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THIN SECTIONS OF DIVIDING *NEISSERIA GONORRHOEAE*

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ABSTRACT

FITZ-JAMES, PHILIP (University of Western Ontario, London, Ontario, Canada). Thin sections of dividing *Neisseria gonorrhoeae*. J. Bacteriol. **87**:1477-1482. 1964.—The fine structure of the gram-negative pathogen *Neisseria gonorrhoeae* is described. Its cell-wall and membrane structures are not unlike those of *Escherichia coli*, possessing periodic points where wall and membrane are approximated. It appears to divide by a process of unequal constriction plus septum formation, preceded by a loop of membrane. Mesosomes are also seen but in the cell periphery away from the division plane. At some of the wall-membrane junctions, the cell wall appears to arise out of the membrane.

Membrane folds and membranous organelles (mesosomes) have been shown in sections of dividing and sporulating gram-positive bacilli in positions which suggest that the membrane structures have some role in these processes (Fitz-James, 1960; Van Iterson, 1961). However, the commonly studied gram-negative organisms, such as *Escherichia coli*, appear to be devoid of complicated membranous organelles, besides having a differently constructed cell wall (Kellenberger and Ryter, 1958). This general difference in membrane form, plus the fact that most gram-positive organisms divide by transverse septation as opposed to the wall-membrane constriction seen in many gram-negative organisms, prompted a study of the peculiar division of the gram-negative pathogen *Neisseria gonorrhoeae*. It was found that cells of this organism divide by a combination of pinching and septation involving a simple type of membranous fold. Mesosomes were found, but not at the site of cell division. A preliminary report of a more comprehensive study of the taxonomic relationship of the *Neisseria* to gram-positive and gram-negative bacteria has appeared elsewhere (Murray, Reyn, and Birch-Andersen, 1963).

MATERIALS AND METHODS

The culture used was a fresh clinical isolate. After 20 hr of growth on blood-glucose-agar, a loopful of cells was scraped from the agar and quickly suspended in an osmium solution; this preparation was then further fixed and embedded as described by Kellenberger, Ryter, and Séchaud, 1958. The sections, cut with glass knives, were in some cases stained with lead by the method of Dalton and Zeigel (1960).

RESULTS AND DISCUSSION

In spite of the peculiar gonococcal profile of many of the cells, the general appearance of the wall-membrane was not unlike that of *E. coli* or of other gram-negative forms (Fig. 1 and 2).

The cell wall possessed a triplex structure which, in regions free from adherent medium contamination, had a minimal overall thickness of some 80 A and showed two dense zones and a lighter central zone, of some 30 A each, as found in *E. coli* (Kellenberger and Ryter, 1958). The cell membrane presented the familiar double-line profile, with an overall width of 95 to 100 A and a center-to-center distance of the parallel lines of about 75 A. Both wall and membrane were slightly convoluted, the wall being more so; hence, the wall-membrane interspace varied in width up to 200 A. At irregular intervals the wall and membrane, as in *E. coli*, appeared adherent; at such junctions the overall width measured 250 A (Fig. 3).

The earliest indication of division in central cuts of cells was a hemispherical constriction deeper on one side than the other (Fig. 1). At the apex of these early constrictions, a delicate single membranous extension can sometimes be seen (arrow, Fig. 1). In the deeper constrictions this membrane fold often appears as a simple membranous pocket, forming well ahead of the completed or mature cell-wall septum (Fig. 3, 5, and 6). These folds occurred at the division

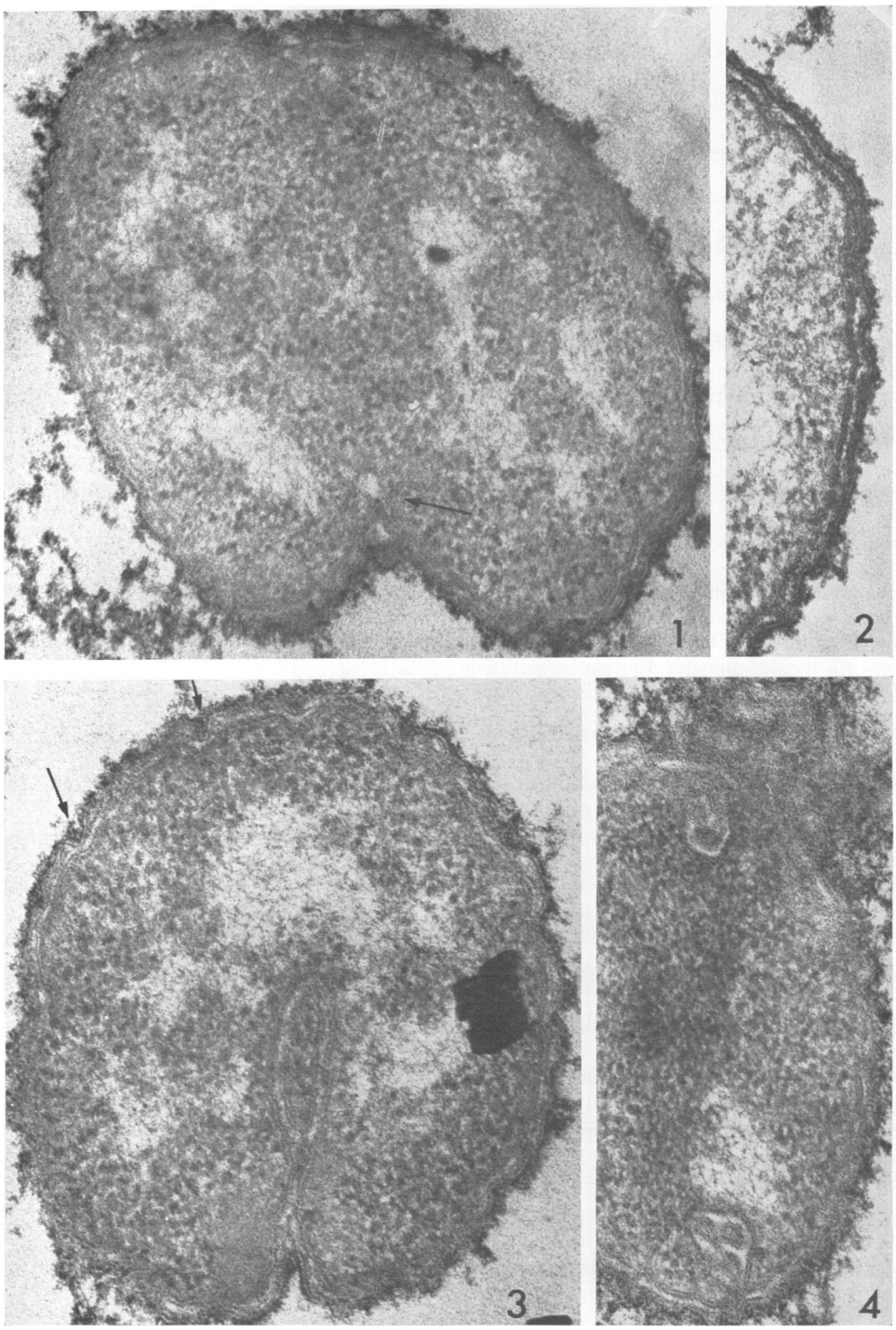
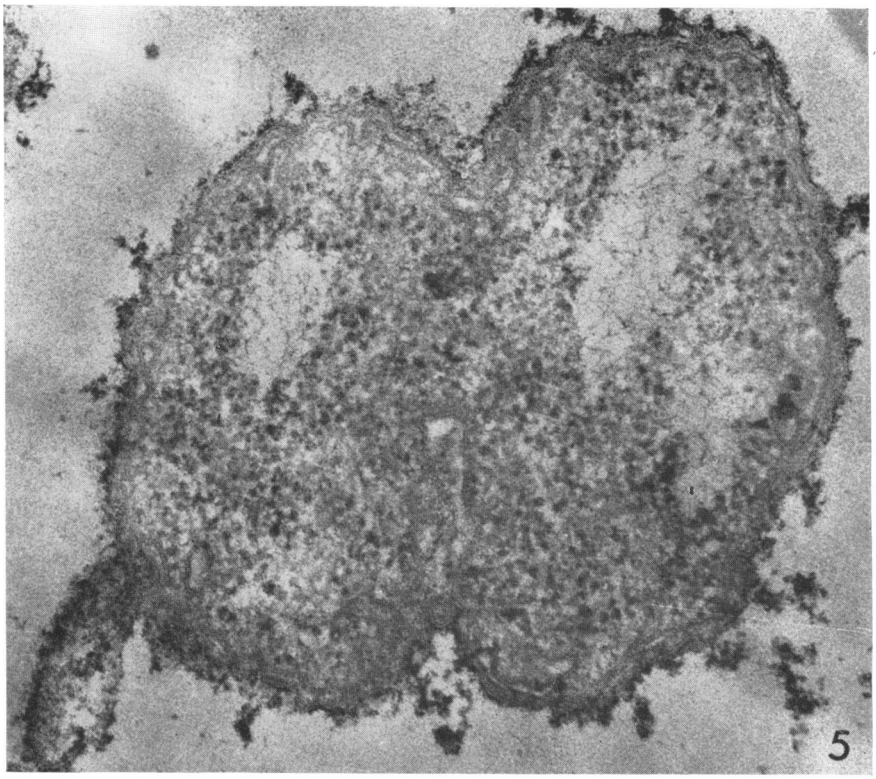


FIG. 1. *Thin section through a gonococcus early in its division process. A poorly contrasting membrane is seen (arrow) at the site of division.* × *100,000.*

FIG. 2. *Wall and plasma membrane of a partly lysed cell. The surface of the cell wall is matted with fixed medium, but shows the triplex pattern of densities similar to that of an Escherichia coli cell wall. The densely stained membrane stands out against the low-density cytoplasm.* × *100,000.*

FIG. 3. *An oblique cut through a partly divided cell showing, at the division site, a double-membraned loop continuous with the plasma membrane. At intervals (arrows) the cell wall appears to approach or touch the membrane.* × *127,000.*

FIG. 4. *Section through the outer edge of a gonococcus, showing two mesosomes, one of which is sectioned near its point of adherence to chromatin and also near its continuity with plasma membrane. The division plane is probably parallel to the plane of this section.* × *100,000.*



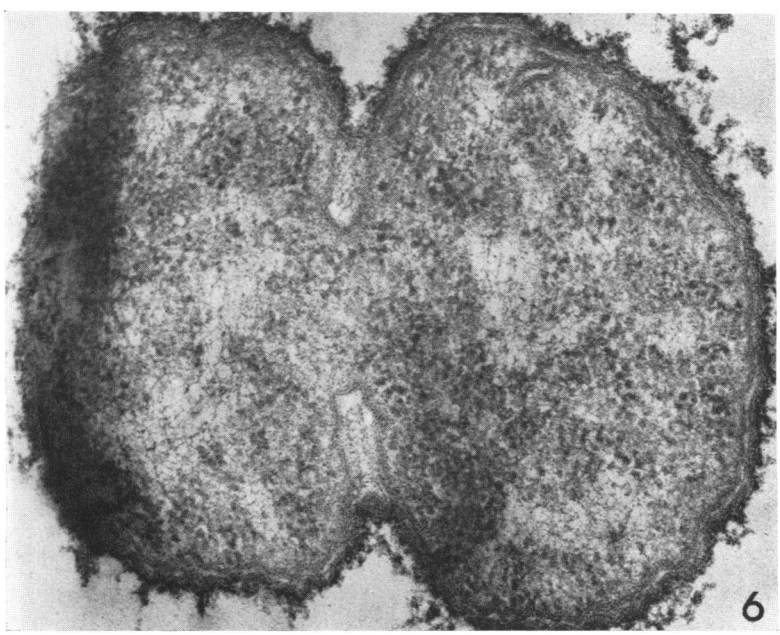
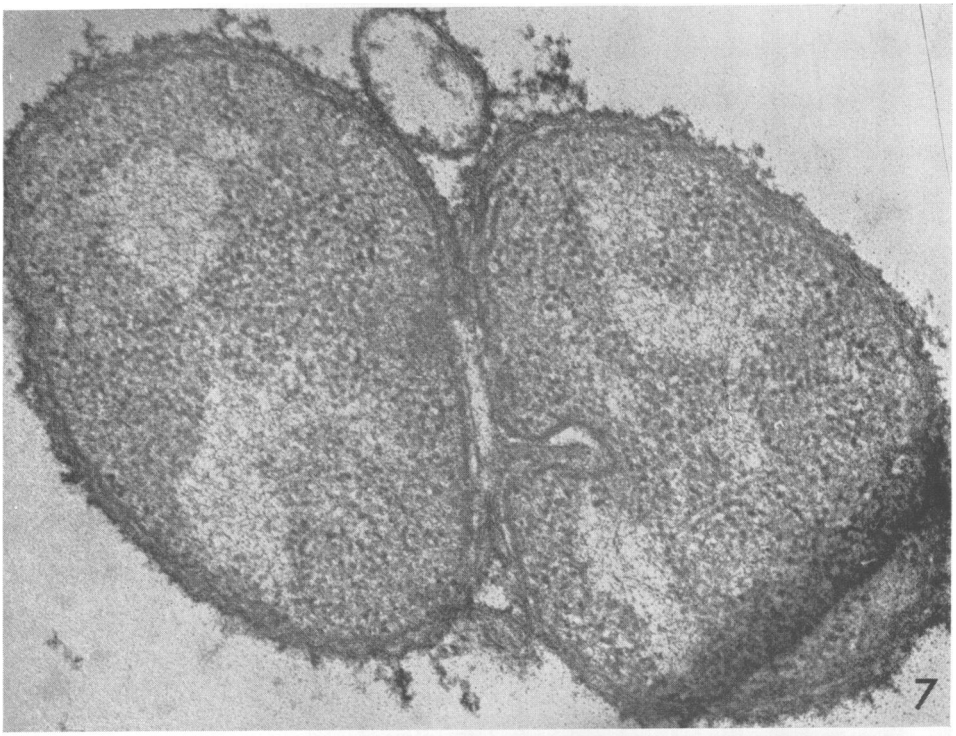


FIG. 5. *An off-center cut through a more completely divided gonococcus. The major cleft (bottom) shows a preceding membrane fold. The minor cleft (top) also possesses membrane swirls.* × *100,000.*

FIG. 6. *A section probably at right angles to those in Fig. 1, 5, and 8, and showing the membrane fold enclosing unstained and probably incomplete cell wall.* × *80,000.*



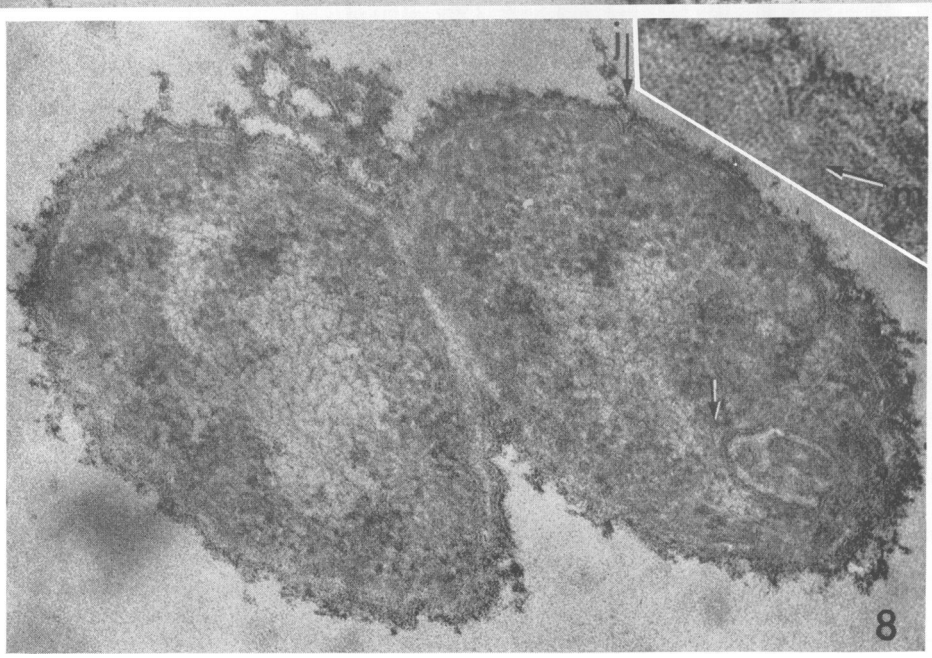


FIG. 7 and 8. *Sections of apparently completely divided gonococci still held together by yet incomplete cell wall. In Fig. 7, division appears to be proceeding in the daughter cells, one of which already shows a major and minor cleft forming. × 80,000. In Fig. 8, mesosomes in one pole of the cell appear to be partly embedded in the nucleoid. × 100,000. A wall-membrane junction (j), showing an apparent break in the wall continuity, is enlarged in the corner insert to show the relationship of inverting wall to membrane (m). × 240,000.*

plane and did not appear to have any close association with chromatin material.

More complex membranous pockets or mesosomes were seen at the poles of the cell away from the dividing plane (Fig. 4, 5, 6, and 8). Their prevalence in sections was sufficient to indicate that at least one and probably two were present in each daughter cell. As in *Bacillus subtilis* (Van Iterson, 1961) and *B. cereus* (Fitz-James, 1960), these organelles, continuous at their base with plasma membrane, often appeared at their apices adjacent to, or entwined with, the chromatin material of the cell. Here their complexity varied from that shown in Fig. 4 and 8 to that indicated in Fig. 5 and 6. The position of the mesosomes in these dividing cells suggests that they may function by holding the separated chromatin bodies away from the plane of division in the growing structure.

In spite of the complete separation of some cells by membranous processes, the completion of the cell wall often appeared considerably behind membrane development. In fact, many of the sections indicated that the cell-wall material laid down between sister cells matures slowly, in that it is not dense like that surrounding the cell. Hence, a delay in separation of already divided cells accounts for the high proportion of apparently dividing duplex forms seen in the cultures of this organism. Although most of the forms were in pairs or single, in a rare pair there was some suggestion that the division was continuing in a daughter cell (Fig. 7).

At some of these wall-membrane junctions already noted, the wall invaginated abruptly and appeared to be discontinuous (arrows, Fig. 3 and 8). However, examination of such junctions at higher magnification (insert, Fig. 8) revealed a faint profile of presumably immature wall extending from a membrane crease or dimple to the stained, mature wall. The interpretive drawing of such a wall-membrane junction (Fig. 9) was made from several micrographs. These sites are conceivably sections through points or regions of peripheral cell-wall synthesis active during the growth of the cell; the membrane loops dividing the cell may function in synthesizing the inner wall by cleaving the growing cell into two halves.

Plasticene models containing an embedded "membrane" zone and constructed from what appear to be central sections (Fig. 1, 5, 7, and 8) of dividing pairs of gonococci were sectioned at various angles. The resulting profiles suggest that that seen in Fig. 6 is at right angles to those in Fig. 1 and 5; Fig. 3, showing a simple membrane loop, was achieved by an oblique cut through an early major cleft of a dividing cell. These profiles suggest that the constriction initially dividing the cells is deeper on one side (bottom) than on the other (top), but equal at each side (front and back).

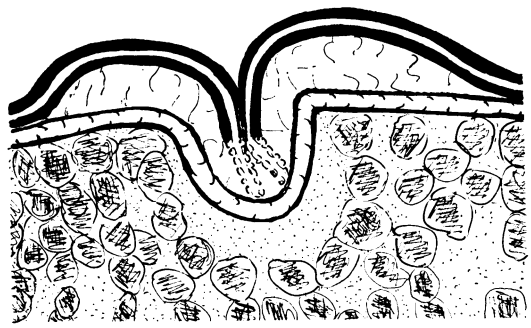


FIG. 9. *Interpretive drawing of a wall-membrane "junction" made from details of several such structures. These regions or zones are possibly sites of wall synthesis or polymerization, enlarging the wall over the periphery of the cell.*

It may be concluded, then, that this gram-negative pathogen possesses a wall-membrane covering not unlike that of *E. coli;* it appears to divide partially by constriction, followed by the slower development of a cell septum along a division plane first indicated by a simple membranous fold. As in many gram-positive bacilli and cocci, more complicated membranous structures, mesosomes, are present but not, as in the gram-positive species, near the plane of cell division.

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LITERATURE CITED

DALTON, A. J., AND R. F. ZEIGEL. 1960. A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy. J. Biophys. Biochem. Cytol. 7:409–410.

FITZ-JAMES, P. C. 1960. Participation of the cytoplasmic membrane in the growth and spore

formation of bacilli. J. Biophys. Biochem. Cytol. **8**:507–528.

KELLENBERGER, E., AND A. RYTER. 1958. Cell wall and cytoplasmic membrane of *Escherichia coli*. J. Biophys. Biochem. Cytol. **4**:323–326.

KELLENBERGER, E., A. RYTER, AND J. SÉCHAUD. 1958. Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. J. Biophys. Biochem. Cytol. **4**:671–678.

MURRAY, R. G. E., A. REYN, AND A. BIRCH-ANDERSEN. 1963. The fine structure of *Neisseria* and possible implication in classification. Can. J. Public Health **54**:46–47.

VAN ITERSON, W. 1961. Some features of a remarkable organelle in *Bacillus subtilis*. J. Biophys. Biochem. Cytol. **9**:183–192.