ELECTRON MICROSCOPY OF CELLULAR DIVISION IN *ESCHERICHIA COLI*

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ABSTRACT

CONTI, S. F. (Brookhaven National Laboratory, Upton, N. Y.) AND M. E. GETTNER. Electron microscopy of cellular division in *Escherichia coli*. J. Bacteriol. **83**:544–550. 1962.—Exponentially growing cells of *Escherichia coli* were fixed in formalin, exposed to uranyl nitrate, dehydrated at low temperatures with ethanol, and embedded in methacrylate. Polymerization was carried out at −70 C, by exposure of specimens to radiation from a cobalt^{60} source. Electron micrographs revealed that cellular division occurs by the centripetal growth of the cell wall. The fine structure of the cytoplasm, nuclear apparatus, cell wall, and cytoplasmic membrane was also studied.

Studies by Knaysi (1941, 1949, 1951) and Robinow (1945), employing the light microscope, indicated that the initial stages of bacterial cytokinesis involve the inward annular growth of the cytoplasmic membrane to form a transverse septum (cell plate), the latter structure subsequently being split by centripetal growth of the cell wall. Electron microscope observations of *Bacillus cereus* (Chapman and Hillier, 1953) indicated that it was inward growth of the cell wall which produced partitioning of the cell. It was also suggested that the cytoplasmic membrane, if such exists, is not involved in the initial partitioning of the cell. More recent electron microscope studies by Chapman (1959*a*,*b*), however, revealed that in some unidentified bacteria cytokinesis occurs in a manner similar to that proposed by Knaysi and Robinow. There are also indications (Chapman, 1960) that *Sarcina lutea* may be bounded by a structure intermediate in complexity between a cell wall and a cell wall with an accompanying cytoplasmic membrane. Although Kellenberger and Ryter (1958) presented conclusive evidence for the presence of a cytoplasmic membrane in *Escherichia coli*, the behavior of this structure during cell division has not been studied.

It is the purpose of this report to present observations on the process of cytokinesis in *E. coli*. The fine structure and behavior of the cytoplasm, nuclear apparatus, cytoplasmic membrane, and cell wall during cell division are illustrated and discussed.

MATERIALS AND METHODS

E. coli strain 15T$^-$ was grown in the enriched medium described by Zamenhof, DeGiovanni, and Rich (1956). During the exponential phase of growth, cells were harvested and resuspended in aqueous 0.85 M NaCl buffered at pH 7.4 with 0.1 M Veronal acetate, and fixed for 2.5 hr in 10% formalin in 0.1 M phosphate buffer (pH 7.4). This was followed by a saline wash and dehydration by passage through a graded alcohol series. The dehydration series entailed the addition of absolute ethanol to the wash solution to obtain the final desired concentration. The wash solution was replaced by absolute ethanol in the final dehydration step. Dehydration was modified by adding 1% uranyl nitrate to the alcohol. The temperature during dehydration was maintained as far below 0 C as the freezing point of the solution allowed. Cells were then placed in a 1% solution of uranyl nitrate in ethanol, stored overnight, and washed in absolute ethanol. Butyl methacrylate was employed as the embedding medium, polymerization being accomplished by exposure of the specimens in gelatin capsules to radiation from a cobalt^{60} source (total exposure of 12 million roentgens in a 48-hr period). Embedding and polymerization were carried out at −70 C. Specific details of the preparative procedures employed will be described elsewhere (Gettner and Bergeron, *in preparation*).

Ultrathin sections were cut on a Porter-Blum microtome employing a diamond knife. Electron

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micrographs were taken with an RCA EMU-2B electron microscope equipped with a 75 μ objective aperture; negatives (Ilford N60) were developed in Promicrol.

RESULTS

Figures 1 to 5 illustrate that the initial stages of cytokinesis in *E. coli* do not involve the inward annular growth of the cytoplasmic membrane to form a cell plate prior to centripetal growth of the cell wall. This contention is supported by the observation of cells (Fig. 2 and 4) in which inward growth of the cell wall is in progress, although nuclear material and cytoplasmic inclusions occupy the area within the region of cross-wall formation. There are no observable indications in any of the micrographs of differentiation of the cytoplasm within the area of the advancing cross wall. Although it appears that the cell wall and cytoplasmic membrane are closely associated and represented in the ingrowing cross wall, other interpretations are possible; e.g., the dense lines at the inner surface of the cross wall may be due to the delineation of the inner surface of the cell wall. The completely developed cross wall (approximately 300 A in width) forms a complete partition across the cell (Fig. 5 and 5a).

Nuclear apparatus. A dispersed network of fibrils is enclosed within an area of low electron density. These branching and anastomosing fibrils are generally thought to represent the deoxyribonucleic acid portion of the chromatin material. In some cells these fibrils appear to be 20 to 30 A in width; in others, the fine fibrillar material disappears, being replaced by somewhat thicker strands, 80 to 100 A wide. Direct contact between the fibrillar material and the granular cytoplasm appears extensive at the interface between the two regions, in agreement with the observation of Caro (1961). The chromatin areas appear to be occasionally invaded by extensions of the cytoplasmic material (Fig. 4 and 5). The significance and nature of these areas within the nuclear region remains a matter of conjecture (Giesbrecht and Piekarski, 1958; Murray, 1960).

Cell wall and cytoplasmic membrane. The outer layer of the cell appears to assume either of two configurations; it appears as a layer of uniform density, approximately 250 A in width, or gives the appearance of two dense lines enclosing an area of lower electron density.

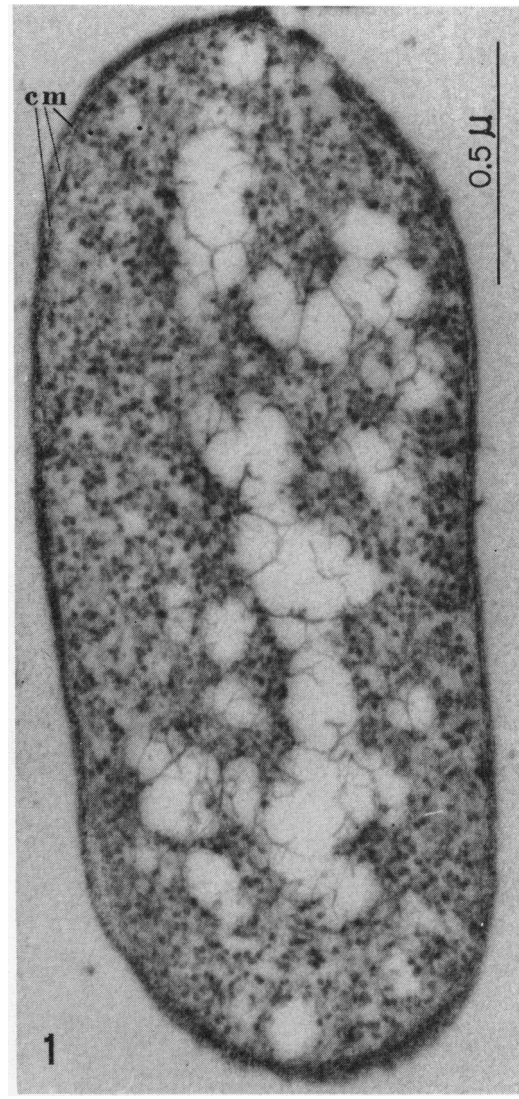

FIG. 1. *Electron micrograph of Escherichia coli 15T⁻ illustrating the appearance of a nondividing cell. The cytoplasm is relatively uniform throughout and contains conspicuous particles (100 to 200 A) which appear somewhat variable in size, shape, and density. The outer layer of the cell is about 250 A thick. The cytoplasmic membrane (cm), approximately 80 to 120 A in thickness, is closely applied to the cell wall, and is most clearly seen in areas of the cell where separation from the cell wall has occurred. The "cytoplasmic membrane" could here be composed of the inner surface of the wall and the true cytoplasmic membrane. A dispersed network of fibers can be observed within the nuclear region.*

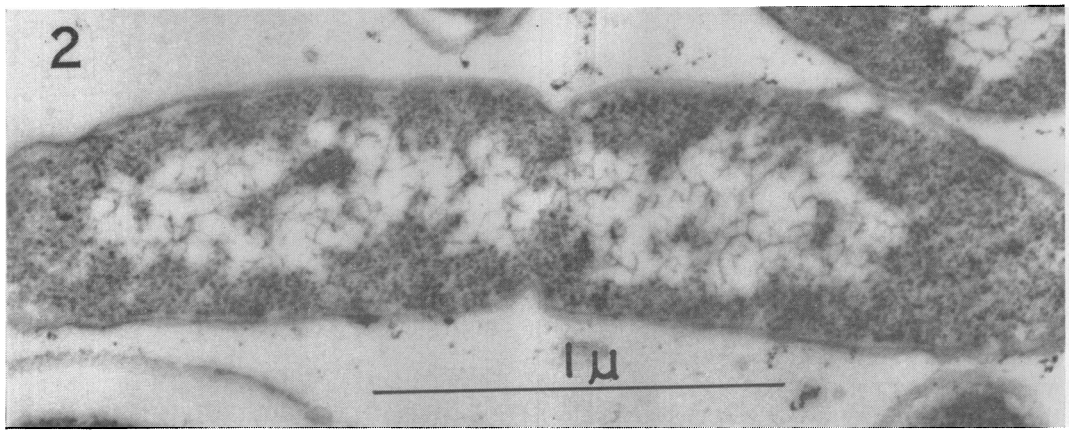

FIG. 2. *Electron micrograph of Escherichia coli 15T⁻ in an early stage of division. Note the presence of nuclear material within the region of the ingrowing cell wall, indicating that cell-plate formation has not occurred.*

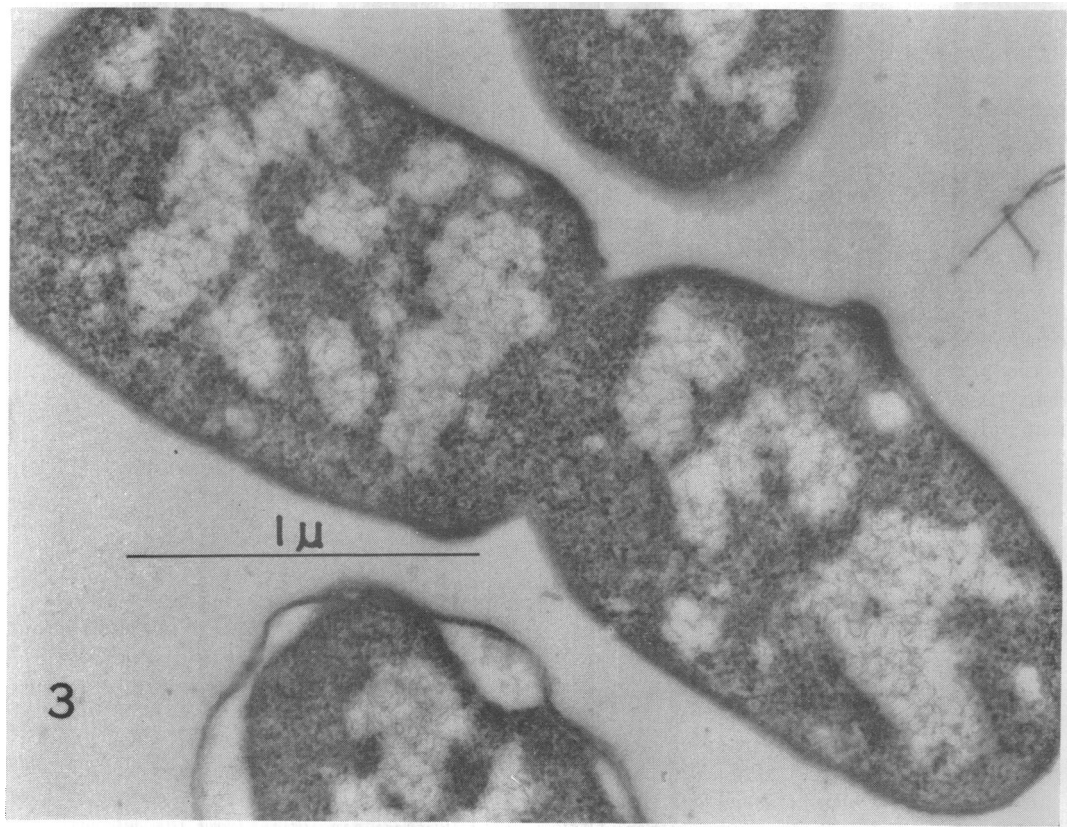

FIG. 3. *Electron micrograph of Escherichia coli 15T⁻. This is a somewhat thicker section of a cell which is in about the same stage division as the cell shown in Fig. 4. Note particularly the fineness of the nuclear fibrils. The appearance of the cell, particularly the nuclear region, is reminiscent of the micrographs presented by Ryter and Kellenberger (1958).*

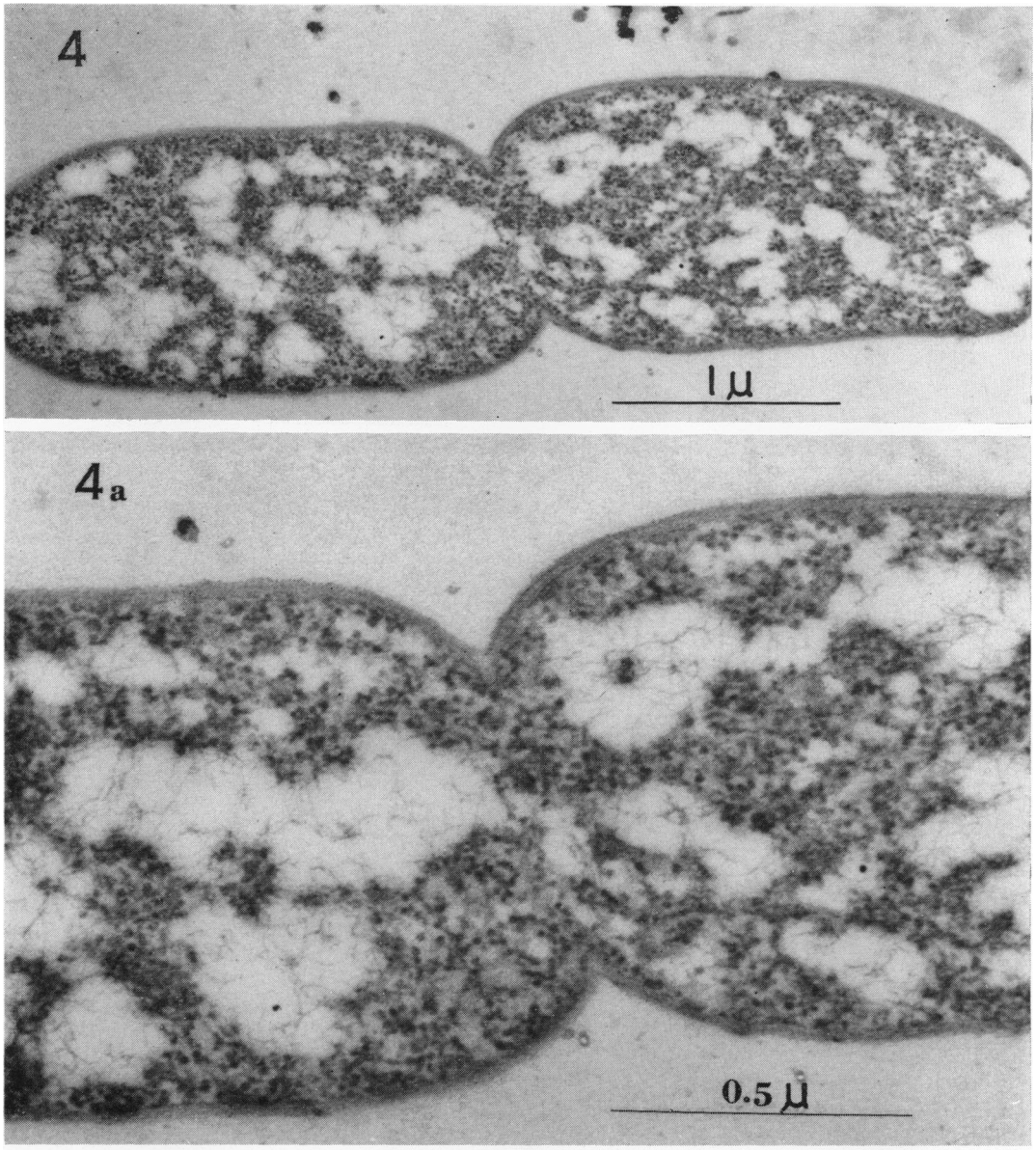

FIG. 4 and 4a. *Electron micrographs of Escherichia coli 15T⁻ in a later stage of cell division than shown in Fig. 2. Cytoplasmic particles and nuclear material occupy the area within the plane of the ingrowing cross wall. Figure 4a is an enlargement of the central region of the cell shown in Fig. 4.*

Although the cytoplasmic membrane (approximately 80 A wide) could not be observed in the majority of the cells, the presence of a structure which is presumed to be the cytoplasmic membrane is indicated in cells where the cytoplasmic membrane has partially separated from the cell wall (Fig. 1). There are also some indications that the cytoplasmic membrane is a unit membrane.

Cytoplasm. The investigations of Bradfield (1956) and others, of a variety of bacterial species, indicate that the major portion of the cytoplasm consists of small granules in the range of 100 to 200 A. Figures 4a and 5a clearly reveal the pres-

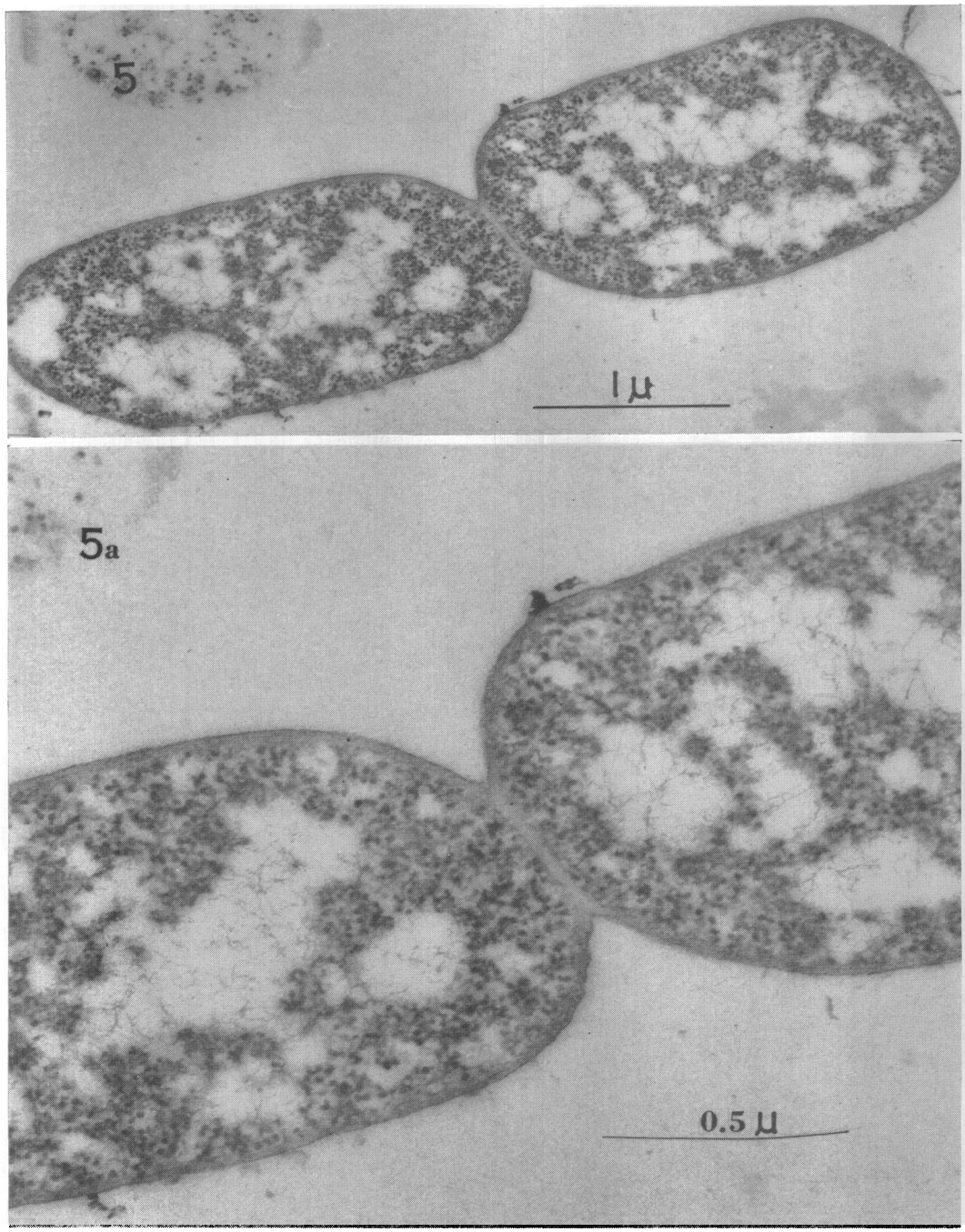

FIG. 5 and 5a. *Electron micrograph of Escherichia coli 15T⁻ after the cross wall (about 300 A wide) has been formed. Figure 5a is an enlargement of the central region of the cell shown in Fig. 5. Note particularly the size, shape, and appearance of the cytoplasmic inclusions. The cytoplasm of the cells is separated from the cross wall by a wide (20 to 30 A) membrane, presumably the cytoplasmic membrane.*

ence of such particles in the cytoplasm of *E. coli*, and, in addition, verify the observation by Murray (1960) that these particles do not appear to be entirely homogenous in size or shape. Although the majority of these particles are spherical, others appear to be rodlets of short length. It can be observed that some of the particles appear to be closely associated, thus giving areas of the cytoplasm a beaded appearance.

DISCUSSION

It is clear that identification of the partitioning structure(s) from these electron micrographs is difficult. The homogeneous appearance of the bounding structure indicates that it is centripetal growth of the cell wall that partitions the cell. Since ultrathin sections of bacterial cells rarely reveal the presence of a cytoplasmic membrane, it may well be that the cell wall and cytoplasmic membrane are represented in the developing transverse wall. The possibility that the apposed membrane surfaces are too close to be resolvable is supported by the appearance of the cytoplasmic membrane in a partially plasmolyzed cell (Fig. 1), and by the demonstration by Kellenberger and Ryter (1958) of the presence of a cytoplasmic membrane in electron micrographs of *E. coli*.

The elegant studies of Chapman (1959*a*, *b*) indicate that, in some bacteria, centripetal growth of the cytoplasmic membrane forms a cell septum which partitions the cytoplasm, whereas in others, the cytoplasmic membrane is not involved (Chapman and Hillier, 1953). It was also suggested that *S. lutea* is "bounded by a structure intermediate in complexity between a cell wall and a cell wall in addition to a cytoplasmic membrane" (Chapman, 1960).

Although the problems encountered in visualizing the cytoplasmic membrane in electron micrographs of bacterial cells render the understanding of its role in cellular division quite difficult, the mass of indirect evidence for its existence suggests that only two basic mechanisms are involved in cell partitioning. The cell septum is formed either by the annular centripetal growth of the cytoplasmic membrane, as described by Chapman (1959*b*) in a study of an unidentified bacterium, or by the cell wall with a closely apposed cytoplasmic membrane, as in *E. coli*, *S. lutea*, and perhaps *B. cereus*. The "peripheral bodies," associated with cross-wall formation in *B. cereus* and *S. lutea*, were not observed. Also, supernumerary transverse cell-wall formation was not noted, despite continued efforts to detect its presence. The suggestion (Chapman, 1959*b*) that "peripheral bodies" and aberrant transverse cell-wall formation are associated with cell-wall septation and not with cytoplasmic-membrane septation is therefore questionable, in view of the results obtained to date with *E. coli*.

Although accurate measurements are difficult, it should be noted that there is a close correspondence, in size, shape, and number of cytoplasmic particles observed in sections of intact cells of *E. coli*, to the ribonucleic acid particles isolated and described by Tissieres et al. (1959), Huxley and Zubay (1960), Beer, Highton, and McCarthy (1960) and others. These particles were most clearly seen in cells exposed to uranyl nitrate.

Detailed discussions and observations on the fine structure of bacterial nuclei have been presented by Chapman (1959*a*,*b*), Caro, Van Tubergen, and Forro (1958), Murray (1960), van Iterson and Robinow (1961), and others. Although the micrographs presented here enable one to follow the over-all behavior of the nucleus during cell division, little can be added to what has been said by these investigators in regard to its structure.

Since most electron microscope studies of bacterial ultrastructure involve the use of osmium tetroxide as the fixative, it is encouraging to obtain results similar to those presented by others without exposure of the cells to osmium. The difficulties and subjective approaches involved in investigations of bacterial ultrastructure, as stated by Murray (1960), should be borne in mind. Unfortunately, at the present time our only criteria for good fixation are apparent retention of shape, form, and structure, and cellular appearance in agreement with preconceived ideas as to what a cell should look like.

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