*MINIATURE ESCHERICHIA COLI CELLS DEFICIENT IN DNA**

By H. I. Adler, W. D. Fisher, A. Cohen, and Alice A. Hardigree

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY

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A newly isolated strain of *Escherichia coli* K12 regularly produces a large number of unusually small anucleate cells during the logarithmic phase of growth. These small cells do not divide. They may be isolated from the normal, rod-shaped cells by density gradient centrifugation and have properties that may make them useful in a variety of biological studies. In this report we communicate information regarding some of the basic properties of these *minicells.* A preliminary report of this work has been made.[1]

Materials and Methods.—(1) *Organisms and culture methods:* *E. coli* K12 P678 was obtained from Dr. F. Jacob of the Institut Pasteur, Paris, approximately six years ago and has been maintained on nutrient agar slants. The minicell-producing strain, P678-54, was derived from P678 after treatment of a log-phase nutrient-broth culture with triethylenemelamine (0.5 mg/ml). Both organisms are F^- strains that require threonine and leucine. They are unable to utilize lactose, galactose, xylose, maltose, and mannitol as sole carbon sources.

For most experiments, these organisms were cultivated in a nutrient broth prepared as described previously.[2] In some experiments, appropriately supplemented synthetic medium was used.[3]

(2) *Separation of minicells:* Cultures of P678-54 were centrifuged for 20 minutes at 10,000 × *g*. The pellet from 1 liter of culture medium was resuspended in approximately 20 ml of 0.067 *M* potassium phosphate buffer, pH 6.8.

Both minicells and normal cells were separated on sucrose gradients by centrifugation for 45 minutes at 2000 rpm (1000 × *g*) in a no. 253 swinging bucket rotor in an International model PRII centrifuge. A 2.0-ml sample of the cell suspension was layered over a 40-ml linear gradient ranging from 5 to 20 per cent sucrose buffered with 0.067 *M* phosphate at pH 6.8. The sample zones were withdrawn from the gradient, pelleted by centrifugation at 15,000 × *g* for 15 minutes, and resuspended in the phosphate buffer. All separations were done at approximately 4°C.

(3) *RNA, DNA, and protein analysis:* Samples of 10^{10} cells or 10^{11} to 10^{12} minicells were extracted in 5 ml of cold 10 per cent trichloroacetic acid for 30 minutes. After centrifugation the precipitate was resuspended in 3–5 ml of 5 per cent TCA and hydrolyzed at 100° for 30 minutes. The supernatant was collected after centrifugation, and the pellet digested in 2 ml of 1 *N* NaOH for 30 min. DNA was determined on the supernatant by Burton's modification of the diphenylamine reaction,[4] RNA on a 5–10× dilution of the supernatant by the orcinol reaction,[5] and protein on a 10–20× dilution of pellet digest by the Lowry method.[6]

(4) *Respiration and enzyme induction:* Oxygen consumption was determined in a Warburg apparatus at 37°C;[7] β-galactosidase was induced by methyl-β-D-thiogalactopyranoside (TMG) and assayed by measuring the rate of hydrolysis of O-nitrophenol β-D-galactoside at 28°C.[8]

Results.—(1) *Isolation of mutant, general properties, and morphology:* The

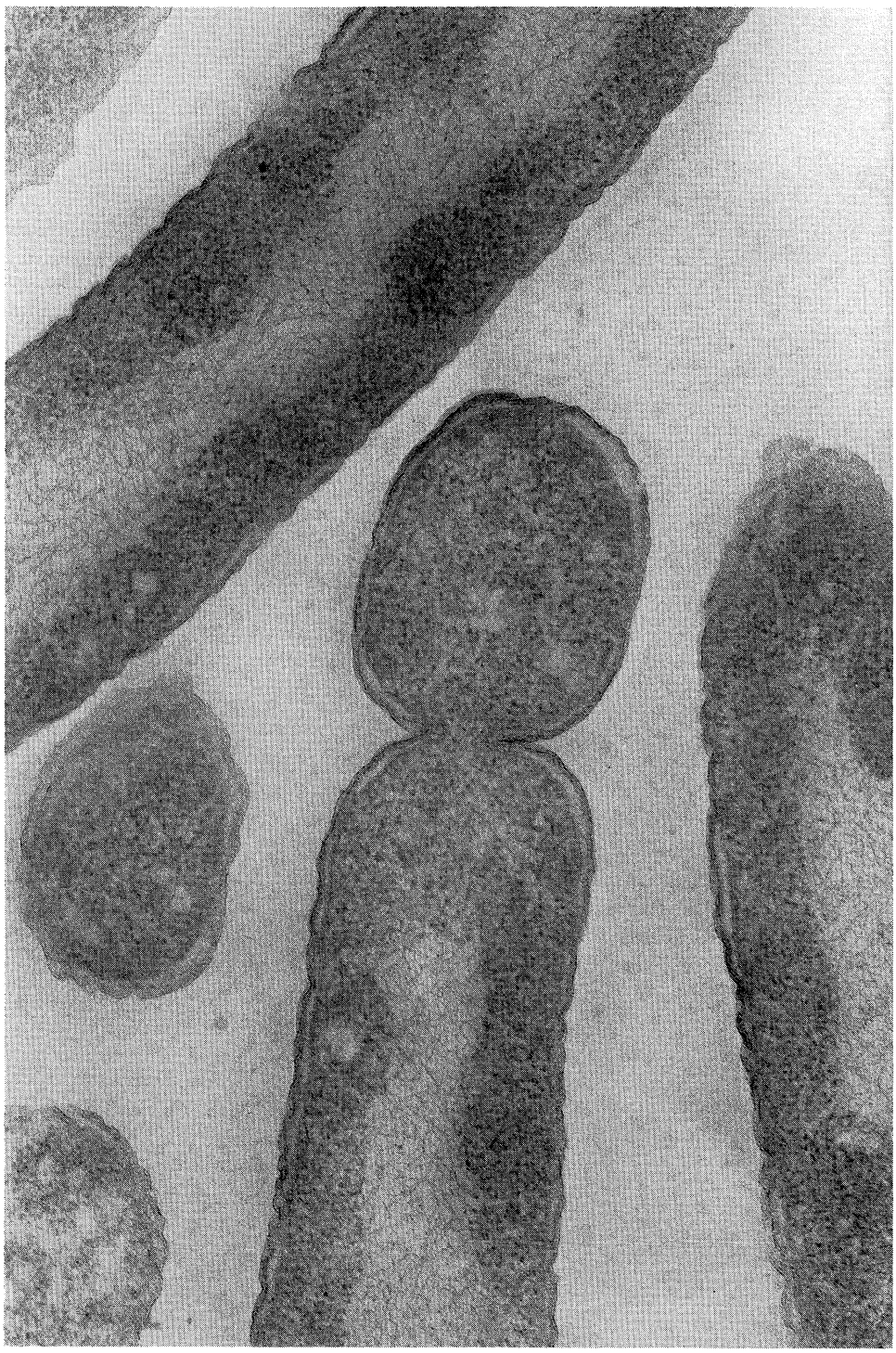

FIG. 1.—Thin-section electron micrograph showing a cell producing a minicell. ×66,000.

mutant strain, P678-54, was isolated in the course of a screening program designed to find strains that are unusually resistant to ionizing but not to ultraviolet (2537 Å) irradiation. The strain does have this combination of properties, and these will be considered in a separate publication. Phase-microscopic observation of stationary-phase cultures grown in either nutrient broth or in synthetic medium revealed a large number of small approximately spherical bodies mixed with the normal rod-shaped *E. coli* cells. These small bodies are approximately $^1/_{10}$ the volume of normal cells, and are not present in cultures of the parental strain, *E. coli* P678. The origin of these small structures, now referred to as minicells, became apparent when we observed by means of time-lapse cinematography the growth of individual cells incubated on an agar-covered microscope slide. The minicells are produced by a process that seems to be very similar to normal cell division except that it occurs near one or both poles of the cell. The production of a minicell does not seem to interfere with normal, median cell division which may occur simultaneously. The minicells have not been observed to undergo further growth or division. They accumulate during the logarithmic phase of growth and persist in stationary phase.

Minicells are produced by cultures growing at 30, 37, or 42°C. They are produced in synthetic as well as complex media, and are produced in actively aerated as well as in deep, stationary liquid cultures and on solid media. The ratio of minicells to normal cells is approximately 1:2 in cultures grown under any of the above conditions.

Preliminary genetic data, obtained from crosses of P678-54 with an HfrH donor strain, indicate that both minicell production and resistance to ionizing radiation are a consequence of genetic alteration in the region of the chromosome that controls lactose and galactose utilization.

Figure 1 is an electron micrograph of a rod-shaped cell producing a minicell. The minicell is surrounded by a wall and membrane and contains a cytoplasm indistinguishable from the parental cell. Nuclear material does not extend into the minicell.

Minicells seem to be relatively stable structures. They may be frozen and thawed without lysis. They persist for at least three hours in growth media at 37°C. Experiments have not been extended to longer periods.

(2) *Separation of minicells and chemical analysis:* Minicells have been separated from the normal rod-shaped cells by centrifugation on sucrose density gradient. Microscopically, the preparation consists almost entirely of small spherical minicells with only a few contaminating rod-shaped cells. Based on viable cell counts, the preparations contain approximately one normal-sized cell per 10^3 to 10^4 minicells. Minicells are not lysed by the sucrose concentrations used in this separation.

A chemical analysis of minicells from nutrient-broth cultures is shown in Table 1. The RNA/protein ratios are similar in minicells and normal cells. Only traces of DNA are present in the minicell preparations. Similar results have been obtained for cultures grown in synthetic medium.

(3) *Physiological properties:* Figure 2 presents respiration data for minicells and normal cells. Since minicells do not reproduce, their oxygen consumption is linear with time in contrast to the exponential increase in oxygen uptake observed for the dividing cell population. If a population of normal, rod-shaped cells is allowed to grow and divide in a medium containing a β-galactosidase inducer, the

TABLE 1

MACROMOLECULAR COMPONENTS OF NORMAL CELLS AND MINICELLS

	$\mu g/10^9$ Normal cells	$\mu g/10^9$ Minicells
DNA	6.5	0.009*
RNA	35.0	2.8†
Protein	110.	7.7†

* DNA due to contaminating cells = 0.008.
† Minicells are approximately $^1/_{10}$ the volume of normal cells. The values for RNA and protein are therefore approximately $^1/_{10}$ the values found for normal cells.

TABLE 2

β-GALACTOSIDASE ACTIVITY IN NORMAL CELLS AND IN MINICELLS PRODUCED BY AN INDUCED CULTURE*

Organism *E. coli* P678-54	Activity (mmoles/mg protein/min) Induced culture	Noninduced culture
Normal cells	0.72	<0.01
Minicells	0.53	<0.01

* Normal cells were separated from minicells by sucrose gradient centrifugation. The normal cells were inoculated at 10^7 cell/ml into minimal media containing 0.4% glycerol ± 4×10^4 *M* TMG. After 4 hr of growth with or without inducer, the cultures were harvested and separated to yield normal cells and minicells.

enzyme can be found both in normal cells and in newly produced minicells (Table 2). However, a population of isolated minicells cannot be induced to produce any detectable β-galactosidase (Fig. 3).

Discussion.—We should like to emphasize that the production of minicells by P678-54 is a consequence of the unusual genetic constitution of this organism and does not require any unusual manipulation of the environment. An earlier attempt to produce anucleate *E. coli* cells by stimulating cytokinesis in mitomycin C treated filamentous forms was unsuccessful.[9] The spontaneous production of a single structure resembling a minicell has been reported but was clearly a rare event in the culture used.[10] P678-54 produces large quantities of minicells under a variety of growth conditions and therefore makes possible a detailed study of the properties of *E. coli* cells that are deficient in DNA.

Chemical analysis cannot preclude the presence of DNA in minicell preparations. However, the average amount of DNA per minicell does not exceed $^1/_{1000}$ the amount in the normal cell. Further, the small amount of DNA in the isolated minicell preparation is approximately that expected from contaminating normal cells.

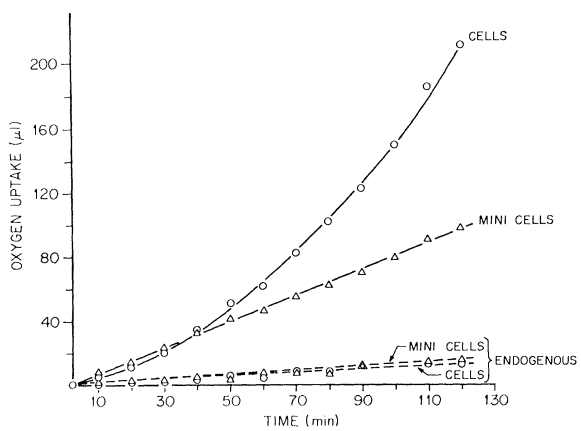

FIG. 2.—Oxygen consumption by cells and minicells of *E. coli* P678-54 culture: Warburg vessels contained in the main compartment 3×10^8 cells (O—O) or 1×10^{10} minicells (Δ—Δ) suspended in 2.6-ml minimal media and 0.2 ml of 1 *N* NaOH in the center well. At time 0, 0.2 ml of 20% glucose (———) or water (— — —) was added to the main compartment.

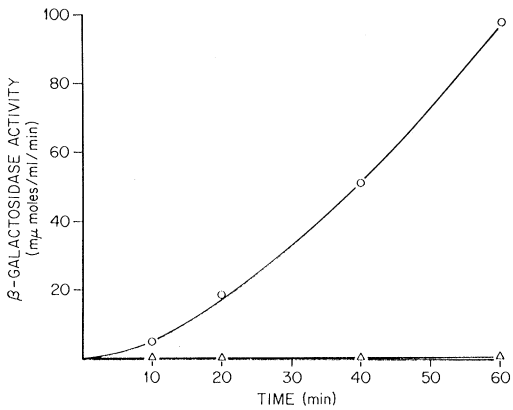

FIG. 3.—Kinetics of β-galactosidase induction in cells and minicells of *E. coli* P678-54 culture. Reaction mixture containing 3×10^8 cells/ml (O—O) or 6×10^9 minicells (Δ—Δ) suspended in a medium containing 0.5% casamino acids, 0.4% glycerol, 40 μg/ml L-tryptophan, and 2 μg/ml thiamine. TMG ((final concentration 4×10^{-3} *M*) was added at time 0. Both cells and minicells were harvested from a culture in the logarithmic phase of growth.

The division process that yields minicells seems to be "normal" in many ways. It involves a simultaneous invagination of wall and membrane and proceeds at a rate comparable with that observed for "normal" cell division. The only striking cytological feature of the minicell-yielding division is that the nuclear region of the cell does not seem to be intimately involved (Fig. 1). DNA is not distributed to both sides of the division plane. This observation should certainly be considered when examining recent models of cell division in *E. coli*.[11, 12]

The data presented on respiration and enzyme synthesis in P678-54 strengthen the impression that minicells may be regarded as small samples of the bacterial cytoplasm that retain many structural and functional features of the normal cell. We anticipate that they will be able to carry out many cellular activities not requiring the direct participation of DNA. We have already made preliminary observations indicating that they can be lysed by bacteriophage T6 but not by T3 and, with the aid of Dr. Roy Curtiss of this laboratory, have observed that they readily become attached to the F-pili of donor K-12 strains.

The minicell-producing strain is derived from a genetically well-characterized F^- parent, and its own genome may be readily altered by conjugation and transduction techniques without the loss of the ability to form minicells. We anticipate that this strain will be valuable in a variety of genetic and biochemical studies.

Summary.—A mutant of an F^- *Escherichia coli* K12 strain frequently undergoes an aberrant cell division near one pole of the normal, rod-shaped cell. This division yields an unusually small cell. These small cells may be separated from the rest of the population and characterized. They contain protein and RNA, but, at most, have only traces of DNA. They are enzymatically active and respire but do not divide.

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