ULTRASTRUCTURE OF SPERMINE-TREATED *ESCHERICHIA COLI*, INCLUDING A POLAR ORGANELLE CONCERNED WITH ENVELOPE SYNTHESIS

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Abstract

Sections of spermine-treated, wild-type *E. coli* W showed intracytoplasmic membranes and tubules, both of which were associated with the nucleoplasm. Ribosomes were frequently arranged linearly or in clusters and linked by fibrils. DNA fibrils were rarely detected.

Spermine treatment of a diaminopimelic acid-requiring auxotroph under conditions of diaminopimelic acid starvation (thereby blocking cell envelope synthesis) resulted in the detection of a polar organization or organelle. This is connected to the plasma membrane and consists of ribosomes in association with a complex of interlocking electron-dense and -light components. We propose and discuss the possibility that this may represent an apparatus for the synthesis and assembly of protein and phospholipid components of the cell envelope.

I. Introduction

Morphological studies of *Escherichia coli* have relied mainly on cells fixed directly with osmium tetroxide (Ryter and Kellenberger 1958) or with successive treatments of glutaraldehyde and osmium tetroxide (Glauert and Thorney 1966). Electron micrographs thus obtained usually show a random distribution of ribosomes, absence of structures associated with the nucleoplasm, and absence of polyribosomes. The extent of structures destroyed or distorted by these fixation methods is not clearly known. Recent biochemical investigations using more gentle extraction methods demonstrate that most of the ribosomes exist as polyribosomes (Schlessinger 1969).

The present paper describes a number of structures previously not reported in *E. coli*. They were detected by growing cells in the presence of spermine, a compound known to stabilize nucleoprotein and phospholipid (Tabor, Tabor, and Rosenthal 1961; Tabor and Tabor 1964). Two strains of *E. coli* were used, wild-type W and the derived mutant 173.25 which requires diaminopimelic acid (DAP) for growth. DAP is required for the synthesis of the mucopeptide component of the cell envelope. The mutant was originally used in an attempt to produce protoplasts as a result of DAP starvation (Cho 1968). In the presence of spermine, this technique did not produce protoplasts but resulted in the detection of previously unknown structures.

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II. Materials and Methods

(a) *Organisms and Growth Conditions*

E. coli W was grown in M9 medium (Adams 1959) to an optical density of 0·5 (1 cm light path at 700 nm). Spermine was added (2×10^{-2}M) and the cell suspension was divided into two equal portions, one with no additions and the other supplemented with chloramphenicol (20 μg/ml). Both portions were incubated with shaking at 37°C for 1 hr before fixation.

E. coli 173.25 was grown to a similar optical density as above in M9 medium supplemented with sucrose (15% w/v), DL-lysine hydrochloride (100 μM), and DAP (20 μg/ml). Cells were centrifuged and resuspended in the same medium minus DAP, but supplemented with spermine (2×10^{-2}M). Half of this suspension was transferred to a flask containing chloramphenicol (20 μg/ml). Both suspensions were further incubated with shaking for 4 hr at 37°C before fixation.

(b) *Electron Microscopy*

Cells were fixed by two methods: (1) a modification of Ryter and Kellenberger's method (1958): pellets used for embedding had no agar and the period of fixation was reduced to 4 hr; (2) a double-fixation method in which cells were pre-fixed with 1% glutaraldehyde in sodium cacodylate buffer (0·05M, pH 6·0) supplemented with 0·01M $CaCl_2$ for 2 hr at room temperature (20–22°C): after washing six times with sodium cacodylate the cells were fixed with osmium tetroxide as in method 1.

Fixed cells were treated with 1% uranyl acetate for 2 hr. Dehydration was carried out in a graded series of mixtures of acetone and water. The specimens were infiltrated with Araldite and acetone (1 : 1 v/v) for 2 hr, with Araldite for 24 hr, and with freshly prepared Araldite at 60°C for 1 hr. Embeddings were done in freshly prepared Araldite and were polymerized at 60°C for 2 days.

Sections were cut with glass knives on a Reichert ultramicrotome, collected on unsupported 400-mesh grids, doubly stained with saturated uranyl acetate in 50% (v/v) ethanol for 1 hr, then on lead citrate for 30 min (Reynolds 1963). Electron micrographs were taken with a Siemens Elmiskop electron microscope model 1A with a double condenser system.

III. Results and Discussion

Differences in fixation techniques and the addition of chloramphenicol had no apparent effect on the morphology of *E. coli* W or *E. coli* 173.25.

(a) *Morphology of Spermine-treated* E. coli *W*

(i) *Nucleoplasm.*—DNA fibrils, shown clearly in normally grown cells, were rarely observed in spermine-treated cells. Instead, the nucleoplasm appears as an electron-light vacuole interwoven by widely scattered coarse threads of low electron density (Figs. 1 and 2). This appearance can be explained by the fact that spermine binds to the phosphate groups present mostly in nucleic acids and phospholipids. The pretreatment of *E. coli* by spermine therefore renders the phosphate groups inaccessible to uranyl acetate which is responsible for the electron-dense appearance of the DNA fibrils in bacteria (Fuhs 1965).

(ii) *Ribosomes.*—The ribosomes are 110–160 Å in diameter, presumably of the 70-S type present in bacteria. However, the ribosomes of spermine-treated cells were frequently arranged linearly (Fig. 3) or in clusters (Fig. 4).

Fig. 4.—Section of spermine-treated *E. coli* W. The structure (arrow *a*) may be a fusion of two "unit membrane" since its thickness is about twice that of a "unit membrane". Ribosomes (arrow *b*) are arranged in clusters. Fixation: osmium tetroxide. ×90,000.

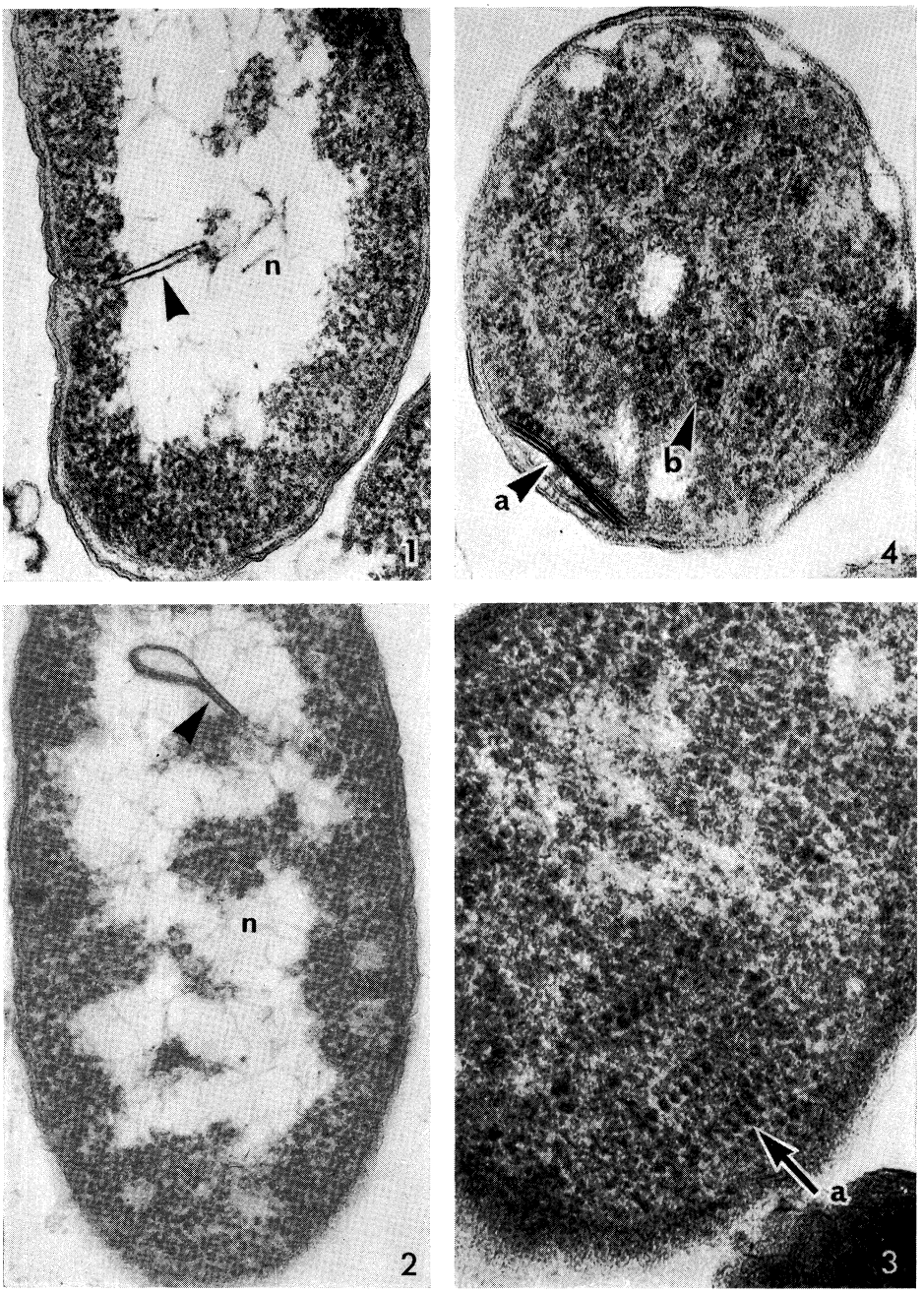

Fig. 1.—Section of spermine-treated *E. coli* W. A tubule (arrow) is shown in association with the nucleoplasm (*n*). Fixation: osmium tetroxide. ×70,000.

Fig. 2.—Section of spermine-treated *E. coli* W. The ends of the "intracytoplasmic membrane" meet to form a five-layered structure (arrow) closely associated with the nucleoplasm (*n*). Fixation: osmium tetroxide. ×75,000.

Fig. 3.—Section of spermine-treated *E. coli* W. Note the linear arrangement of ribosomes (arrow *a*). Fixation: glutaraldehyde and osmium tetroxide. ×125,000.

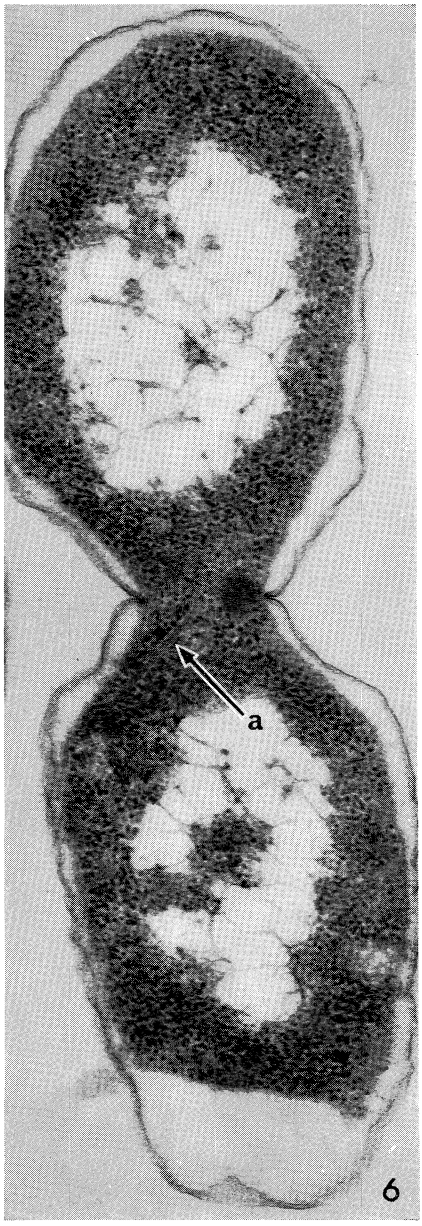

Fig. 5.—Section of spermine-treated *E. coli* 173.25 starved of DAP. Arrays of ribosome (arrow *a*) joined by fibrils are seen running parallel to each other and cut transversely at one end by an electron-dense band. Fixation: osmium tetroxide. ×120,000.

Fig. 6.—Section of spermine-treated *E. coli* 173.25 starved of DAP. A polar organization (arrow *a*) is seen at the dividing plane. Fixation: osmium tetroxide. ×25,000.

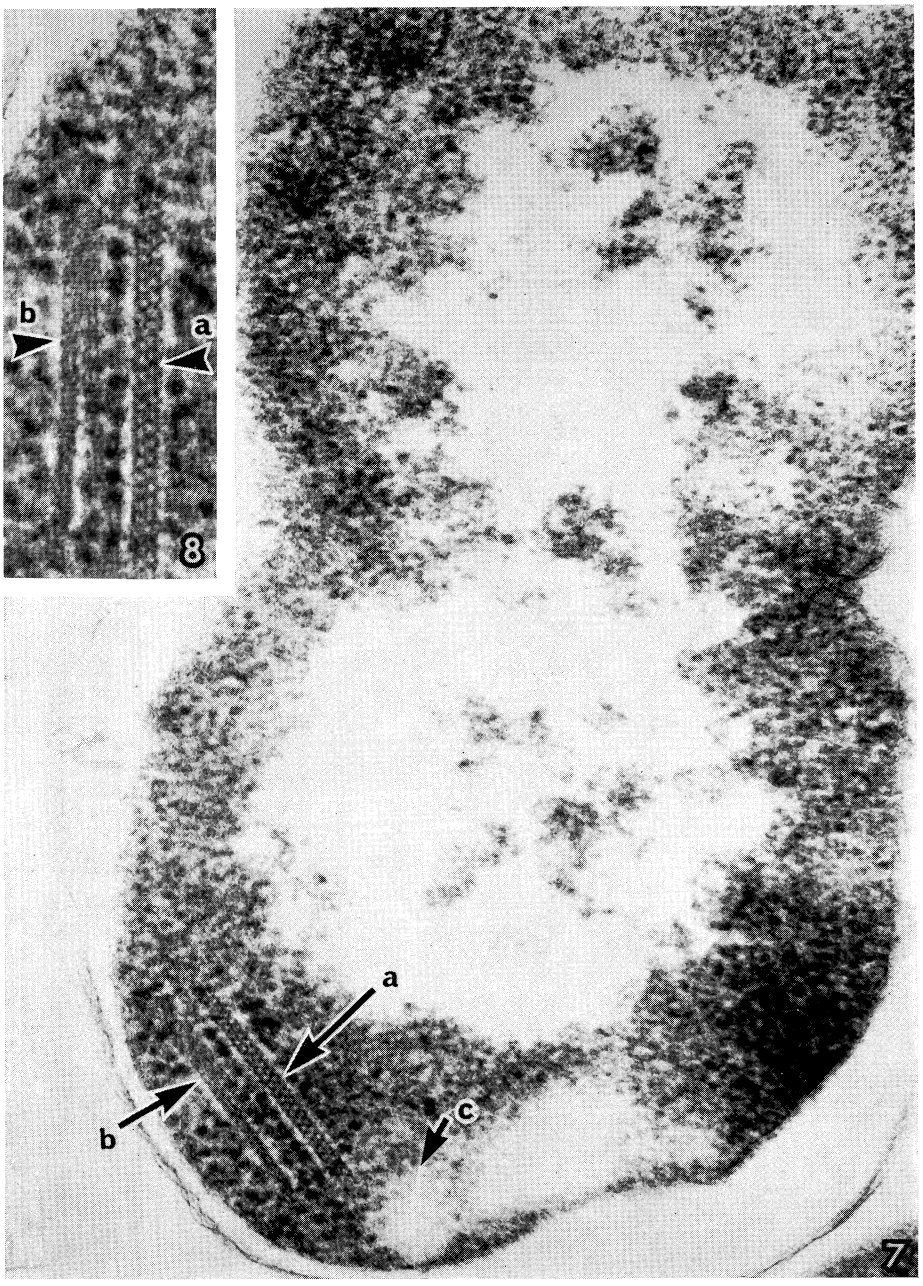

Fig. 7.—Section of spermine-treated *E. coli* 173.25 starved of DAP. A polar organelle (arrow *a*) and a triple-layered strand (arrow *b*) are seen running parallel to each other. Note the electron-light area (arrow *c*) near the polar organelle and the linear arrangement of ribosomes on both sides of the organelle. Fixation: osmium tetroxide. ×90,000.

Fig. 8.—An inset of Figure 7 at a higher magnification. Fixation: osmium tetroxide. ×150,000.

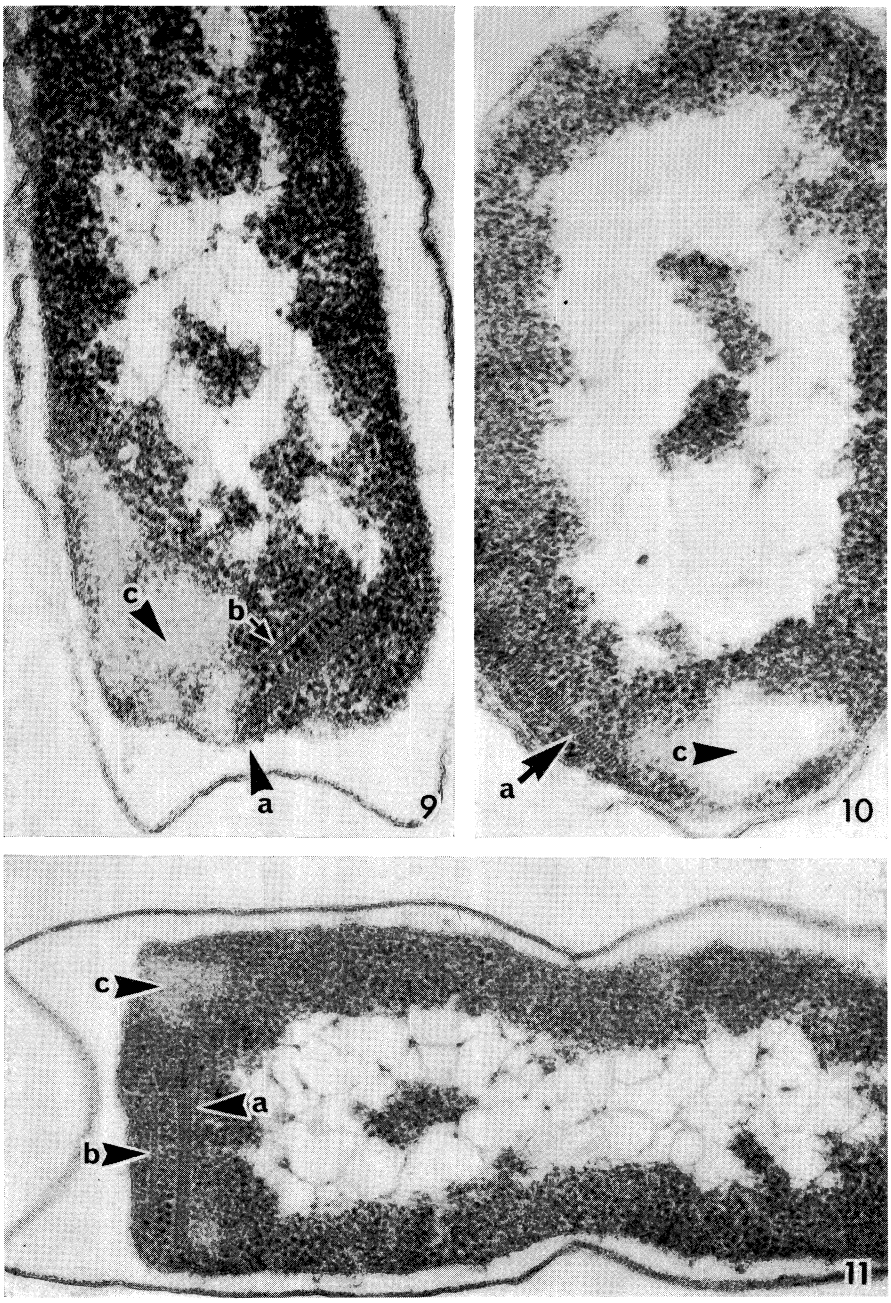

Fig. 9.—Section of spermine-treated *E. coli* 173.25. The polar organelle is seen joining to the plasma membrane (arrow *a*). Note again the frequent association of the triple-layer strand (arrow *b*) and the electron-light area (arrow *c*) to the polar organelle. Fixation: osmium tetroxide. × 70,000.

(iii) "*Intracytoplasmic Membranes*".—Membranes (75–80 Å thick) were present in the nucleoplasm as circular profiles. Sometimes both ends meet "in parallel" and form a five-layered structure (Fig. 2). These membranes are of interest since their location and morphology are different from those observed in normally grown cells (Ryter and Jacob 1966), magnesium-starved cells (Cota-Robles 1966), or phosphate-starved cells (Cho and Doy, unpublished data). On rare occasions, thin sections show a fusion of two extremely electron-dense intracytoplasmic membranes (Fig. 4), with a total thickness of 160 Å.

(iv) *Tubules*.—Tubules associated with the nucleoplasm extend into the cytoplasm (Fig. 1) and consist of two electron-dense layers (each 50 Å) separated by an electron-light layer (100 Å).

(*b*) *Morphology of Spermine-treated* E. coli *173.25*

All observations were made on DAP-starved cells in which envelope synthesis was then halted, with or without the addition of chloramphenicol to inhibit protein synthesis; a technique that in the absence of spermine produced a small percentage of protoplasts (Cho 1968). Spermine inhibited the formation of protoplasts and less than 20% of spheroplasts were produced. It is probable that the envelope is more permeable to spermine under conditions of DAP starvation.

(*c*) *Ribosomes*

Ribosomes are sometimes observed to be arranged linearly or in clusters (similar to those in Figs. 3 and 4). Linear arrangements of ribosomes have been interpreted by other investigators as belonging to a helical polyribosome cut along its length (Kingsbury and Voelz 1969; Nauman, Silverman, and Voelz 1971). When sections of cells are cut in the appropriate plane, parallel arrays of ribosomes are observed (Fig. 5). The ribosomes are separated by a distance of 30 Å and are linked by fibrils (15–20 Å thick). The arrays of ribosomes are about 40–50 Å apart and are traversed by electron-dense strands of about 100–150 Å thick. It is not known if these are, at least in part, associated with the polar organelle described below.

(*d*) *Polar Organelle*

Many examples have been found of an organelle located at a pole or in association with cell division (Figs. 6–11). Ribosomes are arranged linearly in parallel with a complex of electron-dense and electron-light components. In some sections these latter have the appearance of a chain or network of tubules and can occur on both

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Fig. 10.—Section of spermine-treated *E. coli* 173.25 starved of DAP in the presence of chloramphenicol. Arrays of dense filaments or bar-like structures (arrow *a*) (200–250 Å wide and separated by a regular spacing of 55–60 Å) are seen near the pole of the cell and close to the electron-light area (arrow *c*). Fixation: osmium tetroxide. ×90,000.

Fig. 11.—Section of spermine-treated *E. coli* 173.25 starved of DAP in the presence of chloramphenicol. The difference of the arrays of filaments (arrow *a*) as compared with that of Figure 10 is possibly due to difference in the cutting plane. A single-beaded structure (arrow *b*) is seen running parallel to arrays of filaments. Note again the presence of electron-light area (arrow *c*) near the pole of the cell. Fixation: osmium tetroxide. ×62,500.

sides of the ribosomes. In other sections the electron-dense components appear as a series of regularly spaced bars although closer examination shows that there are connections between them. Occasionally the lines of ribosomes are separated by a sheet of particulate electron-dense material which may represent a section along the surface of the electron-dense boundary.

In many electron micrographs (Figs. 7–9) the ribosomes appear to be joined by strands of intermediate density (*b*, Figs. 7, 8, and 9) and themselves to grow in electron density until maximum density is reached adjacent to the system of interlocking electron-light–electron-dense bounded subunits (*a*, Figs. 7, 8, 9). The increase in electron density is consistent with an increase in protein content and we may be witnessing the actual synthesis of protein on ribosomes. Similarly, the electron-light–electron-dense structure is only fully organized opposite the ribosomes and may therefore be picking up protein from them.

The organelle is always close to the plasma membrane and in at least one example can be seen to connect with it directly (Fig. 9). Frequently there is a large electron-light area (*c*, Figs. 7, 9, 10, 11) close to the organelle that is more dense or more structured than the nucleoplasm.

This organization is only observed in spermine-treated, DAP-starved cells, indicating that both conditions are necessary for its detection. The consequence of DAP starvation is that envelope synthesis is stopped and we think that the spermine has preserved either an intermediate stage in a normal sequence of events or else an abnormal accumulation of precursors.

Spermine treatment has the effect of making phosphate-rich macromolecules, such as nucleic acids and phospholipid, appear electron-light, whereas protein is still electron-dense. The association with an interruption of envelope synthesis as well as the location of the organelle strongly suggests that the electron-light components are phospholipid and the relatively large area of electron-light material an accumulated pool of phospholipid with perhaps some protein.

Rothfield and Romeo (1971) have shown that the main component of the cell wall is derived from a lipid carrier via the plasma membrane. We suggest that the polar organelle is involved in the synthesis and transfer of cell-envelope components. The organelle as a whole may represent an assembly of protein and phospholipid with the protein being supplied from the associated ribosomes. This leads to the conclusion that the protein–phospholipid components of the cell envelope are synthesized and at least partly assembled in the cytoplasm and then exported to form the plasma membrane and other components of the envelope.

If the organelle is a natural component of the cell preserved by the technique, rather than an artefact caused by an abnormal accumulation of envelope precursors, it may have a role to play in the organization of cell division including the replication and transfer of DNA. On this view some of the electron-light components could be nucleic acids rather than phospholipid.

Only one organelle has been seen in any one section and although many sections do not have any detectable portion of the organelle this is not inconsistent with there being at least one polar organelle per cell.

A polar membrane has been seen in some specialized Gram-negative organisms (Remsen *et al.* 1968) and assumed to be associated with tufts of flagella, but which could be analogous (though by no means identical) to the structure shown here.

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