Electron Microscope Observations on the Behavior of the Bacterial Cytoplasmic Membrane During Cellular Division*

By GEORGE B. CHAPMAN, Ph.D.

(*From the Biological Laboratories, Harvard University, Cambridge*)

PLATES 118 TO 120

(Received for publication, April 4, 1959)

ABSTRACT

Bacterial cells were fixed in OsO_4, washed, dehydrated, and embedded in a methacrylate mixture. Ultrathin sections were cut on a Porter-Blum ultramicrotome and were examined in an RCA electron microscope, type EMU-2D.

The sections revealed that the cytoplasmic membrane undergoes a centripetal growth to form a membrane septum. This septum is formed as a double structure. Constriction of the daughter cells and deposition of cell wall material lead to the separation of the daughter cells.

The bacterial cytoplasm appears to consist largely of 200 A granules and occasionally reveals arrays of parallel dense lines.

INTRODUCTION

The process of cellular division in *Bacillus cereus* was described by Chapman and Hillier (1953). Essentially, that description depicted the centripetal growth of a ring of cell wall material at each plane of division resulting in the partitioning of the cytoplasm into separate cells. In that paper, and in subsequent papers on the fine structure of *B. cereus* by Chapman (1956) and by Chapman and Zworykin (1957), no cytoplasmic membrane was demonstrated. In so far as *B. cereus* is concerned, the situation remains unchanged. (It has not yet been established whether the absence of a cytoplasmic membrane from *B. cereus* is due to an artifact of preparation or whether this species differs from other species in having no morphologically demonstrable cytoplasmic membrane.)

It has been shown, by preparative procedures similar to those employed in the above studies, that some microorganisms do possess a cytoplasmic membrane. Thus, Chapman and Kroll (1957) demonstrated the presence of a cytoplasmic membrane in *Spirillum serpens*, Murray (1957) showed its existence in cells of a *Spirillum* species, and Kellenberger and Ryter (1958) and Kellenberger *et al.* (1958) published excellent electron micrographs which revealed the presence of a cytoplasmic membrane in *Escherichia coli*. In addition, Vatter and Wolfe (1958) have demonstrated a cytoplasmic membrane in photosynthetic bacteria and Tokuyasu and Yamada (1959) have clearly shown a cytoplasmic membrane to be present in *Bacillus subtilis*. None of these papers showed how the cytoplasmic membrane behaves during the division of bacteria.

It is the purpose of this report to reveal some of the aspects of that behavior.

Materials and Methods

The bacterial cells which were used in this study occurred as unidentified contaminants in an algal culture. Following concentration by centrifugation, the bacteria were fixed by being suspended in several cubic milliliters of a solution with the following composition: 2.5 ml. acetate-veronal (9.714 gm. sodium acetate + 14.714 gm. sodium veronal made up to 500 ml. in distilled water), 1 ml. 8.5 per cent NaCl, 0.25 ml. 0.11 M $CaCl_2$, 2.75 ml. distilled water, 6.25 ml. 2 per cent OsO_4. The pH of this fixative was about 9.2. Fixation was carried out at room temperature for 4 hours and at 10°C. for an additional 8 hours. The cells were then washed in a solution identical to the fixative except for the substitution of 6.25 ml. of distilled water for the 6.25 ml. of 2 per cent OsO_4. Following dehydration through a graded ethyl alcohol series, the cells were suspended in a monomeric mix-

* This investigation was supported by a research grant (E-1720) from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.

ture (60 parts normal butyl methacrylate, 40 parts ethyl methacrylate). Polymerization of the embedding material was carried out at 70°C. in the presence of 1.5 per cent luperco CDB. Sections were cut with a Porter-Blum ultramicrotome set at 25 mμ and were floated onto a surface of 40 per cent acetone in distilled water. They were picked up on collodion-coated 200 mesh copper grids and were examined in an RCA electron microscope, type EMU-2D, which had been fitted with a 0.015 inch Canalco externally centerable platinum condenser aperture and a 50 μ copper aperture in the standard objective pole piece.

OBSERVATIONS AND DISCUSSION

Fig. 1 represents a non-dividing cell. The 500 A thick cell wall, *CW*, 70 A thick cytoplasmic membrane, *CM*, and low density nuclear material, *N*, are readily identified. It is interesting to note that on the right side of the cell the cytoplasmic membrane has remained adherent to the cell wall which has been lifted from the surface of the cytoplasm. On the left side of the cell, under similar conditions of cell wall lifting, the cytoplasmic membrane has remained adherent to the cytoplasm. In Fig. 2, similar cell wall–cytoplasmic membrane relationships exist. At the position marked by the arrow in Fig. 2, a very early stage in the process of septation of the cytoplasm by the cytoplasmic membrane is seen. The nature of this septation process is more clearly revealed by Fig. 3, which shows a later stage in the process. It is seen (arrows) that the cytoplasmic membrane undergoes a centripetal invagination and growth. The apposed membrane surfaces are so close together that they are often not resolvable. In three dimensions, the septum grows as a continuous annular infolding of the membrane.

The marked similarity between this process of membrane septation of the bacterial cytoplasm and the process by which the cell wall septates the cytoplasm, as shown by Chapman and Hillier (1953) should be noted. Except for the difference in the identity of the septating structures, the processes are nearly identical. In the case of cell wall septation, however, the presence of peripheral bodies, presumably with a role in wall formation, was observed. Cell wall septation also differs from cytoplasmic membrane septation in that the former sometimes becomes aberrant with the formation of a supernumerary transverse cell wall. No such aberrations were observed in the process of cytoplasmic membrane septation.

Figs. 4 to 7 represent cells in which the membrane septation is complete. In Figs. 4 and 5, the individual layers of the membrane are not resolved. However, the fact that the septum is twice as thick as the cytoplasmic membrane provides quite conclusive evidence that the septum is composed of two layers of cytoplasmic membrane. In Figs. 6 and 7, which are adjacent serial sections, the two cytoplasmic membrane layers may be resolved (at arrows).

Figs. 8 to 11 reveal various stages in the separation of the daughter cells and the deposit of cell wall material on the cytoplasmic membrane in the region between these two cells. The cytoplasmic membrane which limits the cytoplasm of each daughter cell may be clearly seen in these figures. Figs. 8 to 10 represent early and intermediate stages in the separation process. Fig. 11 represents a very late stage. Actually, deposition of cell wall material is complete—the maximum wall thickness having been attained—and the daughter cells have almost separated.

This report has presented the first high resolution cytological study of cytoplasmic membrane septation of bacteria. It should be pointed out, however, that Knaysi (1941, 1949, 1951), Robinow (1945), and Bisset (1948, 1950), on the basis of light microscope observations, gave remarkably similar descriptions of this phenomenon. Knaysi and Robinow described the inward annular growth of a ring from the cytoplasmic membrane to form a cell plate which was subsequently split by the centripetal growth of the cell wall. In the present study, however, it is clear that the membrane septum is formed as a double structure. It is also of interest that Bisset claimed that the transverse cell wall was secreted by the cytoplasmic membrane plate and that Knaysi described the formation of the transverse cell wall as an independent function of each daughter cell. It seems likely, from electron micrographs not included with this report, that both of the above reports are correct. It has been observed in several electron micrographs that there is an accumulation of material just within the cytoplasmic membrane and that this material resembles in texture and density the cell wall material. This appearance suggests that the cell wall material is produced in the cytoplasm—sometimes accumulating just beneath the cytoplasmic membrane—and gradually diffuses through the cytoplasmic membrane. (The fact that this accumulation is rarely observed may reflect the efficiency of the membrane

in permitting the wall material to pass tnrough. The accumulation might then represent a temporary dysfunction of the membrane.)

Although it has been the object of this report to describe the process of cytoplasmic membrane septation of the bacterial cytoplasm, two brief comments pertaining to the cytoplasm itself may be made. In Figs. 3 and 11, at *X* may be seen what appear as arrays of parallel dense lines in the cytoplasm. Although the proximity of these lines to the nuclear material tempts one to propose an analogy to the mitotic spindle fibers, it must be stated that their significance is unknown.

In several of the figures, particularly Figs. 3 and 11, the cytoplasm exhibits a pronounced granularity. The granules are approximately 200 A in diameter, but, due to their ill defined margins, are difficult to measure accurately. These granules might be thought to represent ribonucleoprotein particles, for it is known that bacterial protoplasm is abundantly supplied with such particles. If they do represent ribonucleoprotein particles, they are somewhat larger than such particles described by Palade (1955) and Palade and Siekewitz (1956) in mammalian cells. Indeed, these particles are similar in size and appearance to the 150 to 300 A particles, or granules, described in the interfibrillar sarcoplasm of the turtle atrium by Fawcett and Selby (1958) and tentatively interpreted by them as representing a particulate form of glycogen rather than ribonucleoprotein. It is interesting to note how similar two particulates which presumably are chemically quite different can appear in the electron microscope.

A few comments concerning the nuclear material are in order. The evidence in support of the interpretation that the low density regions represent the bacterial nuclei has been discussed by Chapman and Hillier (1953). The recent report by Caro *et al.* (1958) substantiates that interpretation, as does the paper by Kellenberger *et al.* (1958). The behavior of the nuclear material during division remains rather enigmatic. All that can be said with certainty is that the nuclear mass appears to undergo a series of changes reminiscent of those undergone by a nucleus in classical amitosis. (See Fig. 2 for the dumb-bell configuration.) Precisely what is happening to the granules and filaments which make up much of the nuclear mass is unclear. However, the interesting and provocative observations of Giesbrecht (1958) and Giesbrecht and Piekarski (1958) should be consulted. Further work on this subject is in progress.

Some nuclear regions (particularly in Figs. 1, 3, 5, 6, 8, 10, and 11) contain intrusions of material which appears quite different in texture from the nucleus proper and slightly different in texture from the cytoplasm. These intrusions are considered to represent extensions of the cytoplasm. Their difference in appearance from the cytoplasm may be due to the fact that they are nearly engulfed by the nucleoplasm and thus may be in an environment of somewhat different pH or other ionic constitution, from the cytoplasm proper.

The author wishes to acknowledge the assistance of Miss Frieda Meier in the preparation of the illustrative material for this report.

Bibliography

1. Bisset, K. A., The cytology of smooth and rough variation in bacteria, *J. Gen. Microbiol.*, 1948, **2,** 83.
2. Bisset, K. A., The Cytology and Life History of Bacteria, Baltimore, The Williams and Wilkins Co., 1950.
3. Caro, L. G., Van Tubergen, R. P., and Forro, F., Jr., The localization of deoxyribonucleic acid in *Escherichia coli*, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 491.
4. Chapman, G. B., Electron microscopy of ultrathin sections of bacteria. II. Sporulation of *Bacillus megaterium* and *Bacillus cereus*, *J. Bact.*, 1956, **71,** 348.
5. Chapman, G. B., and Hillier, J., Electron microscopy of ultra-thin sections of bacteria. I. Cellular division in *Bacillus cereus*, *J. Bact.*, 1953, **66,** 362.
6. Chapman, G. B., and Kroll, A. J., Electron microscopy of ultra-thin sections of *Spirillum serpens*, *J. Bact.*, 1957, **73,** 63.
7. Chapman, G. B., and Zworykin, K. A., Study of germinating *Bacillus cereus* spores employing television microscopy of living cells and electron microscopy of ultra-thin sections, *J. Bact.*, 1957, **74,** 126.
8. Fawcett, D. W., and Selby, C. C., Observations on the fine structure of the turtle atrium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 63.
9. Giesbrecht, P., Zur Struktur des Bakterienzellkerns, *Naturwissenschaften*, 1958, **45,** 473.
10. Giesbrecht, P., and Piekarski, G., Zur Organisation des Zellkerns von *Bacillus megaterium*, *Arch. mikr.*, 1958, **31,** 68.
11. Kellenberger, E., and Ryter, A., Cell wall and cytoplasmic membrane of *Escherichia coli*, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 323.

12. Kellenberger, E., Ryter, A., and Séchaud, J., Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 671.
13. Knaysi, G., Observations on the cell division of some yeasts and bacteria, *J. Bact.*, 1941, **41**, 141.
14. Knaysi, G., Cytology of bacteria. II., *Bot. Rev.*, 1949, **15**, 106.
15. Knaysi, G., Elements of Bacterial Cytology, Ithaca, Comstock Publishing Co., 1951, second edition.
16. Murray, R. G. E., Direct evidence for a cytoplasmic membrane in sectioned bacteria, *Canad. J. Microbiol.*, 1957, **3**, 531.
17. Palade, G. E., A small particulate component of the cytoplasm, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 59.
18. Palade, G. E., and Siekewitz, P., Liver microsomes. An integrated morphological and biochemical study, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 171.
19. Robinow, C. F., Nuclear apparatus and cell-structure of rod-shaped bacteria, *in* Dubos, R., The Bacterial Cell, Cambridge, Harvard University Press, 1945, 353–377.
20. Tokuyasu, K., and Yamada, E., Fine structure of *Bacillus subtilis*. I. Fixation, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 123.
21. Vatter, A. E., and Wolfe, R. S., The structure of photosynthetic bacteria, *J. Bact.*, 1958, **75**, 480.

Explanation of Plates

Abbreviations used on Plates

CM, cytoplasmic membrane.
CW, cell wall.
N, nuclear apparatus.

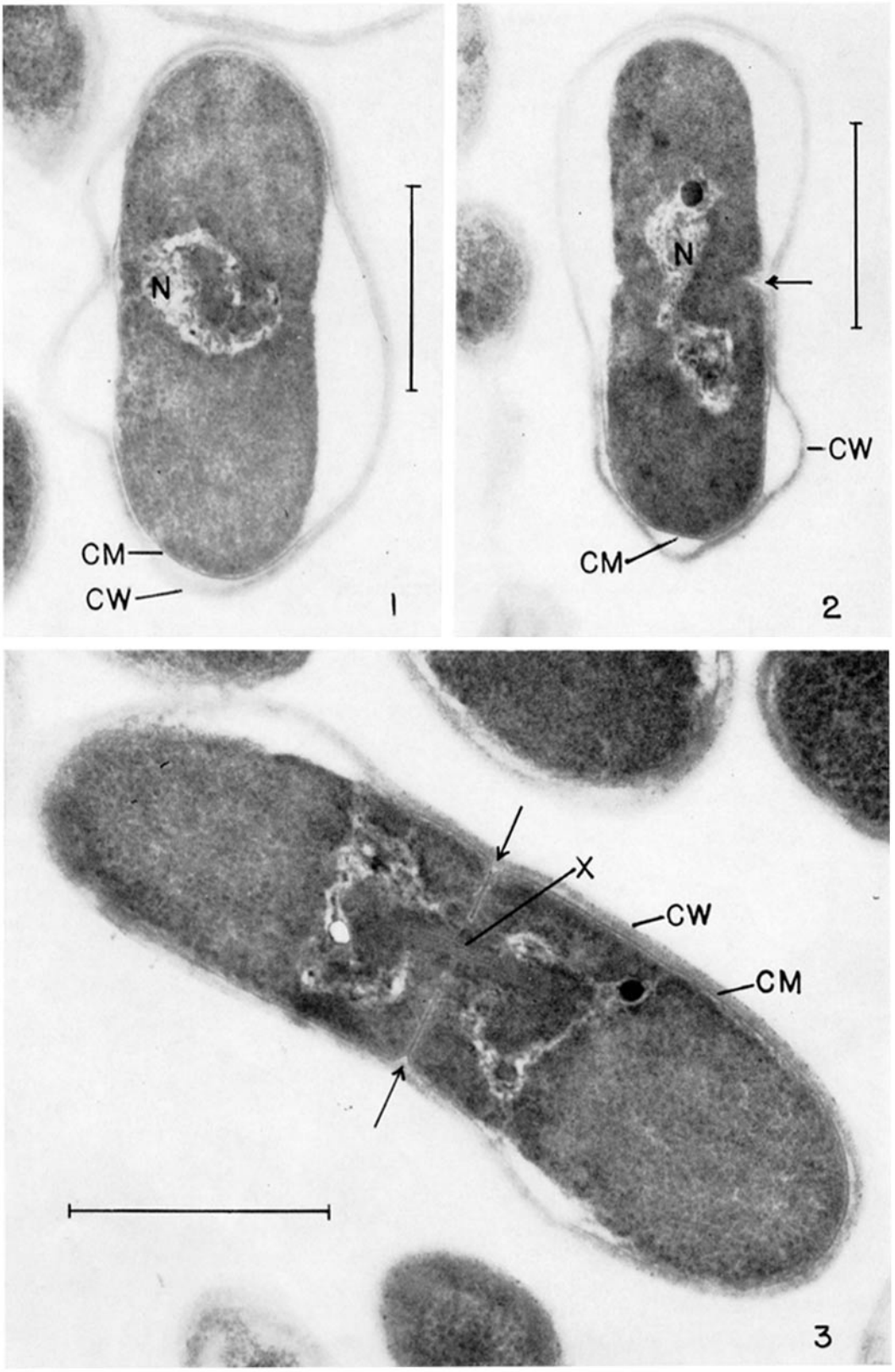
All of the figures are longitudinal ultrathin sections through the bacterial cells.
In each figure, the magnification mark indicates 1 micron.

Plate 118

FIG. 1. A cell which reveals no stage of the division process. Note the ability of the cytoplasmic membrane (*CM*) to adhere either to the cell wall (*CW*) or to the cytoplasm when the cell wall is lifted from the protoplast. × 32,000.

FIG. 2. A cell which reveals (arrow) a very early stage of centripetal growth of the cytoplasmic membrane. Note the dumb-bell configuration of the nuclear apparatus. × 32,000.

FIG. 3. A cell in which the cytoplasmic membrane has nearly septated the cytoplasm (arrows). *X* designates a rarely observed fibrillar arrangement in the cytoplasm. × 41,000.



(Chapman: Bacterial cytoplasmic membrane)

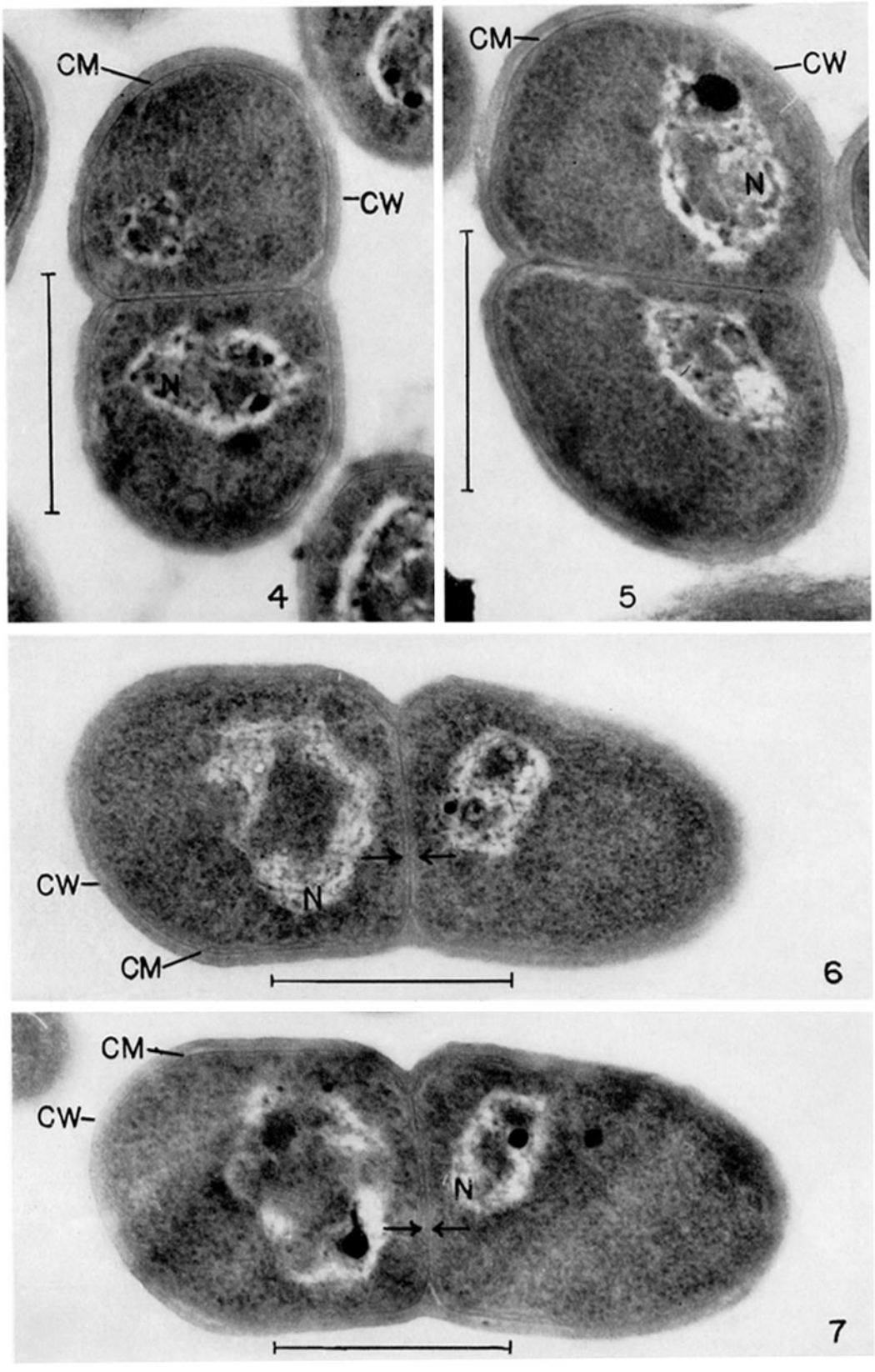
PLATE 119

FIG. 4. The cytoplasmic membrane has septated the cytoplasm of this cell. The two layers of the membrane constituting the septum are so close together that they could not be resolved. Note, however, that the septum is twice as thick as the peripheral cytoplasmic membrane. × 38,000.

FIG. 5. This figure is essentially the same as Fig. 4. × 41,000.

FIG. 6. In this figure, it can be seen that the septum is composed of two layers of membrane (arrows). × 38,000.

FIG. 7. A serial section to Fig. 6. The two layers of membrane are again visible in the septum (arrows). It is of interest that the cytoplasmic membrane is sharply defined at the top of Fig. 7 and at the bottom of Fig. 6. Apparently the change in knife to membrane angle between these two sections has been sufficient to bring about this difference. × 38,000.



(Chapman: Bacterial cytoplasmic membrane)

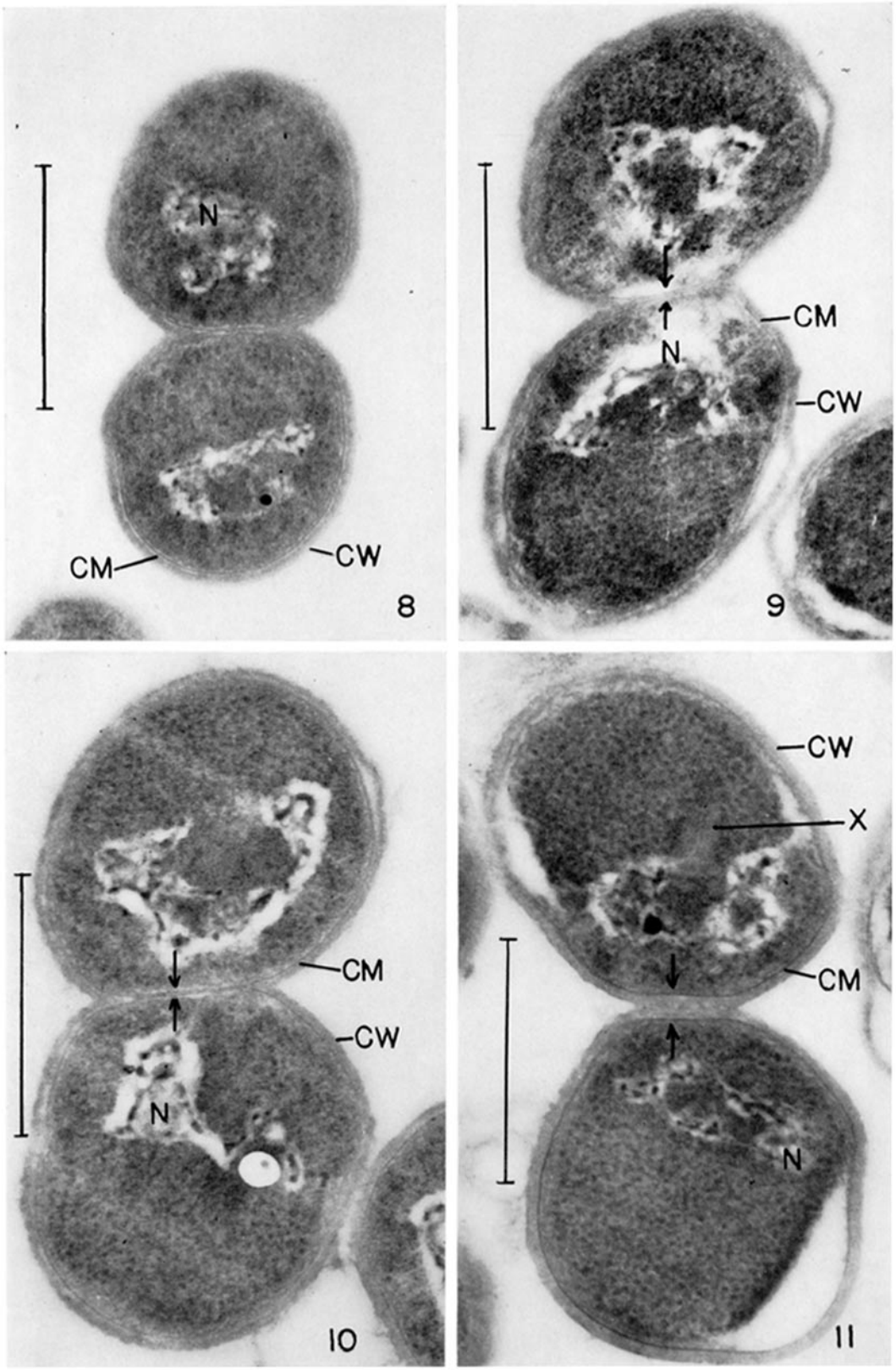
PLATE 120

FIG. 8. The two layers of membrane constituting the septum are clearly seen. The daughter cells have started to separate. × 38,000.

FIG. 9. The two layers of membrane are again clearly seen in the septum (arrows). × 41,000.

FIG. 10. This figure is essentially the same as Fig. 9. × 41,000.

FIG. 11. The cell wall is now complete between the two daughter cells (arrows) and constriction has progressed almost to the point of separation of the cells. *X* designates a rarely observed fibrillar arrangement in the cytoplasm. × 38,000.



(Chapman: Bacterial cytoplasmic membrane)