JOURNAL OF BACTERIOLOGY, July, 1965
Vol. 90, No. 1

Localization of Somatic Antigen on Gram-Negative Bacteria by Electron Microscopy

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Received for publication 16 March 1965

ABSTRACT

SHANDS, J. W. (University of Florida, Gainesville). Localization of somatic antigen on gram-negative bacteria by electron microscopy. J. Bacteriol. **90**:266–270. 1965.—Antisera specific for the somatic antigens of *Salmonella typhimurium* and *Escherichia coli* O113 were prepared, and globulins isolated from these antisera were labeled with ferritin. Micrographs of labeled, sectioned bacteria show that somatic antigen is located in considerable quantities on the surface of the bacteria, and, furthermore, that it can extend up to 150 mμ beyond the confines of the cell wall. The arrangement of the ferritin on the bacteria suggests that the antigenic sites are located on fibrillar structures.

Using a partially rough strain of *Escherichia coli* B, Weidel and Primosigh (1958) and Weidel, Frank, and Martin (1960) reported biochemical evidence that the cell wall of a gram-negative bacterium is a three-layered structure. Their concept of the gram-negative cell wall includes a superficial lipoprotein layer covering a lipopolysaccharide layer and an innermost rigid or "R" layer. Electron micrographs of sectioned *E. coli* are at least consistent with these findings, revealing three layers in the cell wall (Kellenberger and Ryter, 1958).

It is firmly established that the lipopolysaccharides, or somatic antigens, of gram-negative bacteria are part of the cell wall (Ribi, Milner, and Perrine, 1959), but it is less certain that they are confined to the cell wall or much less to one of the three layers. Indeed, some findings indicate that this may be an erroneous concept. Agglutination of bacteria by antibody to the somatic antigen would be impossible if the somatic antigen were covered by a lipoprotein layer. Furthermore, Wardlaw (1964) compared rough and smooth strains of *Escherichia coli* in their susceptibility to the lethal activity of complement. His findings led him to propose that endotoxin (in the smooth strain) is located on the cell-wall surface as well as the middle layer.

The present approach to the problem was at the morphological level, with the use of ferritin-conjugated antibodies specific for the somatic antigens of *Salmonella typhimurium* and *E. coli* O113. Intact cells of both organisms were labeled and studied by electron microscopy.

MATERIALS AND METHODS

Bacteria. S. typhimurium 7, a smooth, virulent organism, was obtained from M. Herzberg (Department of Bacteriology, University of Florida). A smooth strain of *E. coli* O113 was obtained from E. Ribi (Rocky Mountain Laboratories, Hamilton, Mont.). The bacteria were grown for 5 hr in glucose-salts medium prior to labeling.

Antisera. Antisera to the somatic antigens were prepared in New Zealand rabbits. The antigens were whole organisms of *S. typhimurium* or *E. coli* which had been boiled for 2.5 hr in saline to denature flagellar and K antigens. The immunizations consisted of weekly intravenous injections of 5×10^8 to 1×10^9 organisms, and the animals were bled 6 days after the last injection. The globulins were precipitated from the antisera by 37% ammonium sulfate, and were coupled to ferritin by the method of Sri Ram et al. (1963).

Labeling. The culture of bacteria to be labeled was washed once and reconstituted in Hanks' balanced salt solution. Four to six drops of ferritin-conjugated antibody were added to 2 to 3 ml of culture, and the mixture was allowed to stand until agglutinated. The agglutinated bacteria were then washed three times in Hanks' balanced salt solution, fixed by the method of Kellenberger and Ryter (1958), and embedded in Maraglas (Marablette Corp., Long Island City, N.Y.) by the method of Spurlock, Kattine, and Freeman (1963). Thin sections were cut on the Porter-Blum MT-2 microtome (Ivan Sorvall, Inc., Norwalk, Conn.), stained with lead citrate (Reynolds, 1963), and examined with a Siemens Elmiskop I electron microscope.

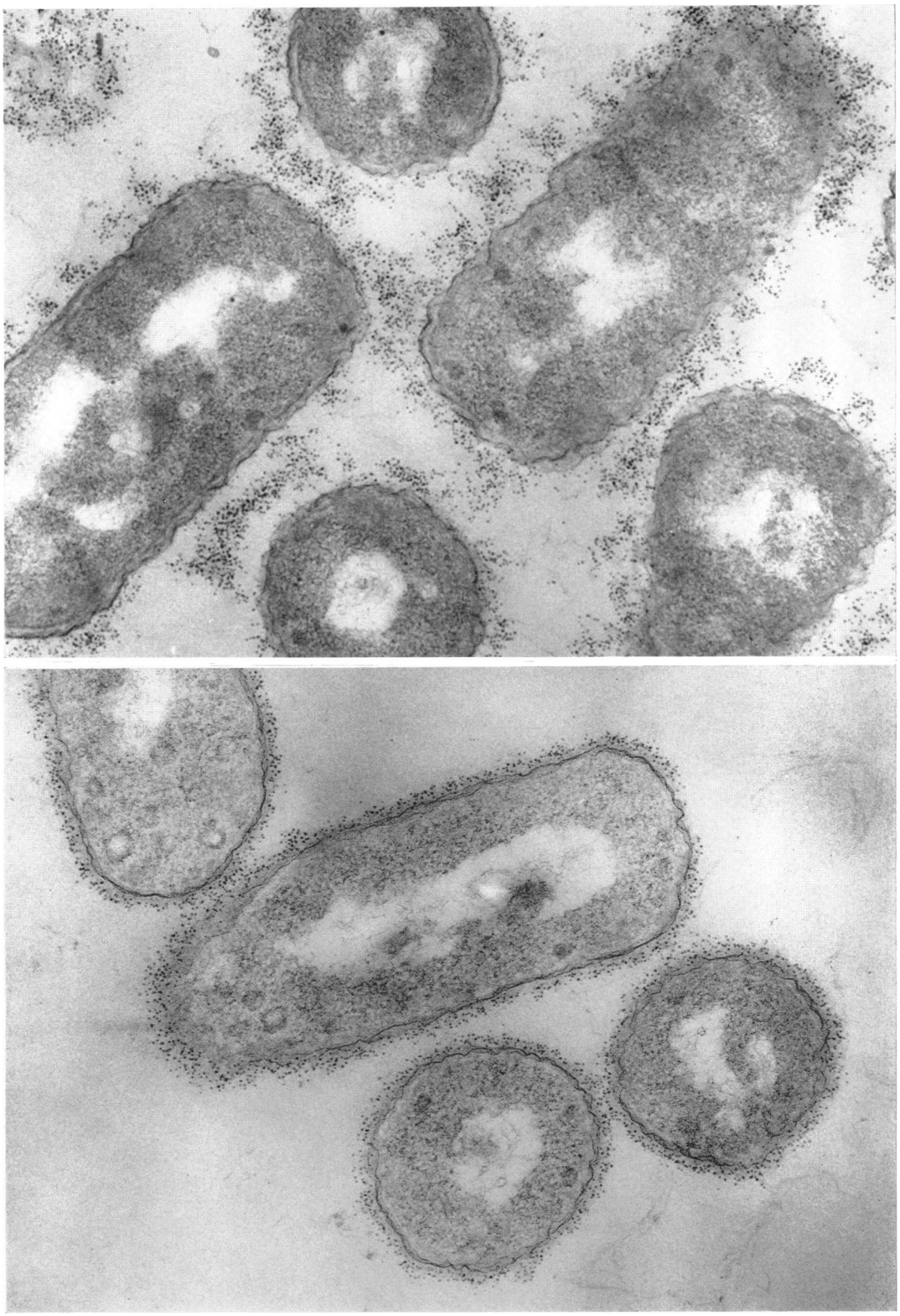

FIG. 1. *Escherichia coli agglutinated by ferritin-conjugated antibody to somatic antigen. The bacteria are widely separated and appear to be connected by bridges of ferritin.* ×*60,000.*

FIG. 2. *Salmonella typhimurium agglutinated by ferritin-conjugated antibody to somatic antigen.* ×*60,000.*

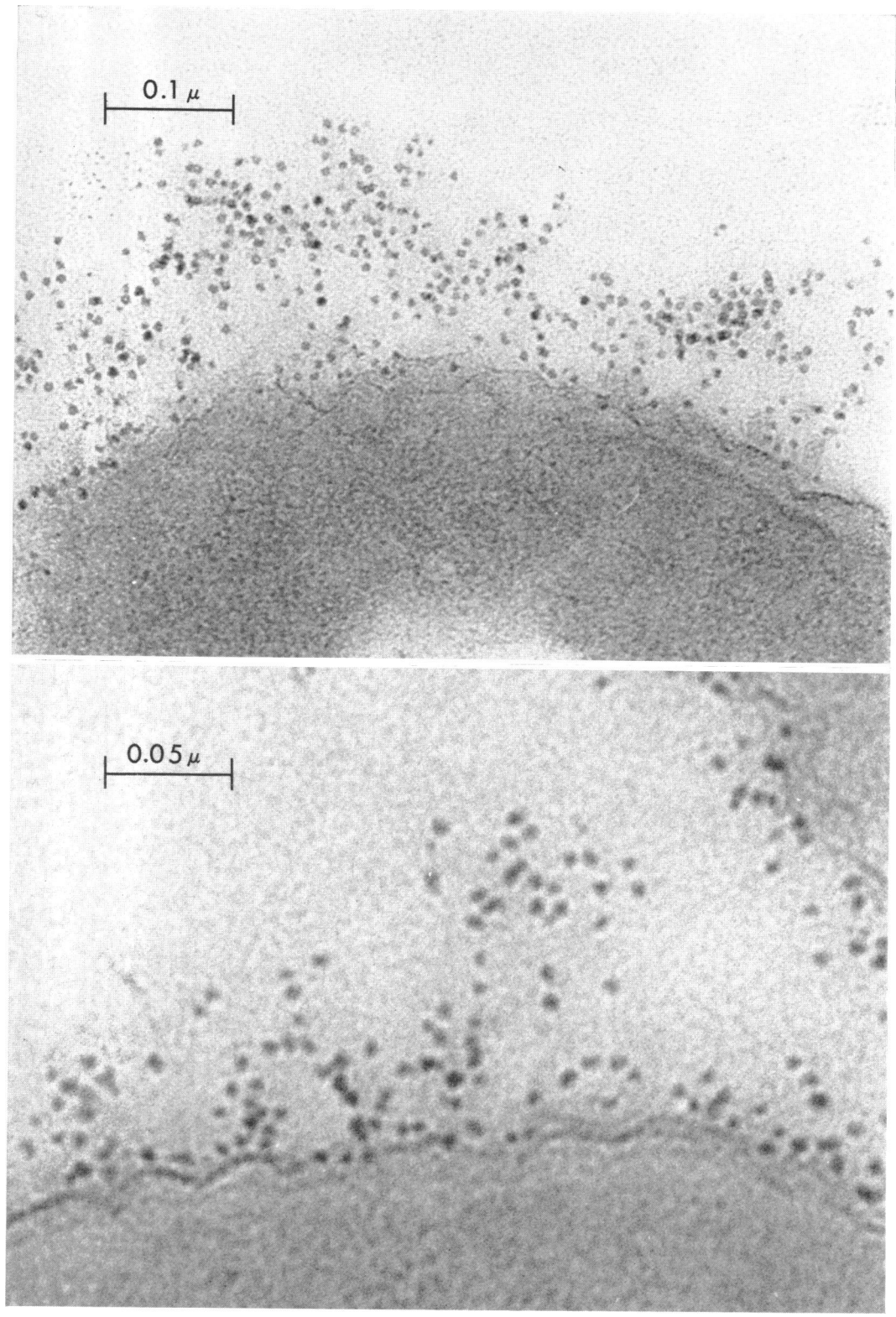

FIG. 3. *Surface of Escherichia coli labeled with ferritin-conjugated antibody showing a fibrillar orientation of the ferritin.* ×*200,000.*

FIG. 4. *Surface of labeled Salmonella typhimurium.* ×*400,000.*

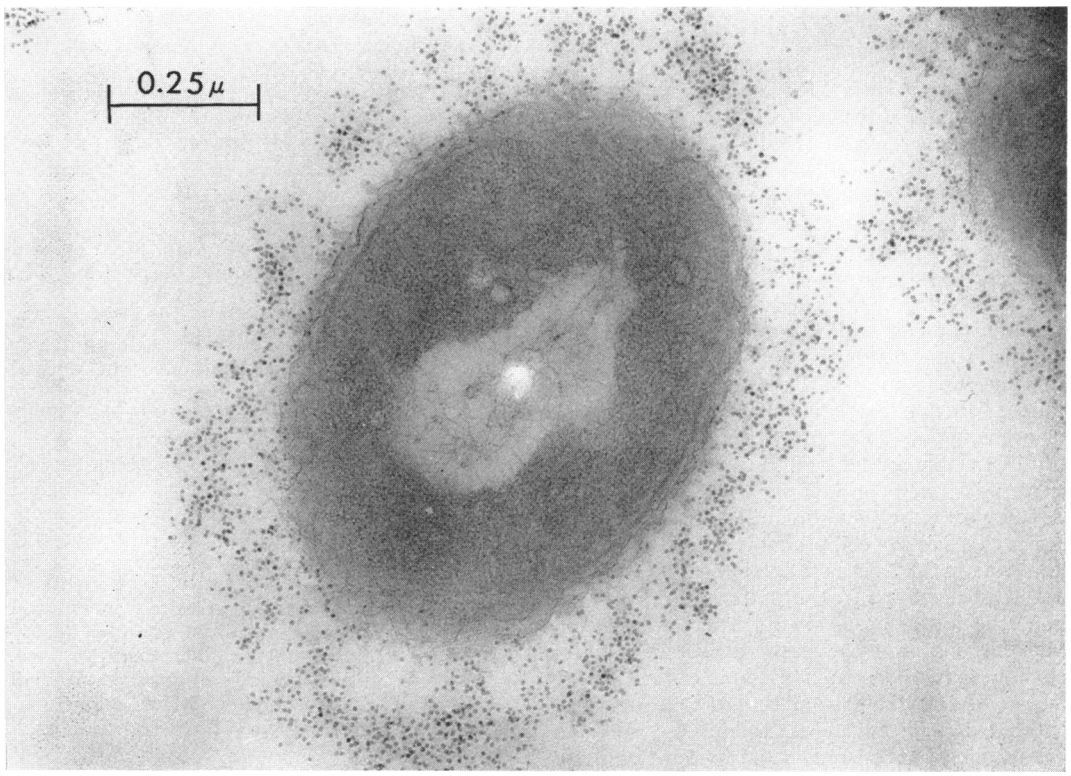

FIG. 5. *Labeled Escherichia coli showing "capsule" of ferritin surrounding the organism.* ×*80,000.*

RESULTS

The specificity of the ferritin-conjugated antibodies was assayed in several ways. To rule out nonspecific labeling of the bacteria, crossed experiments were performed in which *S. typhimurium* was incubated with ferritin-labeled anti-*E. coli* globulin and vice versa. No labeling of the bacteria was found in either experiment. The antibody activity of the *S. typhimurium* antiserum could be completely removed by absorption with a potent endotoxin prepared from the same organism by aqueous ether extraction (Ribi et al., 1959). Similarly, all the activity of the anti-*E. coli* conjugate could be absorbed by the same organism after being killed in alcohol, digested with trypsin, and boiled for 2.5 hr. Consequently, it was concluded that virtually all the antibody activity was directed against the somatic antigens and none against either the flagellar or K antigens.

Study of labeled, agglutinated organisms revealed that the agglutination of these gram-negative organisms almost never involved juxtaposition of the cell wall of one organism against another. The organisms were almost always separated by a distance of at least several hundred Ångstroms and appeared to be held together by bridges of ferritin-conjugated antibody (Fig. 1 and 2). Pictures at higher magnification strongly suggested that the basic structures labeled by the ferritin antibody conjugate were fibrils which extended out from the cell wall, some curling back on themselves or cross-linked to other fibrils by antibody (Fig. 3 and 4). *E. coli* was so densely labeled that the somatic antigen appeared to form a structure analogous to a capsule about the organism (Fig. 5).

DISCUSSION

Although the major portion of the somatic antigen or endotoxin is found in the cell wall of gram-negative bacteria (Ribi et al., 1959), the present findings show that it is certainly not confined to this structure, but rather that it extends for distances up to 150 mμ beyond the confines of the cell wall. It is, therefore, clear that the somatic antigen should not be designated simply as a layer in the gram-negative cell wall.

The somatic antigen also appears to be structurally a fibril. This finding is consistent with

those of Milner et al. (1963), who demonstrated a fibrillar structure in the lipopolysaccharide derived from *Bordetella pertussis*. The agglutination of gram-negative bacteria by antisomatic antibody appears to be effected by cross-linking of these fibrils from individual cells and not by cross-linking of cell walls.

The existence of long fibrils of somatic antigen or endotoxin streaming from the surface of gram-negative organisms implies that quantities of this material may be easily removed without killing and disrupting the integrity of the bacterium. This may be one explanation for the presence of endotoxin in culture filtrates. These fibrils may also be the source of endotoxin obtained by aqueous ether extraction (Ribi et al., 1959), a procedure which does not disrupt the cell, and which requires viable, intact bacteria to produce a good yield.

The portion of endotoxin extending beyond the confines of the cell probably plays an important role in host-parasite relationships of gram-negative infection. In the pathogenesis of infection, the initial host-parasite interaction would take place at the bacterial surface. It is quite possible that at the time of the initial encounter release of endotoxin from the bacterial surface, without damage to the bacterium, may serve to decrease local host defenses and thereby facilitate the establishment of infection. Once infection is established, continued endotoxin production could lead to endotoxemia and its consequent manifestations, even in the absence of death and disintegration of large numbers of organisms.

The superficial localization of somatic antigen may also be one explanation for the relative resistance of smooth gram-negative organisms to the bactericidal activity of normal serum (Michael and Landy, 1961). Where the concentration of antibody is limited, most of it may be taken up by antigenic sites distant from the cell wall. The subsequent fixation of complement at these sites would presumably result in less bactericidal activity.

ACKNOWLEDGMENTS

The technical assistance of Lorenza Brown is gratefully acknowledged.

This investigation was supported by Public Health Service research grant AI 03798-05 from the National Institute of Allergy and Infectious Diseases.

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