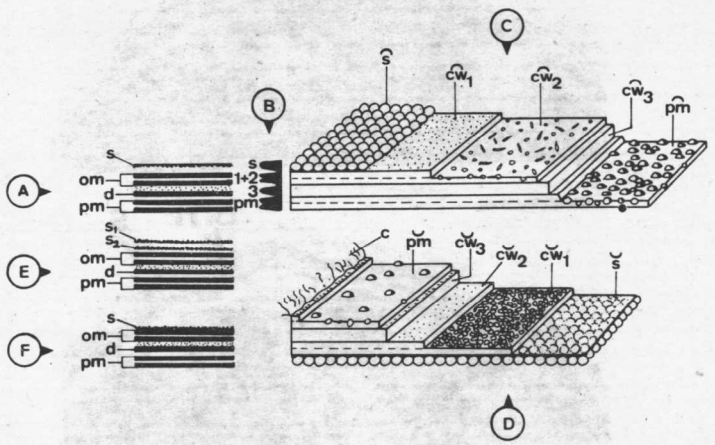

FIG. 4. Diagrams showing the relationship between the layers revealed in thin sections (A) and in freeze-etched preparations (B, C, and D) of the cell envelope of a typical gram-negative bacterium which has an additional S layer of regularly arranged subunits. (A) The cell envelope as seen in thin sections. The innermost layer is the plasma membrane (pm) which is covered by the cell wall. The cell wall consists of a dense layer (d), an intermediate layer, an outer membrane (om), and an S layer (s) composed of regularly arranged subunits. (B) The cell envelope as it frequently appears after cross-fracture in a freeze-etched preparation. The four main ridges represent the plasma membrane (pm) and the layers of the cell wall: the dense layer and the intermediate region between the dense layer and the outer membrane (cw_3), the outer membrane (cw_1 and cw_2), and the additional S layer (s). (C) An obliquely fractured cell envelope seen from the convex side in a freeze-etched preparation. The etched outer surface of the S layer ($\hat{s}$) consists of regularly arranged subunits and lies next to one of the fracture faces ($\widehat{cw}_1$) of which small areas are seen occasionally. Internal fracture of the outer membrane and the plasma membrane reveals the convex faces ($\widehat{cw}_2$) and ($\widehat{pm}$). (D) An obliquely fractured cell envelope seen from the concave side shows the cytoplasm (c), the concave fracture face of the plasma membrane ($\breve{pm}$), and the outer membrane ($\breve{cw}_1$). The fracture faces $\breve{s}$ and $\breve{cw}_2$ are revealed only occasionally. (E) In a few gram-negative envelopes two (or more) separate surface layers (s_1 and s_2) are revealed in thin sections. (F) Diagram showing a gram-negative cell wall profile where a single S layer can be seen as a structured layer in close contact with the outer dense layer of the outer membrane. The diagrams have been modified from Sleytr *et al.* (1974).

Salton and Owen, 1976). Whereas the unit membrane structure of the outer membrane can be demonstrated in almost all gram-negative organisms, the dense layer is frequently less clearly visible as a separate layer. Occasionally the intermediate layer reveals a globular structure and appears to bridge the gap between the dense layer and the outer membrane.

In freeze-etched preparations the cell envelope of gram-negative organisms can fracture along two well-defined planes (Figs. 4C and D,

46980

此为试读,需要完整PDF请访问: www.ertongbook.com

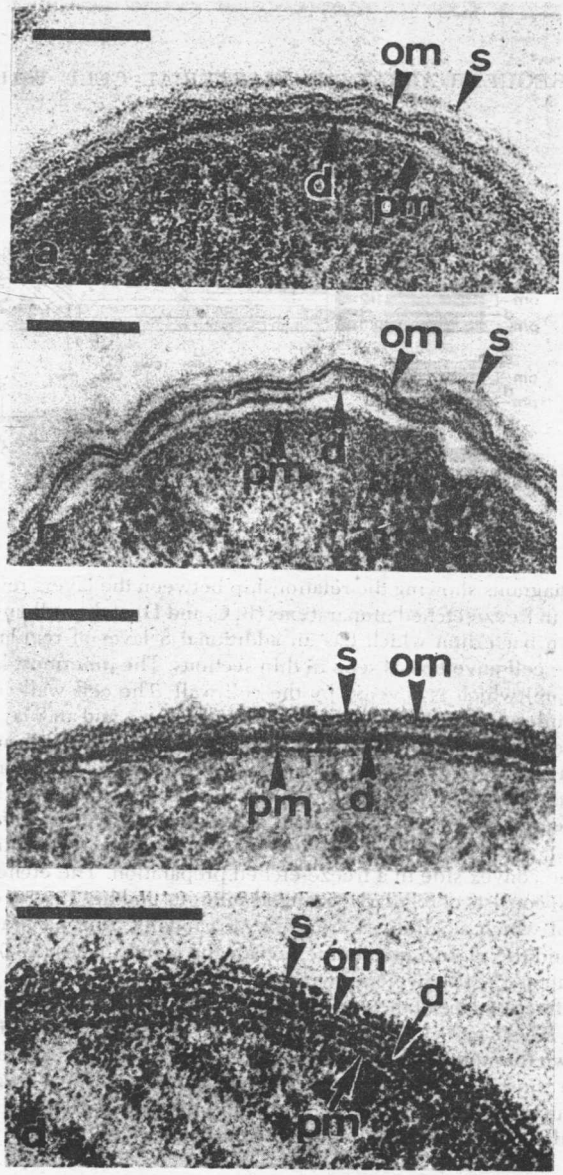

FIG. 5. Ultrathin sections of envelopes of gram-negative bacteria with regularly arranged S layers. pm, Plasma membrane; d, dense layer; om, outer membrane; s, S layer composed of regularly arranged subunits. Bar represents 0.1 μm. (a) Logarithmically grown cell of *Acinetobacter* sp. strain MJT/F5/5. (From Sleytr *et al.*, 1974, by permission from the American Society for Microbiology, Washington.) (b) Heat-treated cell of *Acinetobacter* sp. strain MJT/F5/5, showing a more distinct separation of the layers of the cell envelope. (From Sleytr *et al.*, 1974, by permission from the American Society for Microbiology, Washington.) (c) Cell envelope of *Acinetobacter* sp. MJT/F5/199A. The array of subunits is not visible as a separate layer. (From Sleytr and Thornley, 1973, by permission from the American Society for Microbiology, Washington.) (d) Portion of the cell envelope of *S. serpens* strain VHA on which the regularly arranged S layer can be seen overlying the outer membrane. (Photomicrograph courtesy of R. G. E. Murray.)

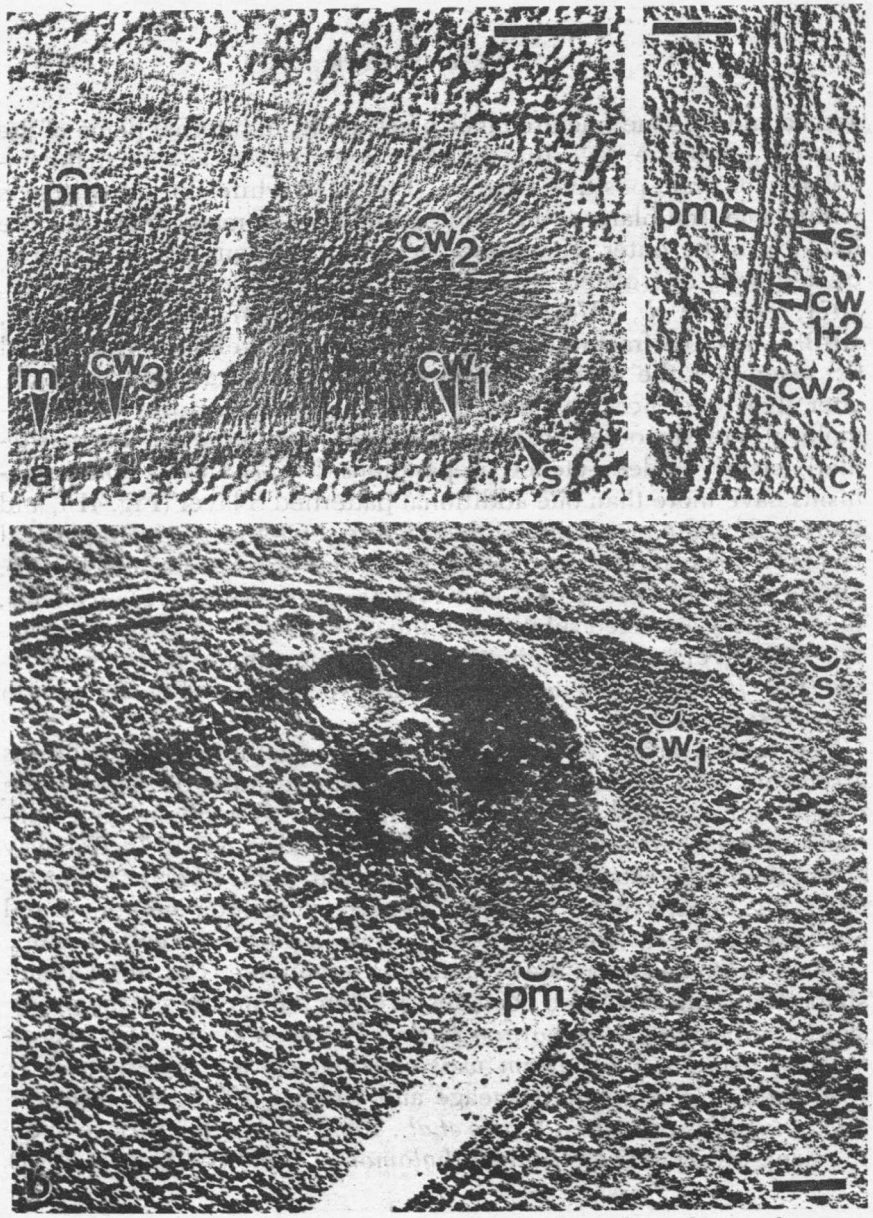

FIG. 6. (a) Convex view of an obliquely fractured cell envelope of *Acinetobacter* sp. strain MJT/F5/5 in a preparation freeze-etched in the presence of glycerol (compare with Fig. 4). Due to the glycerol, only a small area of the outer surface (s) has been exposed by etching; it shows the regular array of subunits. The edge of cw_1 adjoins the main convex fracture face in the cell wall $\breve{cw}_2$. The edge of cw_3 and the outer portion of the plasma membrane (m) lie next to the internal fracture face of the plasma membrane ($\breve{pm}$). Bar represents 0.2 μm. (From Sleytr *et al.*, 1974, by permission from the American Society for Microbiology, Washington.) (b) Concave view of an oblique fracture through the cell envelope of *Acinetobacter* sp. strain MJT/F5/5 freeze-etched in the presence of glycerol (compare with Fig. 4). The concave fracture faces $\breve{pm}$, $\breve{cw}_1$, and $\breve{s}$ are visible.

(*Continued*, see p. 8)

and 6a and b): One fracture takes place along an internal plane of the plasma membrane, revealing the same fracture faces as seen in the envelopes of gram-positive bacteria (Fig. 1D), while the other occurs within a central plane of the outer membrane. In many organisms the fracture in the outer membrane occurs more frequently in glycerol-treated cells or isolated outer membranes (Van Gool and Nánninga, 1971; Sleytr *et al.*, 1974; Thornely and Sleytr, 1974; Gilleland *et al.*, 1973). The cross-fractured, gram-negative cell envelope (Figs. 4B and 6c) resembles the profile seen in thin sections (Fig. 4A), especially after glycerol or heat treatment (Fig. 5b). The regular arrays of macromolecules on the outer surface of the outer membrane are seen particularly clearly in deep-etched preparations (Fig. 7a and b). Some organisms have more than one additional patterned S layer (Fig. 4E), and there is then the possibility of the fracture separating these individual layers (Watson and Remsen, 1970; Beveridge and Murray, 1974, 1975, 1976a; see also Sections III and IV). As in gram-positive organisms, the regularly arranged subunits on the outer surface of the gram-negative cell envelope are frequently better demonstrated by freeze-etching (Fig. 7a and b) and negative-staining techniques (Fig. 7c) than in thin sections (Fig. 5).

The regular arrays of wine glass-, cup-, or goblet-shaped subunits associated with the cell walls of some gram-negative bacteria can be considered a special type of S layer, since the 4- to 50-nm large subunits are attached to the outer membrane (Ridgway, 1977). Ridgway *et al.* (1975) suggested that in the gram-negative *Flexibacter polymorphus* (Fig. 8) parts of the "globlets" penetrate the outer membrane and are linked to the intermediate dense layer complex of the cell envelope or even extend as far as the cytoplasmic membrane (Fig. 8b). Goblet-shaped subunits have also been observed on the surfaces of the photosynthetic bacteria *Chromatium buderi* (Remsen *et al.*, 1970), *Chromatium okenii*, *Chromatium weissei* (Hageage and Gherna, 1971), *Chromatium warmingii* (Hageage and Gherna, 1970), and *Amoebobacter bacillosus* (Cohen-Bazire *et al.*, 1969), and on the surface of the methane-utilizing bacterium *Methylomonas albus* (Wilkinson, 1971).

FIG. 6 (*cont'd*). The bar represents 0.1 μm. (From Sleytr *et al.*, 1974, by permission from the American Society for Microbiology, Washington.) (c) Cell envelope of *Acinetobacter* sp. strain MJT/F5/5 seen in cross-fracture in a preparation of heat-treated cells freeze-etched in the presence of glycerol (compare with Figs. 4 and 5b). Four main ridges are visible: s, cw(1 + 2), cw_3, and pm. And both cw(1 + 2) and the plasma membrane (pm) appear as double ridges (double arrows). Bar represents 0.1 μm. (From Sleytr *et al.*, 1974, by permission from the American Society for Microbiology, Washington.)

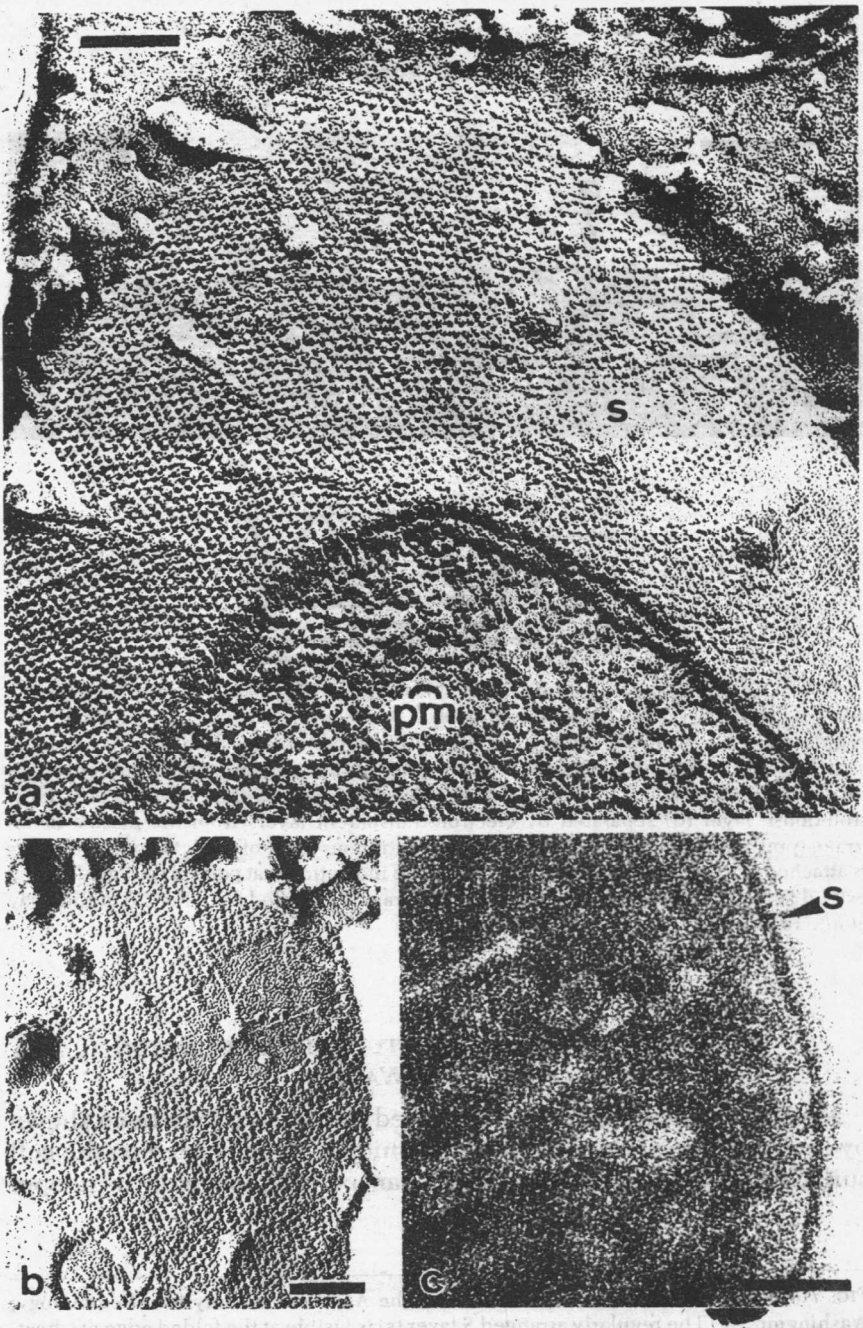

FIG. 7. (a) Electron micrograph of a freeze-etched preparation of *Acinetobacter* sp. strain MJT/F5/5. The outer surface (s) revealed by etching consists of hexagonally arranged subunits. pm, Internal fracture face of the cytoplasmic membrane. Bar represents 0.1 μm. (From Sleytr *et al.*, 1974, by permission from the American Society for Microbiology, Washington.) (b) Cell surface of *Acinetobacter* sp. strain MJT/F5/5 showing an area lacking regularly arranged subunits. Bar represents 0.1 μm. (From Sleytr *et*

(*Continued*, see p. 10)

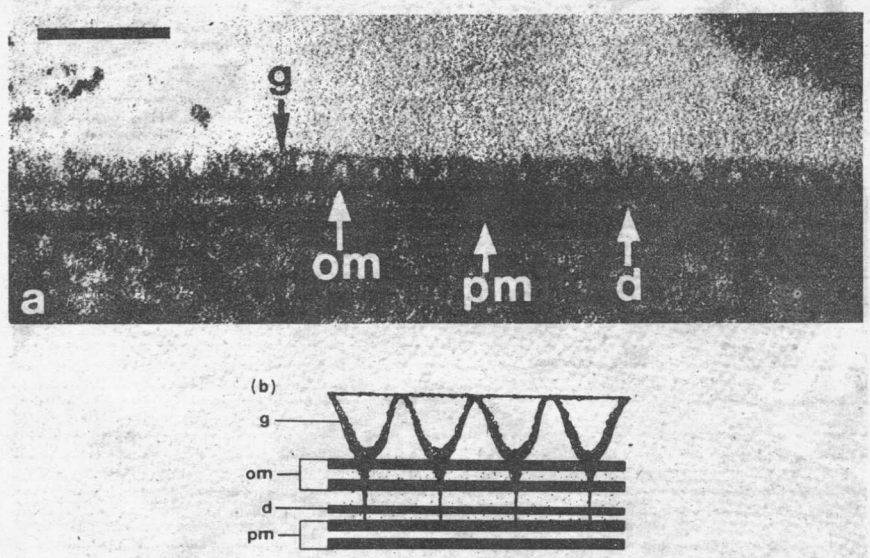

FIG. 8. (a) Longitudinal section of the cell envelope of *F. polymorphus* showing the array of "goblets" (g). om, Outer membrane; d, dense layer; pm, plasma membrane. Bar represents 0.1 μm. (From Ridgway et al., 1975, by permission from the National Research Council of Canada, Ottawa.) (b) Diagram of the fine structure of cell envelope layers of *F. polymorphus* as seen in thin sections. The cell envelope consists of an electron-dense layer (d) separated by electron-transparent layers from the plasma membrane (pm) and the outer membrane (om). A continuous layer of goblet-shaped subunits is attached to the outer membrane. There is some indication that parts of the "goblets" (g) extend to the cytoplasmic membrane. (The diagram is modified from Fig. 25 in Ridgway *et al.*, 1975.)

C. OTHER REGULAR STRUCTURES IN ASSOCIATION WITH CELL WALLS

Most of the regular patterns observed in bacteria have been shown by freeze-etching and shadowing techniques to be located on the cell surface. However, regular patterns can be demonstrated in deeper

FIG. 7 (*cont'd*). *al.*, 1974 by permission from the American Society for Microbiology, Washington.) (c) The regularly arranged S layer (s) is visible at the folded edge of a heat-treated cell of *Acinetobacter* sp. strain MJT/F5/5 in a negatively stained preparation. The cytoplasm has retracted, and the regular array of surface subunits is visible. Bar represents 0.1 μm. (From Sleytr *et al.*, 1974, by permission from the American Society for Microbiology, Washington.)

layers after certain treatments (e.g. heat, enzymes, or detergents). Fischman and Weinbaum (1967) observed a periodic monolayer of macromolecules in *Escherichia coli*. Later a structurally different regular array of subunits in *E. coli* was shown to be composed of one of the major polypeptides that cover the outer surface of the peptidoglycan (Rosenbusch, 1974; Steven *et al.*, 1977). It is possible that similar internal regular arrays of macromolecules are present in other bacteria. The detection of these structures will depend on the development of suitable methods for the selective disintegration and solubilization of the overlying components of the envelope, or on the removal of masking material, such as capsular slime.

Whereas capsular material in bacteria usually has little defined structure (Glauert and Thornley, 1969), a regular pattern has been demonstrated in the sheath of *Lampropedia hyalina* (Chapman *et al.*, 1963; Pangborn and Starr, 1966), which loosely surrounds the flat, squarish aggregates of the gram-negative cells. The envelope consists of two complex layers, both having a hexagonal structure but with different spacings and subunit morphology.

The cell walls of the extremely thermophilic, acidophilic organisms *Caldariella* (Millonig *et al.*, 1975) and *Sulfolobus acidocaldarius* (Weiss, 1974) and a range of extremely halophilic bacteria of the genus *Halobacterium* (Cho *et al.*, 1967; Steensland and Larsen, 1969; Blaurock *et al.*, 1976) do not have a rigid mucopeptide layer of the type found in other gram-negative bacteria. The envelopes of intact cells and isolated cell envelopes reveal a two-layered structure in thin sections (Fig. 9). The inner layer is a cytoplasmic membrane which appears as a typical unit membrane, while the outer cell wall is composed of regularly arranged subunits.

Finally, some species of bacilli and clostridia have regular hexagonal patterns on the exosporium, and the outer surface of the spore coat may have regular arrays of subunits or regularly spaced ridges (Holt and Leadbetter, 1969).

FIG. 9. Schematic drawing of a subunit type of cell wall in thin sections as found in extremely thermophilic, acidophilic, and some halophilic organisms which lack a peptidoglycan layer. pm, Plasma membrane. The cell wall (w) reveals a subunit structure.

此为试读，需要完整PDF请访问：www.ertongbook.com