JOURNAL OF BACTERIOLOGY, June 1975, p. 1283-1292

Vol. 122, No. 3
Printed in U.S.A.

Inhibition of an Early Event in the Cell Division Cycle of *Escherichia coli* by FL1060, an Amidinopenicillanic Acid

RICHARD JAMES,* JULI Y. HAGA,[1] AND ARTHUR B. PARDEE
Department of Biochemical Sciences, Moffett Laboratories, Princeton University, Princeton, New Jersey 08540

Received for publication 3 January 1975

Analysis of exponential and synchronous cultures of *Escherichia coli* B/r after the addition of FL1060 indicates a block point for division by this agent some 15 to 20 min before the end of the preceding cell division cycle, a time corresponding to the beginning of the C period of the cell division cycle. Morphological examination of FL1060-treated synchronous cultures of *E. coli* B/r was consistent with inhibition by FL1060 of a very early event in the cell division cycle. This event appears to be essential for normal cell surface elongation in a rod configuration. Temporary treatment of synchronous cultures of *E. coli* B/r with FL1060 resulted in division delay, the extent of which was a function of the duration of exposure to FL1060. However, even after relatively long times of FL1060 treatment the delayed divisions were still synchronous. Although FL1060 had no direct effect on deoxyribonucleic acid (DNA) synthesis, the synchronous delayed divisions occurring after temporary treatment with FL1060 were accompanied by a delay in the attainment of resistance of cell division to inhibitors of DNA, ribonucleic acid, and protein synthesis. These results suggest that an FL1060-sensitive event initiates at the beginning of the C period of the cell division cycle of *E. coli* and is responsible for normal cell elongation. This cell elongation pathway procedes independently of DNA synthesis, but there is an interaction between this pathway and termination of a round of DNA replication in which a normal rod configuration is necessary to allow a signal for cell division to be generated upon completion of DNA replication.

Bacterial growth is composed of linear sequences of events which can overlap one another in time, the extent of the overlap being dependent upon the growth rate. The most obvious events are thus observed periodically and give the appearance of a cycle. The Helmstetter-Cooper model (8) separates the division cycle into three periods which occur sequentially; the I period of preparation for the initiation of deoxyribonucleic acid (DNA) synthesis, the C period of DNA synthesis, and the D period occurring after the completion of DNA replication and up to the time of cell separation. These three periods are linked by minimal requirements which limit progression from one period to another, but the model does not preclude the existence of other requirements for cell division. There are probably multiple pathways, most of which are completed prior to the end of the period and which are thus not observed as being limiting to division under normal growth conditions. The existence of such nonlimiting pathways may be detected by the use of a specific inhibitor, e.g., protein synthesis required for division has been resolved into three classes by the use of chloramphenicol. The first is required for the initiation of DNA replication and occurs during the I period (15), the second is a 40-min period of protein synthesis which occurs concurrently with DNA replication (22), and the third is a short period of protein synthesis which must take place at the time of the completion of a round of DNA replication (13). Experiments using benzyl-penicillin have similarly indicated a requirement for septum specific murein synthesis at the end of the C period of the cell division cycle (10).

In this paper we report for the first time the existence of an event occurring early in the cell cycle which is essential for normal cell elongation and which is blocked by FL1060, a novel amidinopenicillanic acid. One consequence of this inhibition is the failure of cell division to occur C + D min later.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* B/r (laboratory collection) was grown aerobically in a gyratory shaker at 37 C in a modified M9 medium (6)

[1] Present address: Microbiological Associates, Inc., 4733 Bethesda Avenue, Washington, D.C. 20014.

containing sodium acetate (1 g/liter) or glucose (0.4%, wt/vol) as carbon sources.

Sucrose gradient synchrony. A modification of the method of Mitchison and Vincent (19), as described by Gudas and Pardee (6), was used to synchronize *E. coli* B/r growing in M9-glucose medium. Good synchrony was obtained with this method for a minimum of two generations.

Counting cell number. A formalin-saline solution consisting of 50 ml of 38% formaldehyde and 9.0 g of NaCl per liter of distilled water was filtered through a 0.22-μm membrane filter. Samples of cultures were diluted into 10 ml of formalin-saline, and the cell number was determined with a Coulter Counter model B equipped with a size distribution plotter.

DNA synthesis. DNA synthesis was measured by the incorporation of acid-insoluble radioactivity after 3-min pulses of [^{3}H]thymidine (6).

Electron microscopy. After the addition of FL1060 at 10 min, 8-ml samples of control and treated synchronous cultures of *E. coli* B/r were harvested at 45, 60, and 75 min after synchronization. The volume of each culture was immediately made up to 10 ml with prechilled glutaraldehyde fixative (see below), and the cells were recovered by centrifugation. FL1060 was also added to an exponentially growing culture, and the cells were harvested after 2 h in the same manner.

The cell pellets were fixed in glutaraldehyde (1%)-acrolein (5%) in 0.2 M sucrose-0.67 M Sorensons phosphate buffer (pH 7.1) for 6 h in an ice bath. The cells were then washed in the same buffer and postfixed in 1% osmium tetroxide for 3 h at room temperature, en bloc stained with uranyl acetate, dehydrated, and embedded in Epon 812. The embedded specimens were then thin sectioned on an LKB Ultrotome 111 equipped with a diamond knife and then stained with uranyl acetate and lead citrate. Specimens were examined in a Phillips 300 electron microscope at 60 kV.

Materials. FL1060 (16) was a generous gift of Leo Pharmaceutical Products, Ballerup, Denmark. Cephalexin was a gift of Eli Lilly Co. The minimum inhibitory concentration of FL1060 with *E. coli* B/r was less than 0.05 μg/ml. Penicillinase (Calbiochem) was dissolved in distilled water and stored in the cold.

RESULTS

The timing of action of FL1060. Cultures of *E. coli* B/r growing exponentially in M9-glucose medium (doubling time, 40 min) exhibited a considerable amount of residual division after the addition of FL1060 (Fig. 1A). Data from several experiments suggested that all cells divided once in the presence of FL1060, and that some 40% of all cells divided twice. Utilizing the ideal distribution of cell age in an exponential culture (7), we can calculate that the block point for inhibition of cell division by FL1060 is located some 60 min before division ceases, a point which at this growth rate corresponds with the beginning of the C period of the cell cycle (8). With *E. coli* growing in M9-acetate medium (doubling time, 100 min) the amount of residual division (Fig. 1B) suggested a block point 115 min before division ceased, or again at the beginning of the C period of the cell division cycle (6; see Appendix). The loss of division capacity after these times of treatment with FL1060 is not a result of inhibition of growth per se, since mass increase measured as turbidity (18, 21) and incorporation of radioactive label into total protein (R. James, unpublished observation) is the same as the control for several hours.

These results should be contrasted with the limited residual division of *E. coli* B/r in glucose medium after the addition of cephalexin (Fig. 1C), a β-lactam antibiotic which readily induces filamentation in *E. coli* (20). This compound inhibited up to as little as 15 min before division, a result in good agreement with the reported time of maximum sensitivity to cell killing by benzyl-penicillin and with the time of maximum murein synthesis in *E. coli* B/r (10). The correlation of the timing of action of FL1060 at two different growth rates makes it unlikely that the apparent delay in the effect of FL1060, as compared with cephalexin, is the result of slower penetration.

The apparently unique timing of action of FL1060 was studied further using synchronous cultures of *E. coli* B/r growing in M9-glucose medium. The addition of FL1060 immediately after synchronization had almost no effect on the first synchronous division occurring at 55 min (Fig. 2); however the second synchronous division, occurring at 96 min, was completely inhibited. Delaying the addition of FL1060 until 10 min or later after synchronization abolished the small effect on the first division and, after 30 min, progressively allowed more division in the second division cycle. More than half of the cells could divide in the second cycle when FL1060 was added at 40 min. In this experiment the midpoint of the increase in cell number of the control culture occurred at 96 min. Thus, considering the variation in the interdivision times of individual cells in a synchronous culture, the results indicated that cell division became resistant to FL1060 some 56 min before division, a result in good agreement with the 60-min value calculated from the experiments with exponential cultures.

Morphology of E. coli B/r treated with FL1060. Several authors have reported a gradual rounding up of exponential cultures of *E. coli* treated with FL1060 (5, 17, 18, 21) leading to the formation of greatly enlarged osmotically stable spherical forms; however, the precise

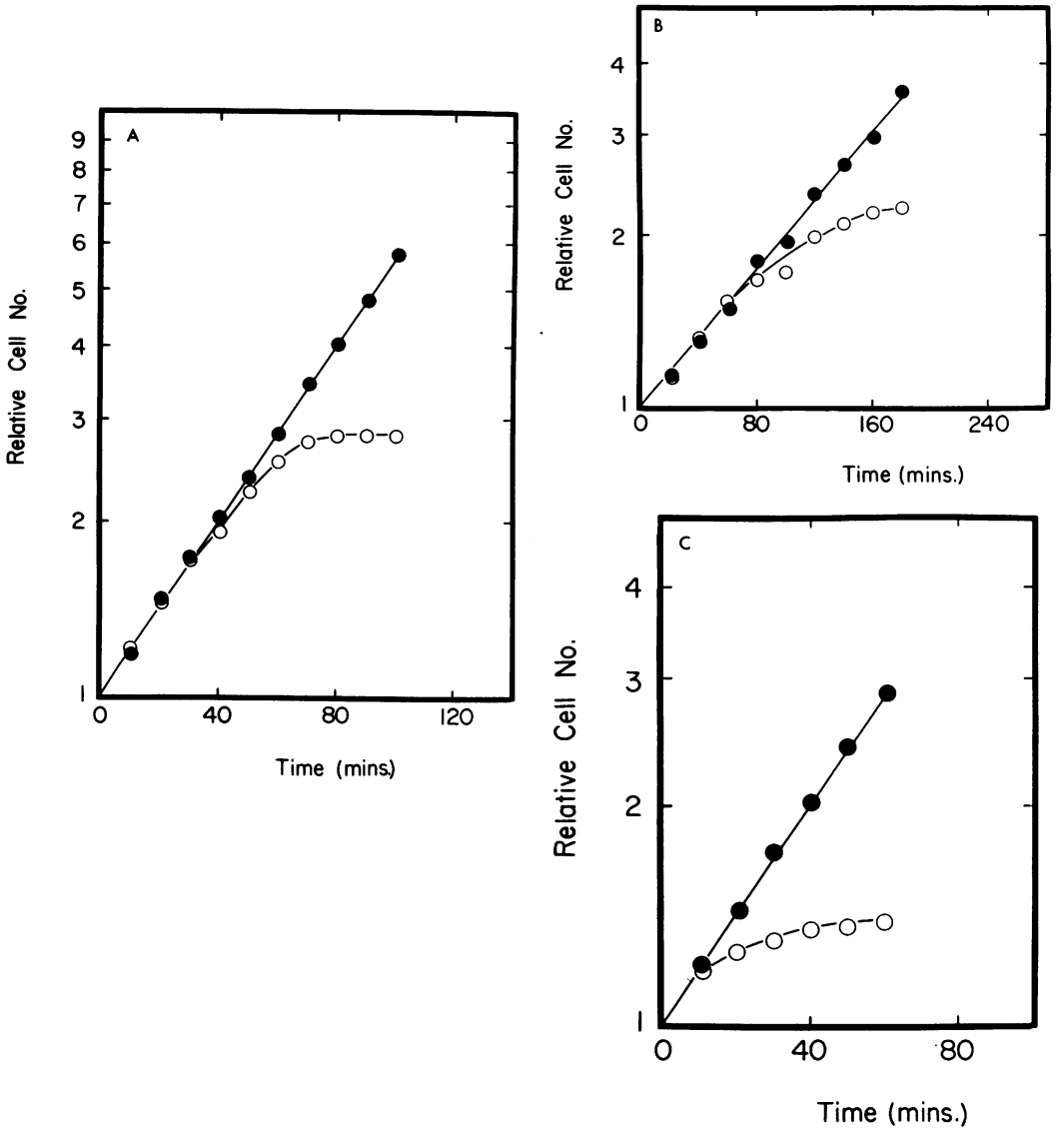

FIG. 1. *Growth of E. coli B/r (A) in M9-glucose after addition of FL1060 (1 μg/ml) at zero time* (O), *(B) in M9-acetate after addition of FL1060 (1 μg/ml) at zero time* (O), *and (C) in M9-glucose after addition of cephalexin (4 μg/ml) at zero time* (O). ●, *No inhibitors added.*

timing of this morphological event was not indicated. We have attempted to fix the timing of the morphological effects of FL1060 on synchronously growing cultures of *E. coli* B/r and to investigate the relationship between the morphological effects of FL1060 and the observed timing of its action.

The size distribution of FL1060-treated synchronous cultures of *E. coli* B/r, measured with a Coulter Counter, was identical to that of an untreated control culture until 55 min after synchronization, at which time there was an apparently rapid increase in the cell volume (data not shown). This effect was readily visualized in electron micrographs of cells fixed at various times in the cell cycle after the addition of FL1060 10 min after synchronization. In samples taken 45 min after synchronization the majority of longitudinally sectioned cells showed various stages of septation, but no significant differences were observed between the control and the FL1060-treated cultures (Fig. 3A and B). Septation appeared to be normal in both cases, with inward growth of the

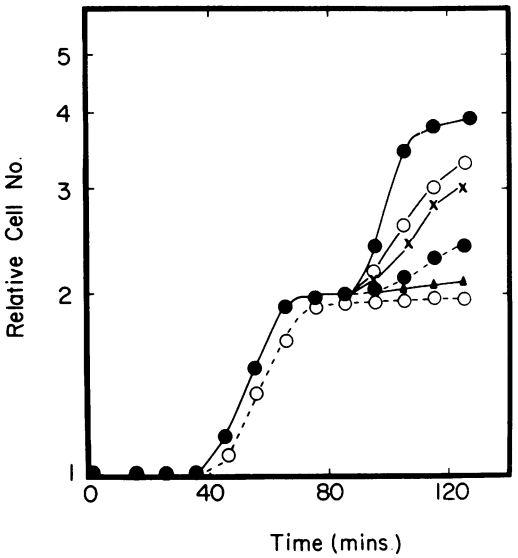

FIG. 2. *Division of synchronous cultures of E. coli B/r in M9-glucose medium. Control* (●——●); *FL1060 (1 μg/ml) added at zero min* (○ - - ○), *10 min* (▲——▲), *30 min* (●- -●), *40 min* (×——×), *and 45 min* (○——○).

inner membrane and the murein layer initiated evenly from both sides of the cell, with exclusion of the outer membrane from the septum until a very late stage (Fig. 3C and D). Our electron micrographs demonstrate that cell division in *E. coli* does occur by septation and not by constriction (23), a process in which all the cell envelope layers infold together. The use of specially adapted fixation conditions allows the visualization of distinct septa in *E. coli*, a result which has also independently been reached by Burdett and Murray (3).

At 60 min after synchronization the FL1060-treated cells had begun to assume an ovoidal shape by expansion outward from the middle of the cell (Fig. 3E); this expansion had become more pronounced at 75 min (Fig. 3F), whereas the control cells were still observed as rods (Fig. 3G). In spite of the enlarged spherical shape of *E. coli* B/r cells after longer times of treatment with FL1060, the initial stages of septation were still observed; however, it was always asymmetric in nature, as reported by Park and Burman (21), and cell separation was not achieved. The fact that FL1060 has no direct effect on at least the early stages of septation, even though cell division is blocked, provides further support for the hypothesis that the site of action of FL1060 is distinct from other β-lactam antibiotics.

We have observed after 2 h of treatment of an exponential culture of *E. coli* B/r with FL1060 that the outer membrane became detached from the cell envelope at many areas of the cell surface (Fig. 3H). Although this apparent physical separation of the outer membrane may be an artifact under these conditions, it is perhaps indicative of some biochemical lesion induced by FL1060.

The results thus far are consistent with inhibition by FL1060 of an early event in the cell division cycle. This inhibition does not affect the normal increase in cell mass; rather it results in loss of the capacity for cell elongation in a rod configuration and finally in loss of the ability to divide. The relationship between this FL1060-sensitive event and the other easily recognizable events of the cell cycle was studied by examination of the effect of temporary inhibition by FL1060 of synchronous cultures of *E. coli* B/r.

FL1060 inhibition and the cell cycle. Addition of an excess of penicillinase allowed the recovery of division by synchronous cultures of *E. coli* B/r to which FL1060 had been added for varying periods of time (Fig. 4). The resultant division delays, measured as the time between the midpoints of the increase in cell number of the control and of the treated cultures, increased with the time of exposure to FL1060. However, the delayed divisions were still synchronous even after long exposures to the drug. Microscopically, the resumption of cell division of the treated cultures was always preceded by return to the rod configuration (data not shown). Further support for the timing of the block point of division by FL1060 may be derived from these results, in that extrapolation back to zero division delay in a plot of division delay against duration of exposure to FL1060 gives a time in the cycle of 42 min as the block point, or 53 min before the second synchronous division.

The synchronous but delayed divisions occuring after temporary exposure to FL1060 suggest that inhibition of the FL1060-sensitive early event prevents further progress along a sequential pathway towards division. Removal of FL1060 appears to initiate recovery of division capacity, an event which was always preceded by return to a rod configuration. This hypothesis was tested by analysis of the timing of an easily recognizable cell cycle event, the attainment of resistance of division to inhibitors of DNA, ribonucleic acid, and protein synthesis, in control and treated synchronous cultures of *E. coli* B/r.

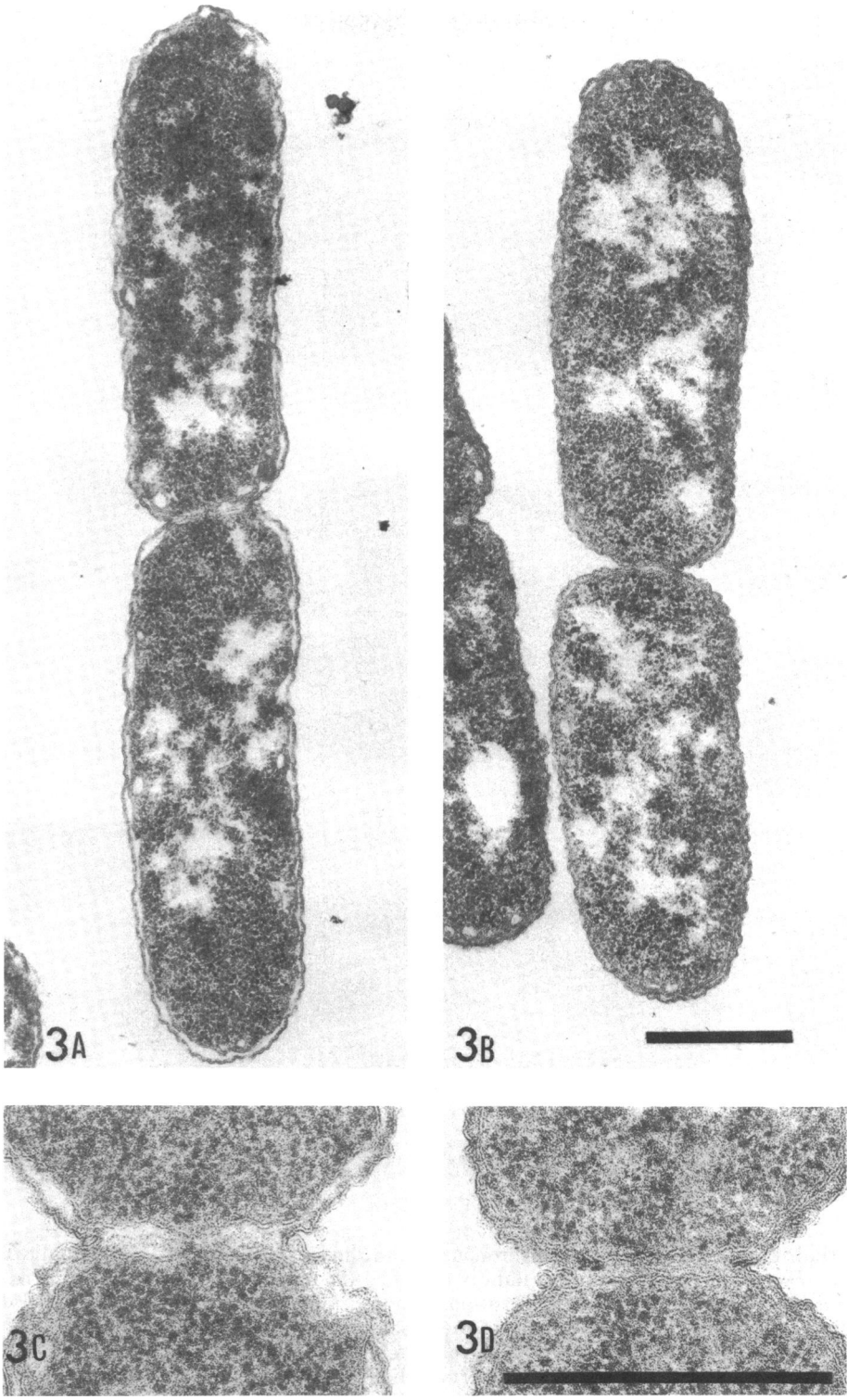

FIG. 3. *Electron micrographs of ultrathin sections of E. coli B/r. Control cells after 45 min of synchronous growth in glucose medium (A); magnified view of septum in control cell (C); FL1060-treated (at 10 min) cell after 45 min of synchronous growth (B); magnified view of septum in FL1060-treated cell (D). FL1060-treated cells at 60 min (E) and at 75 min of synchronous growth (F); control cell at 75 min of synchronous growth (G). FL1060-treated cells after 2 h of exponential growth (H). In all figures the bar represents 500 nm.*

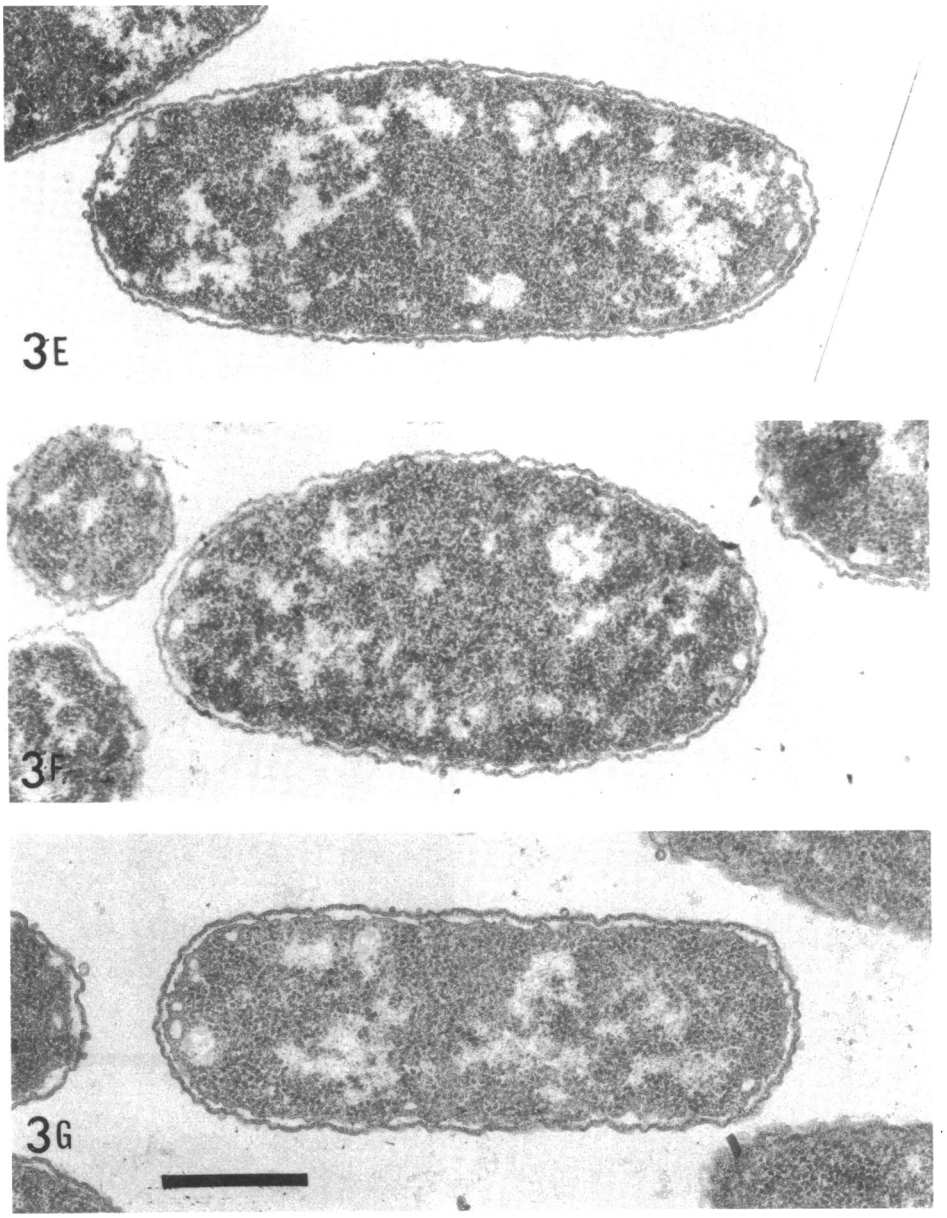

FIG. 3E-G

More than half of the cells of a synchronous culture of *E. coli* B/r divided when nalidixic acid was added at 80 min after synchronization, a time some 13 min before the mid point of the increase in cell number of the untreated control (Fig. 5A). This is consistent with earlier reports that cell division became resistant to this inhibitor at the end of the C period (4). In contrast, the second synchronous division of cultures of *E. coli* B/r treated with FL1060 from 10 to 70 min did not become resistant to nalidixic acid until 105 min after synchronization (Fig. 5B), or again some 15 min before the delayed division occuring after FL1060 treatment. The 25-min delay in the attainment of resistance to nalidixic acid observed in this experiment was little different from the actual division delay induced by FL1060 treatment. Almost identical results were obtained with rifampin and chloramphenicol, further probes for the end of the C period of

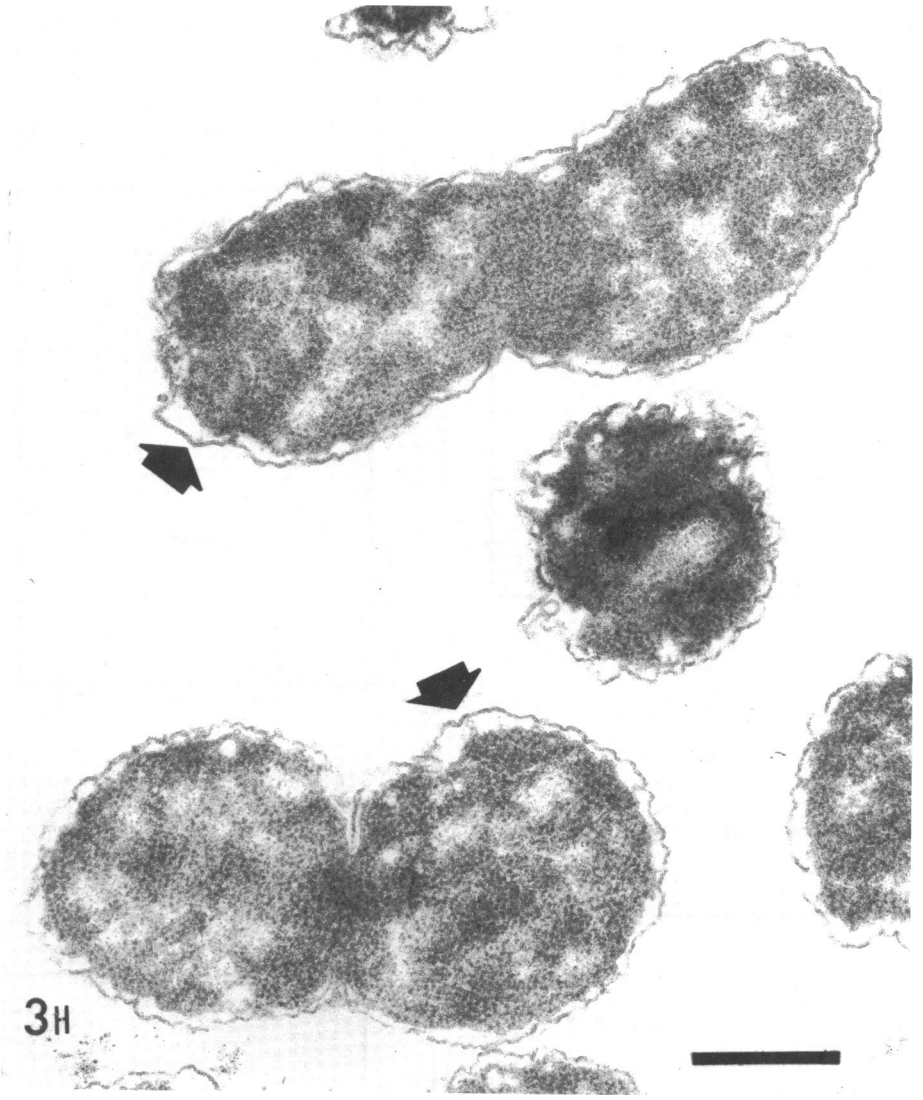

FIG. 3*H*

the cell cycle (data not shown). It is of interest that although the addition of chloramphenicol at any time after FL1060 removal and up to 105 min after synchronization blocked subsequent cell division, it did not prevent the shape transition from sphere to rod. Similarly, nalidixic acid added during this period of time resulted in the formation of short filaments.

One simple explanation for the apparent delay in the end of the C period of the cell cycle after FL1060 treatment is that FL1060 directly inhibits DNA synthesis. However, no detectable effect on the rate of incorporation of [^{3}H]thymidine into the acid-insoluble fraction was observed under these conditions (Fig. 6). The data from this experiment with synchronous cultures of *E. coli* B/r confirms that the block point of FL1060 for division, located at approximately 40 min after synchronization, does indeed correspond with the time of the initiation of DNA synthesis.

DISCUSSION

We have shown that FL1060 inhibits an early step in the cell division cycle of *E. coli* B/r. For *E. coli* B/r cells, growing in minimal salts-glucose medium with a generation time of 40 min, it was located some 55 to 60 min before division, or at the beginning of the C period. The precise timing of this event varied some-

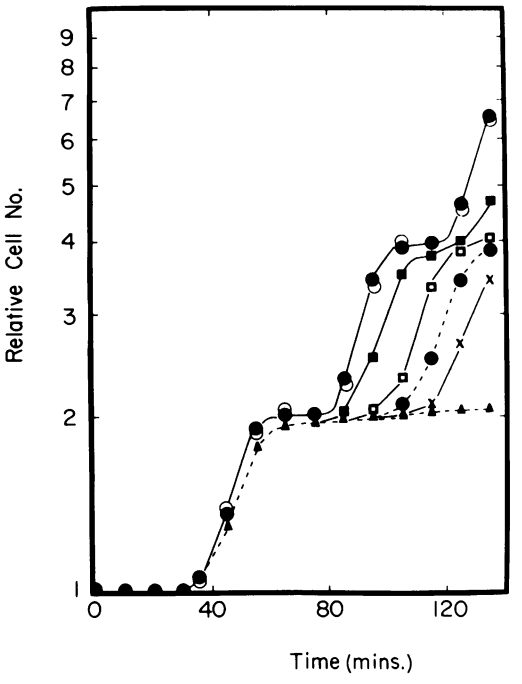

FIG. 4. *Division delay of cultures of E. coli B/r growing synchronously in M9-glucose after exposure to FL1060 for varying times. No additions (●——●); FL1060 added at 10 min (▲--▲); FL1060 added at 10 min, penicillinase (1,000 U/ml) added at: 10 min (○——○), 50 min (■——■), 60 min (□——□), 70 min (●--●), and 80 min (×——×).*

what depending upon the experimental technique used. This timing of action of FL1060 should be contrasted with cephalexin action, where the block point occured some 15 min before division, or approximately at the end of the C period. This difference in the timing of

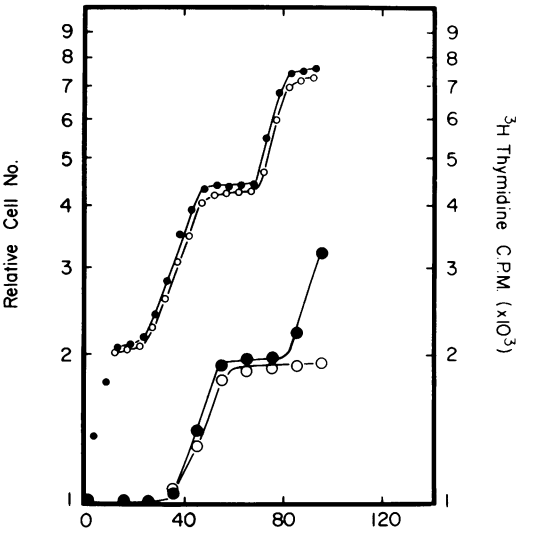

FIG. 6. *Rate of [^{3}H]thymidine incorporation into (small symbols) and cell number (large symbols) of control (●) and FL1060 (1 μg/ml) treated at 10 min (○) E. coli B/r growing synchronously in M9-glucose.*

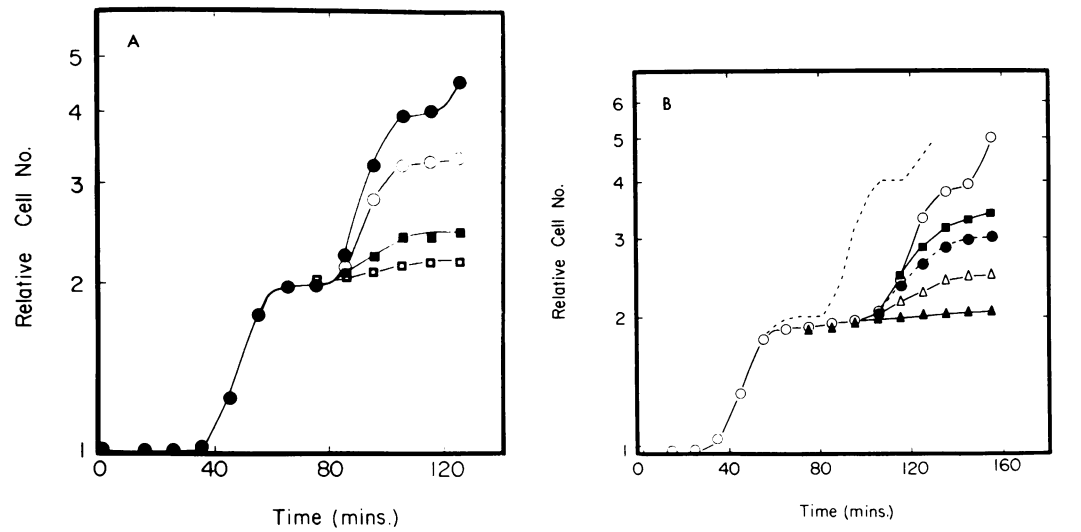

FIG. 5. *Effect of nalidixic acid (15 μg/ml) (A) on the division of synchronously grown cultures of E. coli B/r. No additions (●); nalidixic acid added at 70 min (□), 75 min (■), and 80 min (○). (B) Effect of nalidixic acid on the division of synchronously grown cultures of E. coli B/r which had been exposed to FL1060 (1 μg/ml) from 10 to 70 min. No further additions (-○-); nalidixic acid added at 70 min (-▲-), 90 min (-△-), 100 min (-●-), and 105 min (-■-). The untreated control culture is shown as a dotted line.*

action of these two β-lactam antibiotics correlates well with their morphological effects on *E. coli* B/r; FL1060 seemingly inhibits an early step in the cell cycle and prevents normal cell surface elongation in a rod configuration, resulting in the formation of spherical forms, whereas cephalexin blocks a late step in the division cycle which inhibits septation directly and results in filament formation. Since all cells divide normally at least once in the presence of FL1060, this drug does not affect cell surface elongation or cell division in progress at the time of addition. The apparent inhibition only of new rounds of elongation and division shows that there is a special early event specific to the initiation of a round of cell division which is sensitive to FL1060.

This drug thus appears to be the first reported specific inhibitor of the key initiation step for normal cell elongation, occurring at the start of the C period of the bacterial cell division cycle.

The effect on the cell cycle of temporary inhibition of the capacity to elongate in a rod configuration may be seen in the delay of attainment of resistance of division to inhibitors of DNA, ribonucleic acid, and protein synthesis in FL1060-pulsed cultures. Since this delay was not the result of a direct effect on DNA replication, perhaps the event inhibited by FL1060 (ie., normal cell elongation) has become limiting for the termination of DNA replication under these conditions. Treatment with FL1060 may result in a loss of phase of cell elongation with DNA replication such that, upon removal of the drug, a normal rod configuration must be achieved before a small amount of terminal DNA synthesis necessary for cell division can occur. This FL1060-sensitive pathway for normal cell elongation may thus be the pathway, or one of a series of pathways, suggested by Jones and Donachie (13, 14), which are initiated at the beginning of the C period of the cell cycle and which interact with termination of DNA replication to generate a signal for cell division.

We must assume that the mechanism by which FL1060 inhibits normal cell elongation and cell division involves some modification of murein structure, as do other β-lactam antibiotics. However, the murein structure altered must be essential for initiation of cell surface elongation in a rod configuration, but not for cell surface elogation in progress, or for the initial stages of septation, since these are unaffected by FL1060. At this time there are no known differences between murein synthesized for elongation or for septation; indeed it is the largely circumstantial evidence of differential sensitivity to β-lactam antibiotics (10) and to inhibition of DNA synthesis (26) that leads us to suspect that septation and elongation are different processes. The demonstration of several [^{14}C]benzyl-penicillin binding proteins in *E. coli* membranes (25), and of several penicillin-sensitive enzymatic activities involved in murein methabolism (24), tends to provide support for the existence of different modes of murein synthesis for elongation and septation.

That FL1060 may indeed have a completely novel effect on murein synthesis is suggested by the observations that in vivo FL1060 does not inhibit the transpeptidase, the D-alanine carboxypeptidase, or the endopeptidase of *E. coli* (17, 21), enzyme activities which are sensitive to benzyl-penicillin and which have an assumed role in the terminal steps of murein synthesis (1, 11, 12). Furthermore, using a very sensitive assay system, it has been demonstrated that the proteins for which FL1060 competes with [^{14}C]benzyl-penicillin for binding are different from those for which cephalexin competes for binding (B. G. Spratt, manuscript in preparation).

A potential clue to the mode of action of FL1060 may be that, unlike other β-lactam antibiotics, it is especially active against gram-negative strains of bacteria (16). Such a spectrum of activity could be the result of a target in the outer membrane found only in gram-negative cells, a possibility which is easily reconciled with our observation of an effect of FL1060 on the physical arrangement of the *E. coli* outer membrane. We have preliminary evidence that FL1060 does indeed inhibit the appearance of a major outer membrane protein (R. James, unpublished observation). The fact that the rate of appearance of this same protein increases very rapidly under filament-inducing conditions may be indicative that this protein plays a major role in the regulation of cell elongation in *E. coli*.

The relationship between this outer membrane protein and murein, the assumed target of FL1060, can only be speculative at this time. However, there is a precedent for an actual chemical connection in the cross-linking of murein to the lipoprotein component of the outer membrane of *E. coli* (2, 9). It is an intriguing possibility that linkage between murein and the outer membrane could be the means by which coordinated synthesis of murein and the outer membrane layer of the cell envelope is achieved while maintaining a rod configuration. Initial inhibition of this linkage by FL1060 would then create a defect that prevents subsequent cell surface elongation.

It is anticipated that detailed analysis of the

biochemical action of FL1060 in *E. coli* may provide clues to the unique pathways of murein synthesis necessary for cell elongation and cell shape, as well as shedding some light on some of the less obvious components of the bacterial cell division cycle.

APPENDIX

In glucose media every cell divides once and 40% divide twice; thus the cell age (expressed as a fraction of the total cycle) at which a cell escapes inhibition by FL1060 is $(1 - X) + 1$.

Extrapolating from a graph of idealized age distribution in an exponential culture containing no dispersion in generation times for individual cells (7), a division frequency of 40% corresponds to an X value of 0.5. Thus for a generation time of 40 min, $(1 - X) + 1 = 60$ min.

Similarly, in acetate medium every cell divides once and 10% divide twice. Thus by extrapolation X is 0.85 and $(1 - X) + 1 = 115$ min when the generation time is 100 min.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant CA-AI-11595 from the National Cancer Institute and National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Bogdanovsky, D., E. Bricas, and P. Dezelee. 1969. Sur l'identite de la mucopeptidase et de la carboxypeptidase I d'*Escherichia coli*, enzymes hydrolysant des liasons de configuration D-D et inhibee par le penicilline. C. R. Acad. Sci. Ser. D. **269**:390-393.
2. Braun, V., and H. Wolff. 1970. The murein-lipoprotein linkage in the cell wall of *E. coli*. Eur. J. Biochem. **14**:387-391.
3. Burdett, I. D. J., and R. G. E. Murray. 1974. Septum formation in *Escherichia coli*: characterization of septal structure and the effects of antibiotics on cell division. J. Bacteriol. **119**:303-324.
4. Dix, D. E., and C. E. Helmstetter. 1973. Coupling between chromosome completion and cell division in *Escherichia coli*. J. Bacteriol. **115**:786-795.
5. Greenwood, D., and F. O'Grady. 1973. FL1060: a new beta-lactam antibiotic with novel properties. J. Clin. Pathol. **26**:1-6.
6. Gudas, L. G., and A. B. Pardee. 1974. Deoxyribonucleic acid synthesis during the division cycle of *Escherichia coli*: a comparison of strains B/r, K-12, 15, and 15T under conditions of slow growth. J. Bacteriol. **117**:1216-1223.
7. Helmstetter, C. E. 1967. Rate of DNA synthesis during the division cycle of *Escherichia coli* B/r. J. Mol. Biol. **24**:417-427.
8. Helmstetter, C. E., and S. Cooper. 1968. DNA synthesis during the division cycle of rapidly growing *Escherichia coli* B/r. J. Mol. Biol. **31**:507-518.
9. Hirashina, A., H. C. Wu, P. S. Venkateswara, and M. Inouye. 1973. Two forms of a structural lipoprotein in the envelope of *Escherichia coli*. J. Biol. Chem. **248**:5654-5659.
10. Hoffman, B., W. Messer, and U. Schwarz. 1972. Regulation of polar cap formation in the life cycle of *Escherichia coli*. J. Supramol. Struct. **1**:29-37.
11. Izaki, K., M. Matsuhashi, and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. XIII. Peptidoglycan transpeptidase and D-alanine carboxypeptidase: penicillin sensitive enzymatic reaction in strains of *Escherichia coli*. J. Biol. Chem. **243**:3180-3192.
12. Izaki, K., and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan component of bacterial cell walls. XIV. Purification and properties of two D-alanine carboxypeptidases from *Escherichia coli*. J. Biol. Chem. **243**:3193-3201.
13. Jones, N. C., and W. D. Donachie. 1973. Chromosome replication, transcription and control of cell division in *Escherichia coli*. Nature (London) New Biol. **243**:100-103.
14. Jones, N. D., and W. D. Donachie. 1974. Protein synthesis and the release of the replicated chromosome from the cell membrane. Nature (London) **251**:252-253.
15. Lark, K. G., and H. Renger. 1969. Initiation of DNA replication in *Escherichia coli* 15T$^-$: chronological dissection of three physiological processes required for initiation. J. Mol. Biol. **42**:221-235.
16. Lund, F., and L. Tybring. 1972. 6β-Aminopenicillanic acids—a new group of antibiotics. Nature (London) New Biol. **236**:135-136.
17. Matsuhashi, S., T. Kamiryo, P. M. Blumberg, P. Linnett, E. Willoughby, and J. L. Strominger. 1974. Mechanism of action and development of resistance to a new amidino penicillin. J. Bacteriol. **117**:578-587.
18. Melchior, N. H., J. Blom, L. Tybring, and A. Birch-Anderson. 1973. Light and electron microscopy of the early response of *Escherichia coli* to a 6β-amidinopenicillanic acid (FL1060). Acta. Path. Microbiol. Scand. Sect. B **81**:393-407.
19. Mitchison, J. M., and W. S. Vincent. 1965. Preparation of synchronous cell cultures by sedimentation. Nature (London) **205**:987-989.
20. Nishimo, T., and S. Nakazawa. 1973. Cephalexin induced morphological alterations in the surface structures of *S. aureus* and *E. coli* demonstrated by scanning electron microscopy. Jap. J. Microbiol. **17**:383-392.
21. Park, J. T., and L. Burman. 1973. FL1060: a new penicillin with a unique mode of action. Biochem. Biophys. Res. Commun. **51**:863-868.
22. Pierucci, O., and C. E. Helmstetter. 1969. Chromosome replication, protein synthesis and cell division in *Escherichia coli*. Fed. Proc. **28**:1755-1760.
23. Steed, P., and R. G. E. Murray. 1966. The cell wall and cell division of gram negative bacteria. Can. J. Microbiol. **12**:263-270.
24. Strominger, J. L., E. Willoughby, T. Kamiryo, P. M. Blumberg, and R. R. Yocum. 1974. Penicillin sensitive enzymes and penicillin binding components in bacterial cells. Ann. N.Y. Acad. Sci. **235**:210-224.
25. Suginaka, H., P. M. Blumberg, and J. L. Strominger. 1972. Multiple penicillin binding components in *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. J. Biol. Chem. **247**:5279-5288.
26. Ward, C. B., and D. A. Glaser. 1971. Correlation between rate of cell growth and rate of DNA synthesis in *Escherichia coli* B/r. Proc. Natl. Acad. Sci. U S.A **68**:1061-1064.