JOURNAL OF BACTERIOLOGY, Dec. 1971, p. 1390-1401
Vol. 108, No. 3

Giant Cells of *Escherichia coli*: a Morphological Study

DAVID P. ALLISON

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Received for publication 30 August 1971

Bacterial growth without division was observed in a giant cell-producing strain of *Escherichia coli* K-12. Giant cell production is controlled by the *lon*$^-$ (failure of cell division after irradiation) and *mon*$^-$ (formation of irregularly shaped cells) genes. Irradiation of a *lon*$^-$ *mon*$^-$ strain (P678-A_4) with low doses of ultraviolet or ionizing radiation results in the production of large, amorphous giant cells with 500 to 1,000 times the volume of the nonirradiated parents. The concentration of NaCl in the growth medium was found to influence irradiated-cell morphology. Low concentrations (0.2% NaCl) resulted in elongated cells, whereas spherical giant cells were produced in the presence of high salt (1% NaCl) concentrations.

Thin-section electron microscopy revealed an extensive network of intracellular membranes forming vacuoles, vesicles, and cisternae. These structures bear a striking resemblance to the rough and smooth membranes (endoplasmic reticulum, Golgi complex, vacuoles, etc.) found in eucaryotic cells.

Cell division in *Escherichia coli* K-12 is usually an orderly process. The rod-shaped bacteria grow by extending themselves along the long axis. A central constriction of the cell wall and membrane marks the beginning of fission, and, when division is completed, two daughter cells of approximately the same size as the parent cell are produced. However, this orderly correlation of cell division and cell growth is severly distorted in several mutant strains of *E. coli* (3, 9, 11, 14, 18). One mutation found in both *E. coli* K-12 (4, 10) and *E. coli* B (7) involves the *lon* locus. When *lon*$^-$ mutants are subjected to small doses of radiation, they produce long nonseptate filaments incapable of division.

Adler et al. (5) have described a recombinant of *E. coli* K-12 which combines the *lon*$^-$ mutation with the *mon*$^-$ mutation, a presumed cell-wall defect. When they are irradiated with ultraviolet (UV) or X radiation, these double mutants produce large, amorphous giant cells with 500 to 1,000 times the normal cell volume. In previous experiments (5), giant cells have been grown on a rich agar surface after irradiation; however, it is important that they be grown in liquid medium to obtain quantitative and reproducible results in a variety of studies. The purpose of this report is to define optimal conditions for the growth of giant cells in liquid medium and to study the effects of these two mutations on the morphology of *E. coli* cells.

MATERIALS AND METHODS

Organism. The organisms used in these experiments and their properties are listed in Table 1. Strain P678-7 is a radiation-resistant mutant that was isolated from the rod-shaped P678 after treatment with triethylene melamine; an interesting feature of the mutant is that cells appear in a variety of shapes, most commonly spherical. This property is thought to represent a defect in the cell wall and is attributed to the unmapped *mon* mutation(s) (5). Strain P678-A_4 is the giant cell-producing strain, which incorporates the *lon*$^-$ mutation from 3.300-M6 into the *mon*$^-$ strain of P678-7.

Growth of cells. Cells were grown in 10 ml of liquid culture in 50-ml Delong culture flasks on a gyrotory water-bath shaker at 37 C with moderate agitation.

Media. Minimal media used were tris(hydroxymethyl)aminomethane (Tris) medium (8), M-9 medium (1), and Vogel and Bonner medium E (22), supplemented with the growth requirements of the organism or with 1% final concentration Casamino Acids (Difco). L-broth (15) and nutrient broth (2) were used as complex media.

A rich broth medium containing 35 g of nutrient broth (Difco), 4 g of yeast extract (Difco), and 0 to 30 g of NaCl (*see below*) dissolved in 900 ml of sterile water was completed by adding 100 ml of sterile 0.2 M potassium phosphate buffer (39 ml of 0.2 M KH_2PO_4 plus 61 ml of 0.2 M K_2HPO_4), *p*H 7.0. This medium was devised in this study by varying, one at a time, the ingredients of nutrient broth medium to obtain the largest giant cells.

Irradiation of cells. UV irradiation was accomplished by using a General Electric germicidal lamp emitting primarily at 254 nm. The output of the UV

source was 6.66 ergs per mm^2 per sec as measured by a Jagger meter (12) after the lamp had warmed for 1 hr. Cells were collected by centrifugation and suspended in phosphate buffer at 2×10^8 cells/ml. A 10-ml sample of the cell suspension was then placed in a sterile 5-cm Pyrex glass petri dish and agitated with a Teflon magnetic stir bar. In most experiments, the cells were irradiated with a 30-sec (200 ergs per mm^2) dose from the prewarmed UV source.

Electron microscopy. Exponential cultures were grown in M-9 plus thi plus 1% Casamino Acids to 2×10^8 cells/ml, suspended in phosphate buffer, and irradiated with 200 ergs of UV per mm^2 as described. A 1-ml sample of irradiated cells was used as the inoculum for 20 ml of rich broth medium in each 50-ml culture flask. A series of flasks was inoculated at time zero and placed in a shaker bath at 37 C, and samples were taken at 0, 30, 60, 90, 120, 150, 180, 240, and 300 min by pipetting 9 ml of a flask's contents into 1 ml of fixative. Fixation was completed by the Ryter-Kellenberger method (13). Specimens were dehydrated in ethanol at room temperature and embedded in Epon by the method of Luft (16).

The polymerized Epon blocks were sectioned with a Porter-Blum MT-2 ultramicrotome by using a diamond knife. Sections were stained for 1 hr by placing them, section side down, on drops of 2% uranyl acetate in water. After rinsing in water, the grids were further stained for 5 min with lead citrate (19). A Siemens Elmiskop I electron microscope was used.

RESULTS

Selection of a liquid medium. The liquid medium in which large numbers of giant cells could be obtained was selected in the following way. Irradiated cells were incubated in the medium to be tested and periodically examined in a Reichert Zetophan light microscope (with a micrometer ocular included) to give an estimate of size. Cells grown in L-broth and nutrient broth were large, but they tended to be elongate instead of spherical. A curious phenomenon was observed with both M-9 and Tris media supplemented with the growth requirements of the organism or with Casamino Acids. A large blister or vacuole formed on the surface of the cell 90 min after irradiation and grew at a rate greater than that of the cytoplasm. At later times, cells seemed to have formed a halo, with the cytoplasm forced to one side or the cell.

Nutrient broth medium was selected for further studies, and variations in the concentrations of the ingredients were tested for their effects on the shape and size of the giant cells. The devised medium which resulted from these studies and which produced the largest giant cells was termed rich broth. The effects of various additives to the rich broth on shape and final size were then studied. The additives tried were agar, glycerin, methocel, Ficoll, sucrose, and NaCl. The cultures were examined periodically in the light microscope, and all but NaCl showed little or no effect. However, the NaCl concentration produced a marked effect on giant cell morphology. Elongation was produced by NaCl concentrations from 0 to 0.8%, whereas a 1:1 ratio of elongated to round cells was found from 0.8 to 1.6%; 3.2% NaCl completely inhibited growth. A concentration of 1% NaCl in the medium gave the highest fraction of large, round giant cells. Thus, rich broth medium with a 1% NaCl concentration was adopted for morphological studies.

Growth was measured spectrophotometrically in a Beckman model B spectrophotometer at 600 nm. In rich broth, the generation time for nonirradiated cells was 28 min at 1% NaCl concentration and 25 min at 0.2% NaCl. The typical growth pattern of an irradiated culture can be seen in Fig. 1. Initially the mass increase is exponential, reaching a maximum at the equivalent of 5.5 generations of the nonirradiated control. Finally lysis begins and there is a decline in absorbance.

Morphology of giant cells. The morphological development of the giant cell-producing strain P678-A_4 was studied all the way from nonirradiated cells capable of division to irradiated giant cells just prior to lysis. Light microscopy revealed that, unlike normal rod-shaped *E. coli* K-12, these cells were most often round before irradiation. However, a variety of other shapes were also present, suggesting that the cells do not have a rigid cell wall. Cell septation usually occurred from both sides of the cell and division was usually equatorial, resulting in two daughter cells of equal size. However, frequent examples of nonequatorial division and asymmetrical septation were observed. These abnormalities of division have been confirmed by using the electron microscope (Fig. 2 and 3).

Thin-section electron microscopy revealed two morphological types in the culture (Fig. 2). In one type (smooth), the cells are normal in appearance except for shape; the cell membrane

TABLE 1. *Bacterial strains*

Strain	Mating type	Genotype	Source
P678	F^-	*thr⁻ leu⁻ thi⁻ lac⁻ str^r lon⁺ mon⁺*	F. Jacob
P678-7	F^-	*thr⁻ leu⁻ thi⁻ lac⁻ str^r lon⁺ mon⁻*	H. I. Adler
P678-A_4	F^-	*thr⁺ leu⁺ thi⁺ lac⁺ str^r lon⁻ mon⁻*	H. I. Adler
3.300-M6	F^+	*thr⁺ leu⁺ thi⁺ lac⁺ str^s lon⁻ mon⁺*	A. Markowitz

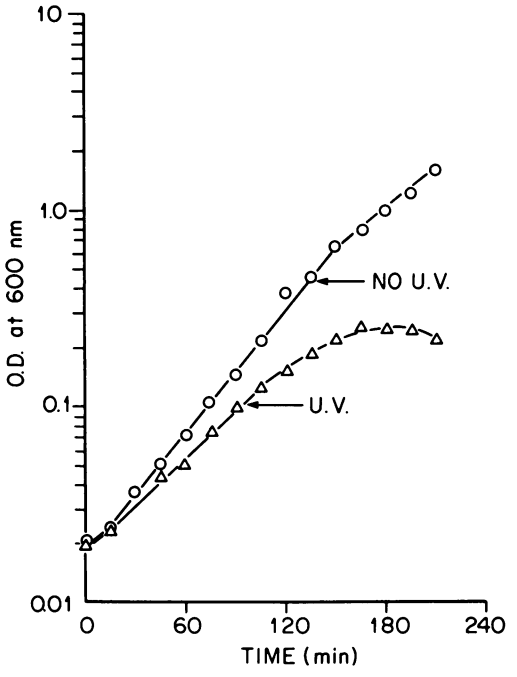


FIG. 1. *Growth of irradiated and nonirradiated P678-A$_4$ in rich broth plus 1% NaCl. Samples were taken at 15-min intervals, and the optical density was read at 600 nm.*

and wall can easily be distinguished; the cytoplasm appears normal, and individual ribosomes are easily seen; and the lightly stained nuclear areas are well-defined, and the threadlike deoxyribonucleic acid (DNA) fibrils can be distinguished. In the second type (rough), the cell wall and membrane are shriveled and blended together so that they are hard to differentiate; the cytoplasm is dense, and ribosomes cannot readily be distinguished; and the nuclear areas are poorly defined and very compact, and DNA fibrils cannot be seen. Generally both cell types are 1 to 1.5 μm in diameter.

Sixty minutes after irradiation several changes can be observed. The cells stop dividing but continue to increase in size. Their appearance becomes more normal, and the shriveled cells observed at zero time lose their grainy appearance, with nuclear areas becoming more pronounced and DNA fibrils more easily seen (Fig. 4).

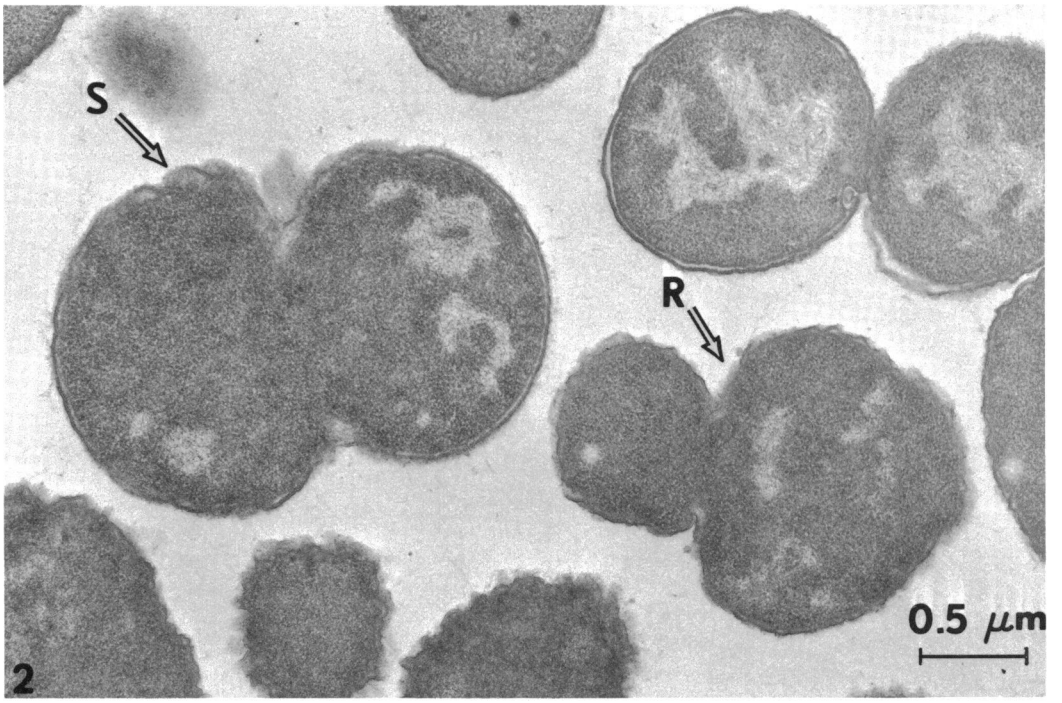
After the cells have been growing for 120 min, membrane systems appear within the cytoplasm. These membranes are in the form of groups of vesicles, vacuoles, and cisternae. The cytoplasm appears to be normal, and ribosomes are readily seen. The DNA is obviously replicating, as evidenced by the observed increase in DNA which follows the increase in cell mass. Again, the cells continue to grow larger without dividing (Fig. 5).

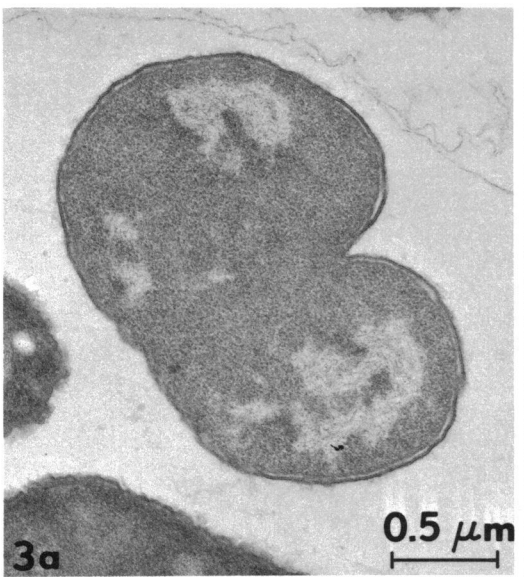
At 180 min, the cells have increased further in size. Approximately 50% of the cells are spherical in shape; although a variety of other shapes are present. An example of a cell which has not kept the spherical morphology is shown in Fig. 6. A filamentous shape is apparent from the ends of the cell. However, the center of the cell has formed a pocket and appears to be growing round. This often happens when irradiated rod-shaped cells start to grow as filaments but give way to the round morphology at late postirradiation times. (Rounding out of irradiated cells is apparently an effect of high NaCl concentration in the medium.) Membrane structures are present throughout the cytoplasm. At higher magnification these structures are found to be unit membranes very much like the cisternae of the endoplasmic reticulum found in eucaryotic cells.

An illustration of the effect of NaCl on giant cell morphology is shown in Fig. 7. Irradiated cells incubated in rich broth plus 0.2% NaCl, a low salt concentration, tend to grow into elongated forms. Cells incubated in higher salt concentrations (rich broth plus 1% NaCl) tend to assume a spherical morphology.

The giant cell shown in Fig. 8 was harvested 300 min after irradiation and is representative of the largest cells grown in liquid medium. Since the lytic response begins 180 min after irradiation (Fig. 1), only a small percentage of cells attain this size. This cell is approximately 12 μm in diameter, which represents a volume of 905 μm^3. If the diameter of nonirradiated cells averages 1.5 μm with a volume of 1.76 μm^3, the cell in this figure represents an increase in volume of approximately 500 times. The amount of membrane material seen is typical for cells of this age, since they all have some intracytoplasmic membranes. A characteristic common to giant cells before lysis is the dilution of the ribosomes within the cytoplasm and a relatively large increase in nuclear area.

The internal membranes form special types of structures, which are found in most giant cells. These structures can be divided into categories of vacuoles, vesicles, and flattened, bag-shaped cisternae (Fig. 9). The vacuoles range in size from those which could better be called vesicles to structures over 1 μm in diameter (Fig. 10). They are devoid of cellular cytoplasmic elements such as ribosomes and DNA, and the density of their contents is similar to that outside the cell. A higher magnification of the vacuolar area is shown in Fig. 11. It is morphologically apparent that the membrane surrounding the vacuole is the same thickness as the plasma membrane of





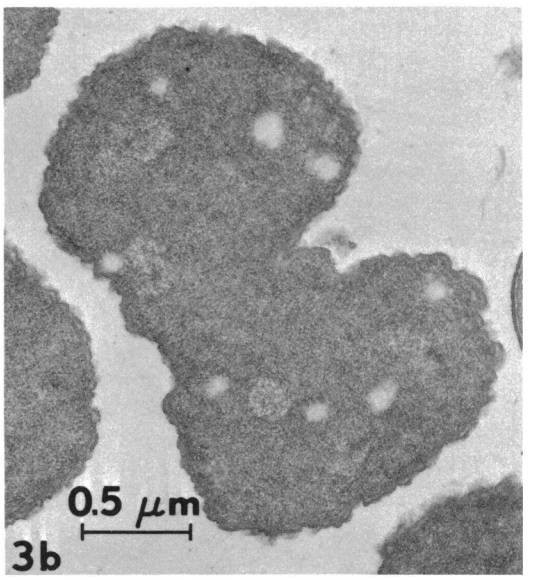


FIG. 2. *Nonirradiated P678-A$_4$. Thin-section electron micrograph prior to irradiation (zero time). Note the smooth (S) and rough (R) forms; also, equatorial and nonequatorial cell division.* FIG. 3. *Nonirradiated P678-A$_4$ showing asymmetrical septation. (a) Asymmetrical septation in the smooth form; (b) asymmetrical septation in the rough form.*

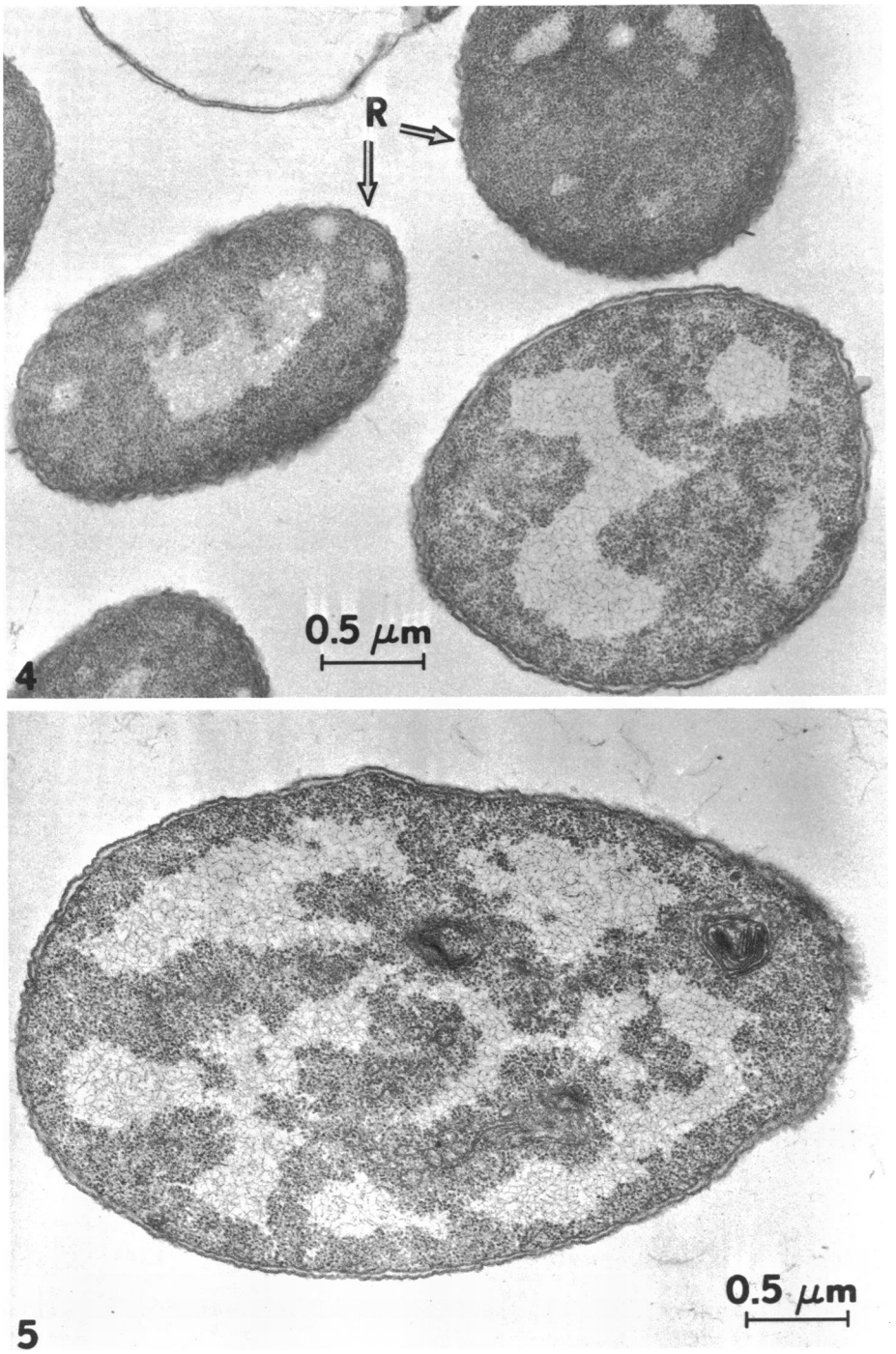


FIG. 4. *Giant cells 60 min after irradiation. There is no evidence of cell division. The rough form (R) is not so prominent. Note the cell membrane and wall of a lysed cell in upper left of the micrograph.* FIG. 5. *After 120 min, the irradiated cells develop membranes within the cytoplasm. These membranes often take the form of stacked vesicles.*

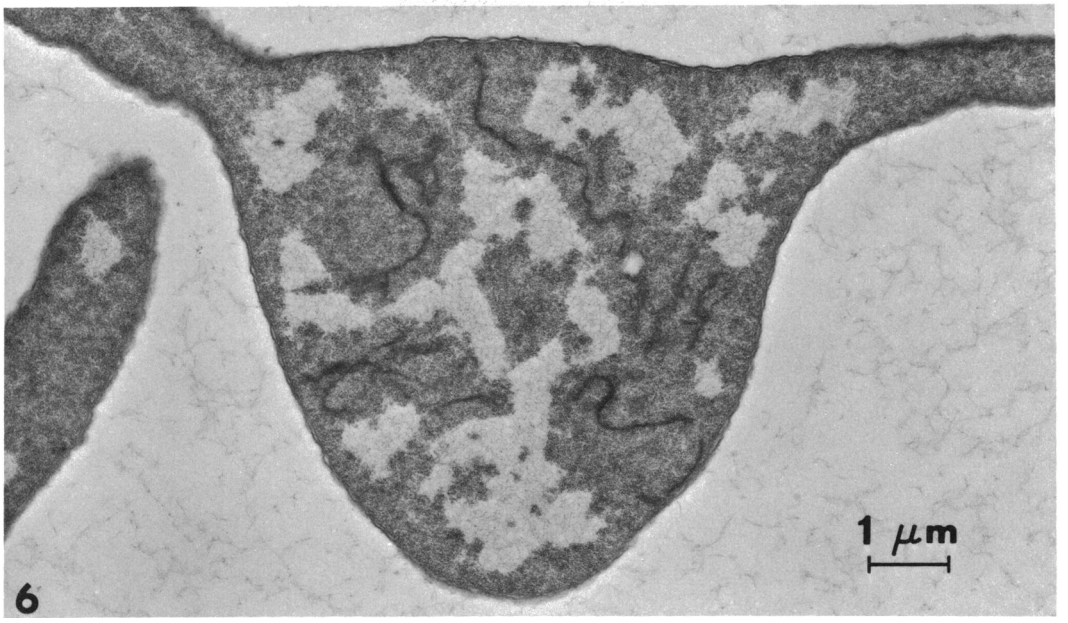


FIG. 6. *Giant cell 180 min after irradiation. The cell has not maintained the spherical morphology. Extensive membrane involvement is similar to the endoplasmic reticulum found in eucaryotic cells.*

the cell. Measurements made on giant cell membranes and on the plasma membranes of morphologically normal P678 indicate that all are of the same thickness. A higher magnification of vesicles is shown in Fig. 12. Vesicles are membrane bound and roughly 30 nm in diameter. Again, the membrane is morphologically indistinguishable from the plasma membrane of the cell.

The origin of these membrane structures is uncertain. At least some, possibly all, are invaginations of the plasma membrane of the cell. Two examples of such invaginations, which result in the invasion of membranes into the cytoplasm, are shown in Fig. 13.

DISCUSSION

Rich broth medium was chosen for studies on the morphology of giant cells. In this medium supplemented with 1% NaCl, the irradiated cells attained volumes up to 500 times larger than normal and were spherical in shape.

The morphological changes observed in irradiated P678-A_4 mediated by different NaCl concentrations (Fig. 7) are as yet unexplained. Perhaps, as Siccardi et al. have suggested in studies on the thermosensitive mutant *DnaB*-BT313 (21), there is a membrane protein which contains a functional defect responsive to NaCl concentrations. However, experiments which substantiate this possibility or eliminate alternative explanations have not been done.

The most interesting observation made during the morphological studies on giant cells concerned the development of extensive intracytoplasmic membrane systems. These membranes were found in most, if not all, cells. Other investigators have reported membranes within the cytoplasm of *E. coli*, and in rare cases the plasma membrane has been observed to invaginate into the cytoplasm, forming a mesosome. Ryter and Jacob (20) have suggested that the difficulty in seeing this type of membrane involvement is due to the simple nature of the *E. coli* mesosome compared to the easily seen, well developed mesosomes of gram-positive bacteria. Although they established that the plasma membrane does invaginate to form a mesosome and that membrane material is associated with the nucleus of the cell, they did not establish a continuity between the mesosome and the nucleus. Pontefract, Bergeron, and Thatcher (17) established this continuity and further proposed a mechanism for physically dividing the nucleus based on mesosomal attachment.

In other mutants of *E. coli*, more extensive membrane involvement has been observed. Weigand, Shively, and Greenawalt (23) have described a thermosensitive mutant, *E. coli* 0111a, which continues to produce membranes at 40 C when cell division has ceased, resulting in a series

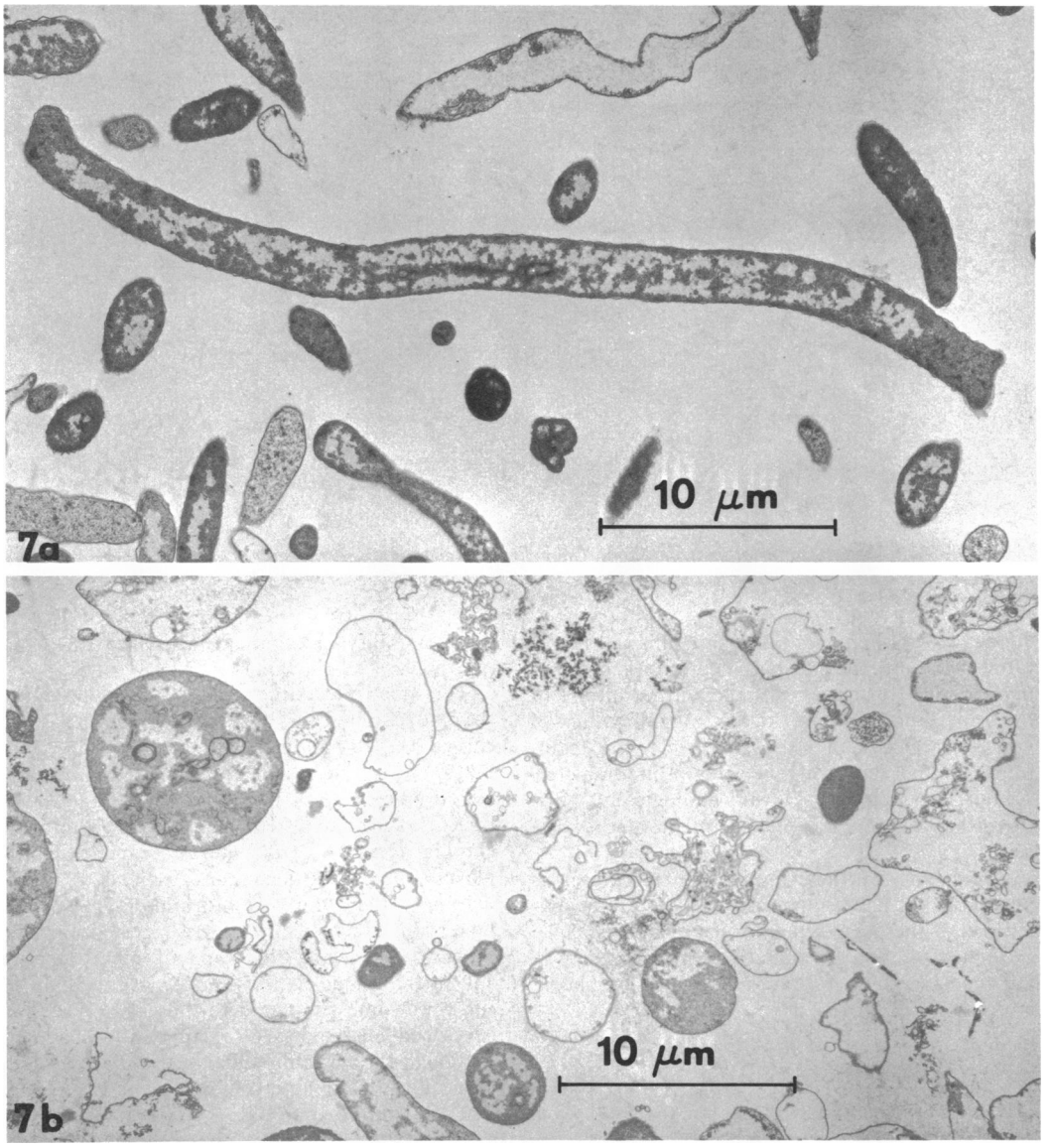


FIG. 7. *Effect of NaCl concentration on giant cell morphology. (a) Irradiated cells incubated in rich broth plus 0.2% NaCl. Note the elongated shapes of both intact and lysed cells. (b) Round irradiated giant cells produced with rich broth plus 1% NaCl.*

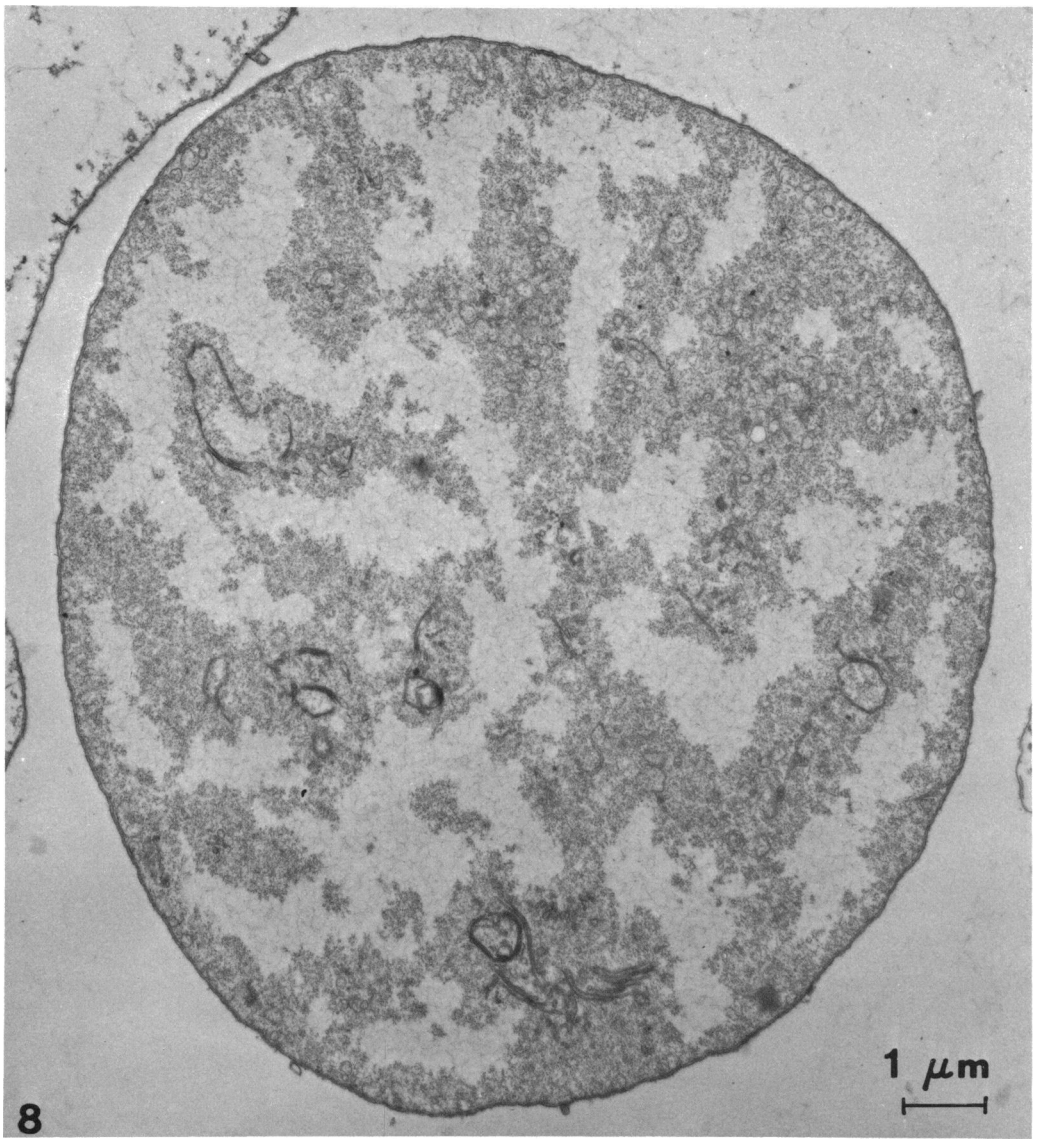


FIG. 8. *Large giant cell after irradiation. Note the extensive membrane involvement and the high ratio of nuclear to cytoplasmic material.*

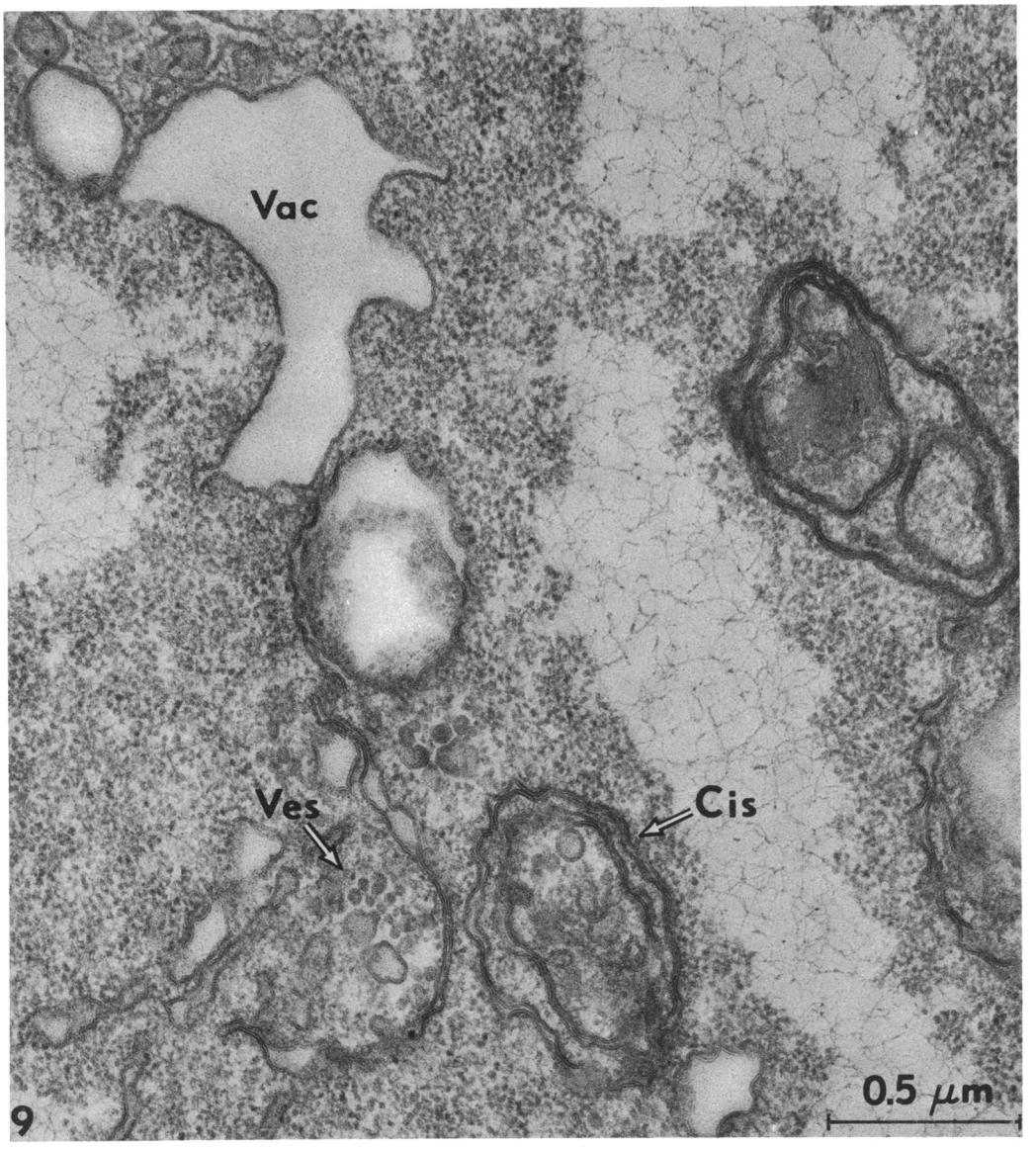


FIG. 9. *Vacuoles, vesicles, and flattened, bag-shaped cisternae found within giant cells.*

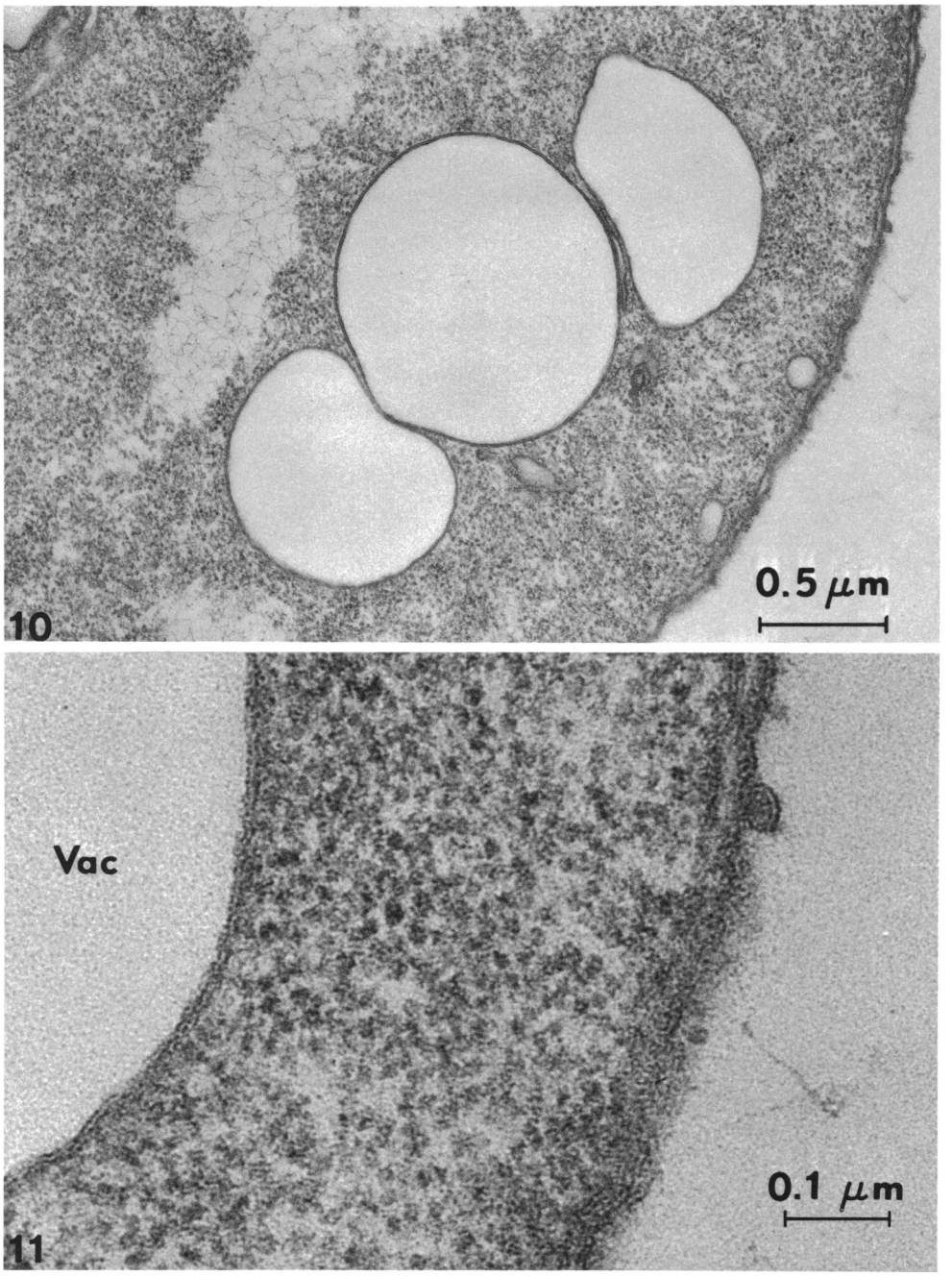


FIG. 10. *Large membrane-bound vacuoles devoid of cytoplasmic material.* FIG. 11. *A portion of the vacuole and plasma membranes of the cell shown in Fig. 10. No differences between the two membranes are apparent.*

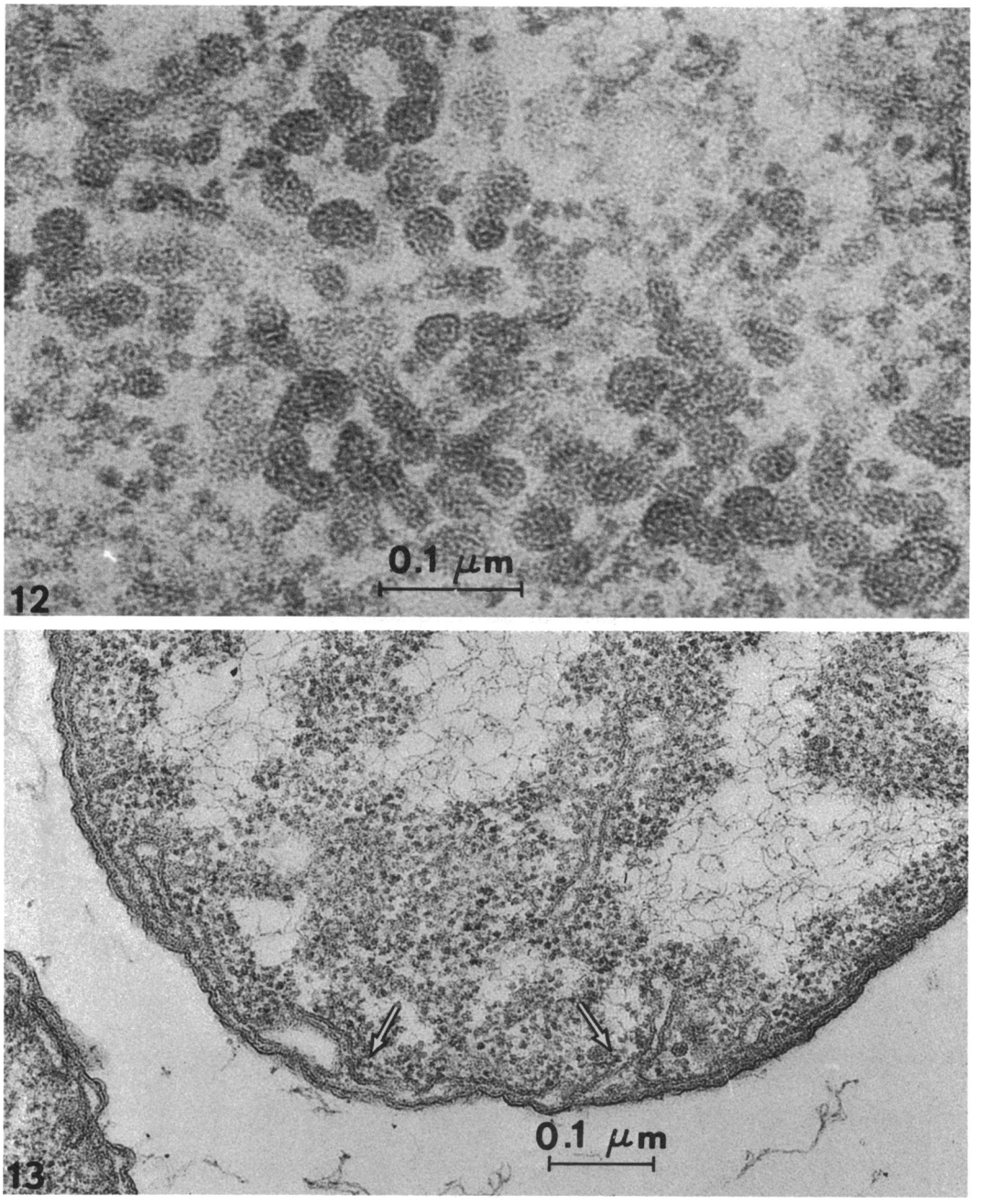


FIG. 12. *A higher magnification of membranous vesicles found within the cytoplasm.* FIG. 13. *A high-magnification micrograph showing two invaginations of the plasma membrane into the cytoplasm.*

of vesicles and whorls of "extra membrane" that in some cases occupy two-thirds of the cell volume. Hirota, Ryter, and Jacob (9) have described a thermosensitive mutant of *E. coli* K-12, CRT 257, which accumulates membrane structures within the cytoplasm after cessation of cell division. And Fischman and Weinbaum (6) have found that *E. coli* B subjected to a rich medium forms nonseptate filaments containing extra membranes within the cytoplasm.

Giant cells have features in common with thermosensitive mutants and with the *E. coli* B mutant. All accumulate extensive quantities of intracytoplasmic membranes which are, unlike the mesosomes of *E. coli*, readily seen in the electron microscope, and all are in some way defective in the cell division mechanism.

Evidence that the plasma membrane of giant cells does invaginate into the cytoplasm has been presented (Fig. 13). If the intracytoplasmic membranes are produced by invaginations of the plasma membrane, any section through a structure that is bordered by a continuous membrane, such as a vacuole (Fig. 9 and 10) or a vesicle (Fig. 9 and 12), should be devoid of cytoplasmic elements, i.e., ribosomes. This was found to be the case in all giant cell sections examined. However, the possibility that complicated membrane systems arise from de novo membrane synthesis within the cytoplasm cannot be ruled out unless a direct continuity between the intracytoplasmic membrane system and the plasma membrane of the cell is established. At the present time this effort is being undertaken through the study of serial sections of giant cells.

ACKNOWLEDGMENTS

I am grateful to Lucien G. Caro for his many helpful suggestions.

This investigation was sponsored by the United States Atomic Energy Commission under contract with the Union Carbide Corporation.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York, p. 446.
2. Adler, H. I., and J. C. Copeland. 1962. Genetic analysis of radiation response in *Escherichia coli*. Genetics **47**:701-712.
3. Adler, H. I., W. D. Fisher, and A. A. Hardigree. 1970. Cell division in *Escherichia coli*. Trans. N.Y. Acad. Sci. **31**:1059-1070.
4. Adler, H. I., and A. A. Hardigree. 1964. Analysis of a gene controlling cell division and sensitivity to radiation in *Escherichia coli*. J. Bacteriol. **87**:720-726.
5. Adler, H. I., C. E. Terry, and A. A. Hardigree. 1968. Giant cells of *Escherichia coli*. J. Bacteriol. **95**:139-142.
6. Fischman, D. A., and G. Weinbaum. 1967. The formation of multiple layers of membrane-like structures in *Escherichia coli* B. J. Cell Biol. **32**:524-528.
7. Greenberg, J. 1964. A locus for radiation resistance in *Escherichia coli*. Genetics **49**:771-778.
8. Hershey, A. D. 1955. An upper limit to the protein content of the germinal subtance of bacteriophage T2. Virology **1**:108-127.
9. Hirota, Y., A. Ryter, and F. Jacob. 1968. Thermosensitive mutants of *E. coli* affected in the process of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. **33**:677-693.
10. Howard-Flanders, P., E. Simson, and L. Theriot. 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K-12. Genetics **49**:237-246.
11. Inouye, M., and A. B. Pardee. 1970. Requirement of polyamines for bacterial division. J. Bacteriol. **101**:770-776.
12. Jagger, J. 1961. A small and inexpensive ultraviolet dose-rate meter useful in biological experiments. Radiat. Res. **14**:394-403.
13. Kellenberger, E., A. Ryter, and J. Sechaud. 1958. Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoides in different physiological states. J. Biophys. Biochem. Cytol. **4**:671-678.
14. Kohiyama, M. D., D. Cousin, A. Ryter, and F. Jacob. 1966. Mutants termosensibles d'*Escherichia coli* K-12. Ann. Inst. Pasteur (Paris) **110**:465-479.
15. Lennox, E. A. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190-206.
16. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409-414.
17. Pontefract, R. D., G. Bergeron, and F. S. Thatcher. 1969. Mesosomes in *Escherichia coli*. J. Bacteriol. **97**:367-375.
18. Reeve, J. N., D. J. Groves, and D. J. Clark. 1970. Regulation of cell division in *Escherichia coli*: characterization of temperature-sensitive division mutants. J. Bacteriol. **104**:1052-1064.
19. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**:208-212.
20. Ryter, A., and F. Jacob. 1966. Étude morphologique de la liaison du noyau à la membrane chez *E. coli* et les protoplastes de *B. subtilis*. Ann. Inst. Pasteur **110**:801-812.
21. Siccardi, A. G., B. M. Shapiro, Y. Hirota, and F. Jacob. 1971. On the process of cellular division in *Escherichia coli*. IV. Altered protein composition and turnover of the membranes of thermosensitive mutants defective in chromosomal replication. J. Mol. Biol. **56**:475-490.
22. Vogel, J. H., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
23. Weigand, R. A., J. M. Shively, and J. W. Greenawalt. 1970. Formation and ultrastructure of extra membranes in *Escherichia coli*. J. Bacteriol. **102**:240-249.