ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1993, p. 559–565
0066-4804/93/030559-07$02.00/0

Vol. 37, No. 3

Mechanisms of Action of Cephalosporin 3′-Quinolone Esters, Carbamates, and Tertiary Amines in *Escherichia coli*

N. H. GEORGOPAPADAKOU* AND A. BERTASSO

Roche Research Center, Nutley, New Jersey 07110

Received 18 August 1992/Accepted 6 January 1993

Cephalosporin 3′-quinolone esters, carbamates, and tertiary amines are potent antibiotics whose antibacterial activities reflect the action of both the β-lactam and the quinolone components. The biological properties of representative compounds from each class were compared in *Escherichia coli*. All compounds bound to the essential PBP 3, inhibited DNA gyrase, and caused filamentation in growing cells. To distinguish between cephalosporin- and quinolone-induced filaments, nucleoid segregation was also examined, as quinolones disrupt nucleoid segregation while the β-lactams do not (N. H. Georgopapadakou and A. Bertasso, Antimicrob. Agents Chemother. 35:2645–2648, 1991). The cephalosporin quinolone esters Ro 23-9424 and Ro 24-6392, at concentrations causing filamentation in *E. coli* ATCC 25922, did not affect nucleoid segregation after 1 h of incubation (cephalosporin response) but did affect it after 2 h (quinolone response), indicating the release of free quinolone. Accordingly, only the quinolone response was produced in a strain possessing TEM-3, an expanded-spectrum β-lactamase. The cephalosporin carbamate Ro 24-4383 and the tertiary amine Ro 24-8138 produced a quinolone response in *E. coli* ATCC 25922, though they produced a cephalosporin response in a quinolone-resistant strain. Carbamate and tertiary amine linkages are chemically more stable than the ester linkage, and both cephalosporin 3′-quinolone carbamates and tertiary amines are more potent inhibitors of DNA gyrase than are the corresponding esters. The results suggest that, while intact cephalosporin 3′-quinolone esters act as cephalosporins, carbamates and amines may possess both cephalosporin and quinolone activity in the intact molecule.

In recent years, a number of compounds which consist of a cephalosporin covalently linked at the 3′ position to a quinolone via an ester, carbamate, or tertiary amine bond (1–3, 6) have been synthesized. The compounds have a dual mechanism of action (hence the name dual-action cephalosporins [DACs]), reflecting both β-lactam and quinolone components: they bind to penicillin-binding proteins (PBPs) and inhibit DNA gyrase (1–3). In the case of the cephalosporin 3′-quinolone ester Ro 23-9424, the latter action arises upon quinolone release (11). Ro 23-9424 is active against *Escherichia coli* resistant to both cephalosporin and quinolone components, suggesting that the intact molecule may also have antibacterial activity (25). Nevertheless, the mechanism of action of cephalosporin 3′-quinolones of different linkage types has not been adequately elucidated.

Cephalosporins act by specifically inhibiting bacterial transpeptidases involved in cell-wall biosynthesis, assayed by their ability to bind penicillin G (hence the name PBPs) (9). In *E. coli*, most cephalosporins bind primarily to PBP 3, a peptidoglycan transpeptidase involved in septation, whose inhibition results in filamentation (17, 30). The current consensus is that quinolones act by inhibiting DNA gyrase, a unique bacterial enzyme involved in DNA replication and decatenation of the chromosomes at the end of replication (4, 8, 15, 31–33). The key feature of gyrase-catalyzed reactions is the formation of a transient double-strand break in DNA, termed cleavable complex (19). Quinolones stabilize this cleavable complex, thus inducing DNA breaks and, in *E. coli*, filamentation (19). We previously reported (10) that the mechanism of action of β-lactams and quinolones can be differentiated in intact, growing *E. coli* cells by the use of 4′,6-diamidino-2-phenylindole (DAPI), a DNA-specific fluorescent dye (18, 20) used to visualize nucleoid partitioning in *E. coli* (14). While both classes of compounds cause filamentation after 1 h of incubation, only quinolones affect nucleoid segregation; β-lactams do not.

In this study, the effects of cephalosporin 3′-quinolones of three linkage types were compared in *E. coli*. Binding to PBPs and effects on DNA gyrase and nucleoid segregation were examined. The goal of the study was to determine whether DACs (i) act as intact molecules, (ii) act as cephalosporins or quinolones, and (iii) release quinolones after β-lactam hydrolysis.

MATERIALS AND METHODS

Chemicals. Calf thymus topoisomerase I, plasmid pBR322, and *Eco*RI endonuclease were purchased from Bethesda Research Laboratories, Grand Island, N.Y.; [8-^{14}C]penicillin G (51 μCi/μmol) and [*methyl*-^{3}H]dTTP (50 mCi/μmol) were from Amersham Corp., Arlington Heights, Ill.; all other biochemicals were obtained from Sigma Chemical Co., St. Louis, Mo.; reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis were from Bio-Rad Laboratories, Richmond, Calif.; and Whatman 3MM paper, trichloroacetic acid, glycine, bromophenol blue, and all solvents (analytical grade) were from Fisher Scientific Co., Pittsburgh, Pa.

Antibiotics. Desacetyl cefotaxime (Ro 24-2414), ceftriaxone (Ro 13-9904), fleroxacin (Ro 23-6240, AM833), Ro 23-9424, Ro 24-6392, Ro 24-4383, and Ro 24-8138 were obtained from Roche Laboratories (Nutley, N.J.). Cefotaxime was from Hoechst-Roussel Pharmaceuticals Inc. (Somerville, N.J.), and ciprofloxacin was from Miles Inc., Pharmaceutical Division (West Haven, Conn.).

Bacterial strains and growth conditions. *E. coli* ATCC 25922, a reference strain for susceptibility testing, was

* Corresponding author.

purchased from the American Type Culture Collection (Rockville, Md.). Other *E. coli* strains were kindly donated as follows: UB1005 (DC0) and its permeability mutant DC2 (28), D. Clark of Southern Illinois University (Carbondale); TE18, a K-12 strain overproducing the chromosomal AmpC β-lactamase (24), S. Normark of Washington University (St. Louis, Mo.); CF204, a K-12 C600 strain containing the plasmid-encoded CTX-1 (TEM-3) β-lactamase (29), D. Sirot of Clermont-Ferrand Hospital (Clermont-Ferrand, France); EN225, a ciprofloxacin-resistant K-12 strain (16), D. Hooper of Massachusetts General Hospital (Boston); and MK47, a DNA gyrase-overproducing strain (22), M. Gellert of the National Institutes of Health (Bethesda, Md.). *E. coli* MK47 was grown in modified Luria broth according to reference 22. All other strains were grown in Antibiotic Medium 3 (Difco Laboratories, Detroit, Mich.) at 37°C.

MIC determinations. Susceptibility of strains to DACs and reference antibiotics was determined by the broth microdilution method (300 μl [10^5 CFU/ml] per well). The MIC was the lowest concentration which inhibited visible growth after 18 h of incubation at 37°C.

PBP binding. Binding to *E. coli* PBPs was determined in Triton X-100-solubilized membranes as inhibition of [^{14}C] penicillin G binding by the test compound (13); proteins were separated by polyacrylamide gel electrophoresis, and PBPs were detected by fluorography. The concentration of the test compound required to reduce [^{14}C]penicillin G binding to each of the PBPs by 50% (IC_{50}) was determined by microdensitometry of the X-ray fluorograms with a Molecular Dynamics Model 300A computing densitometer (Molecular Dynamics, Sunnyvale, Calif.).

DNA supercoiling. DNA supercoiling was performed with partially purified DNA gyrase from *E. coli* MK47 (22) and plasmid pBR322, relaxed with calf thymus topoisomerase I, as the substrate. The reaction products were separated by electrophoresis on a 0.8% agarose gel and stained with ethidium bromide as previously described (7, 12). The IC_{50} was the concentration required to inhibit DNA supercoiling by 50%.

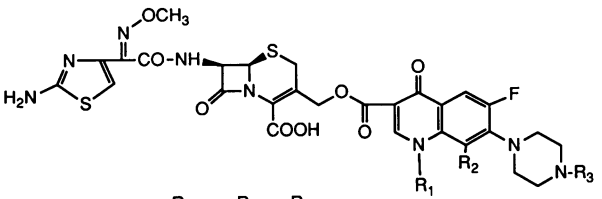
Replicative DNA biosynthesis. Replicative DNA biosynthesis, an indicator of gyrase activity, was measured as the ATP-dependent incorporation of [^{3}H]thymidine into trichloroacetic acid-insoluble material by toluenized cells (23, 26). The IC_{50} was the concentration required to inhibit replicative DNA biosynthesis by 50%.

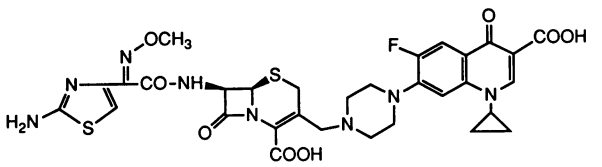
Nucleoid partitioning. Mid-log-phase cells were grown in the presence or absence of a concentration equal to the MIC for the test compound, attached to microscope slides with poly-L-lysine, stained with DAPI, and observed in a fluorescence microscope as previously described (10).

RESULTS

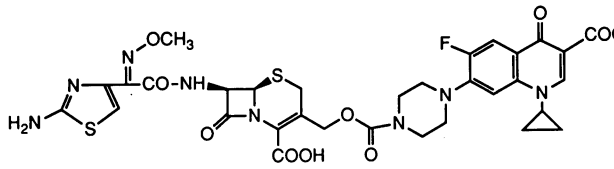
The structures of the cephalosporin 3'-esters Ro 23-9424 and Ro 24-6392, the carbamate Ro 24-4383, and the tertiary amine Ro 24-8138 are shown in Fig. 1. The cephalosporins used for comparison were cefotaxime, ceftriaxone, and desacetyl cefotaxime, the last representing the cephalosporin produced after simple chemical cleavage of the cephalosporin-quinolone linkage. Fleroxacin and ciprofloxacin represented the quinolones released from Ro 23-9424 (fleroxacin) and Ro 24-6392, Ro 24-4383, and Ro 24-8138 (ciprofloxacin).

E. coli ATCC 25922 was susceptible to all compounds tested, while strains CF204 and EN225 were resistant to cephalosporins and quinolones, respectively (Table 1).





Ro 24-8138



Ro 24-4383

FIG. 1. Structures of cephalosporin 3'-quinolones used in this study.

CF204 was susceptible to all four DACs, while EN225 was partially resistant.

Binding to PBPs. Table 2 shows the binding of the four DACs and related cephalosporins to the PBPs of *E. coli*. All compounds bound to PBP 3 at ≤0.1 μg/ml. Ro 23-9424, Ro 24-6392, and Ro 24-4383 also bound to PBPs 1a and 1b at <1 μg/ml. All four compounds produced exclusively filaments at concentrations equal to their MICs.

Effects on DNA gyrase. Table 3 shows the effects of the four DACs and related quinolones on replicative DNA biosynthesis and DNA supercoiling. The former assay measures DNA gyrase activity indirectly, while the latter measures it directly. The esters Ro 23-9424 and Ro 24-6392 were

TABLE 1. Susceptibility of quinolone- and cephalosporin-resistant *E. coli* strains to compounds used in this study

Compound	MIC (μg/ml) for *E. coli* strain:		
	ATCC 25922	CF204	EN225
Ro 23-9424	0.2	0.2	5
Ro 24-6392	0.1	0.1	1
Ro 24-4383	0.5	0.1	5
Ro 24-8138	1	0.2	20
Cefotaxime	0.1	5	0.5
Ceftriaxone	0.1	5	0.5
Desacetyl cefotaxime	0.5	20	1
Fleroxacin	0.1	0.1	20
Ciprofloxacin	≤0.05	0.05	20

TABLE 2. Binding of cephalosporin 3'-quinolones and related cephalosporins to *E. coli* UB1005 PBPs

Compound	IC_{50} (μg/ml) for [^{14}C]penicillin G binding to PBP:						Morphology[a]	MIC (μg/ml)[b]
	1a	1b	2	3	4	5/6		
Ro 23-9424	≤0.1	0.25	30		1.3	>100	F	0.1 (0.03)
Ro 24-6392	0.1	0.9	30		1.5	>100	F	0.03 (0.003)
Ro 24-4383	0.5	0.6	100	0.1	13	>100	F	0.5 (0.03)
Ro 24-8138	12	2.8	30	0.1	13	>100	F	1 (0.06)
Cefotaxime	≤0.1	4	1.4	<0.1	1.6	100	F, L	0.03 (0.02)
Ceftriaxone	0.5	1.3	1.5	<0.1	2.0	100	F, L	0.06 (0.02)
Desacetyl cefotaxime	2.0	10	100	1.0	30	>100	F	0.5 (0.2)

[a] F, filaments; L, lysis.
[b] Numbers in parentheses refer to MICs for DC2, a permeability mutant of strain UB1005.

poorer inhibitors of DNA gyrase than was the carbamate Ro 24-4383 or the tertiary amine Ro 24-8138. As expected, all compounds were less inhibitory in *E. coli* EN225.

Nucleoid segregation. The effects of the four DACs on nucleoid segregation in *E. coli* ATCC 25922 are shown in Fig. 2. With the esters Ro 23-9424 and Ro 24-6392, nucleoids initially appeared to be of normal size, regularly spaced within the filaments (cephalosporin response). However, after 2 h of incubation in the presence of the two compounds, large, irregularly spaced nucleoids appeared (quinolone response). The carbamate Ro 24-4383 and the tertiary amine Ro 24-8138, on the other hand, produced exclusively a quinolone response after 1 or 2 h of incubation.

The cephalosporin contribution to the mode of action of the carbamate Ro 24-4383 and the tertiary amine Ro 24-8138 was examined by looking at the effects of the two compounds on nucleoid segregation in the quinolone-resistant *E. coli* EN225 (Fig. 3). Both Ro 24-4383 and Ro 24-8138 produced exclusively a cephalosporin response after 1 or 2 h of incubation.

Expulsion of the 3'-quinolone substituent by the chromosomal AmpC (24) and the plasmid-mediated TEM-3 (29) β-lactamases was examined by comparing the effects of the two esters in *E. coli* TE18 and CF204. In *E. coli* TE18, Ro 23-9424 and Ro 24-6392 produced effects identical to those produced in *E. coli* ATCC 25922; a cephalosporin response after 1 h and a quinolone response after 2 h of incubation (data not shown). In *E. coli* CF204, however, both compounds produced exclusively a quinolone response after 1 or 2 h of incubation (Fig. 4).

DISCUSSION

Cephalosporin 3'-quinolone esters, carbamates, and tertiary amines are potent antibiotics whose antibacterial activity reflects the action of both β-lactam and quinolone components. Chemically, the cephalosporin-quinolone linkage is least stable in the esters and most stable in the tertiary amines. The esters Ro 23-9424 and Ro 24-6392 hydrolyze in phosphate buffer (pH 7.0) at 37°C (1) with a half-life of 3 h, while the carbamate Ro 24-4383 and the tertiary amine Ro 24-8138 have half-lives of 10 and 120 h, respectively, under the same conditions (3). The cephalosporin 3'-quinolone linkage can also hydrolyze after chemical (5, 27) or enzymatic (3) opening of the β-lactam ring.

TABLE 3. Effects of cephalosporin 3'-quinolones and related quinolones on DNA supercoiling and replicative DNA biosynthesis in *E. coli* strains

Compound	IC_{50} (μg/ml) for:		
	DNA supercoiling in MK47	DNA biosynthesis in strain:	
		ATCC 25922	EN225
Ro 23-9424	20	17	140
Ro 24-6392	5	5	35
Ro 24-4383	2	3.6	38
Ro 24-8138	2	5.2	72
Fleroxacin	0.05	0.3	5.5
Ciprofloxacin	0.02	0.05	1.4

In the present study, compounds having different linkage types but the same cephalosporin (cefotaxime side chain) and quinolone (ciprofloxacin) components were compared in *E. coli*, together with the free components. In the ester-linked compounds Ro 23-9424 and Ro 24-6392, the 3-carboxyl group of quinolones, which is essential for activity (11), is involved in the linkage to the cephalosporins and the linkage is chemically unstable. Accordingly, Ro 23-9424 and Ro 24-6392 acted as cephalosporins and as quinolone prodrugs. As cephalosporins, they bound to PBPs 1a, 1b, and 3 and produced filaments with regularly spaced nucleoids. The absence of cell lysis indicates binding to PBP 3 only in intact, growing cells and thus low concentration in the periplasmic space and poor outer-membrane permeation (11). As quinolone prodrugs, Ro 23-9424 and Ro 24-6392 released fleroxacin and ciprofloxacin, respectively, which then inhibited DNA gyrase and affected nucleoid segregation. Chemical release of quinolones was supplemented by enzymatic release in cells containing the cefotaxime-hydrolyzing TEM-3 β-lactamase but not in cells containing the AmpC enzyme (21).

In the carbamate Ro 24-4383 and the tertiary amine Ro 24-8138, the 3-carboxyl group of quinolones is free; linkage occurs through the piperazinyl group. Further, the half-life of the cephalosporin-quinolone linkage precludes substantial release of free quinolone during the course of the assays, and thus, these compounds must act as intact molecules. Ro 24-4383 and Ro 24-8138 acted primarily as quinolones; they inhibited DNA gyrase, though at concentrations higher than those of the free quinolones, and affected nucleoid segregation. The two compounds also acted as cephalosporins, binding to the essential PBP 3, but in growing cells, cephalosporin activity emerged only in the absence of quinolone activity, as in the quinolone-resistant *E. coli* EN225, where it was accompanied by a 100-fold increase in MICs. The observed cephalosporin activity is also consistent with an intact molecule and precludes quinolone release secondary to β-lactam hydrolysis.

In summary, the mode of action of cephalosporin 3'-quinolone esters, carbamates, and tertiary amines in *E. coli* has been examined. A key finding involved the effects of

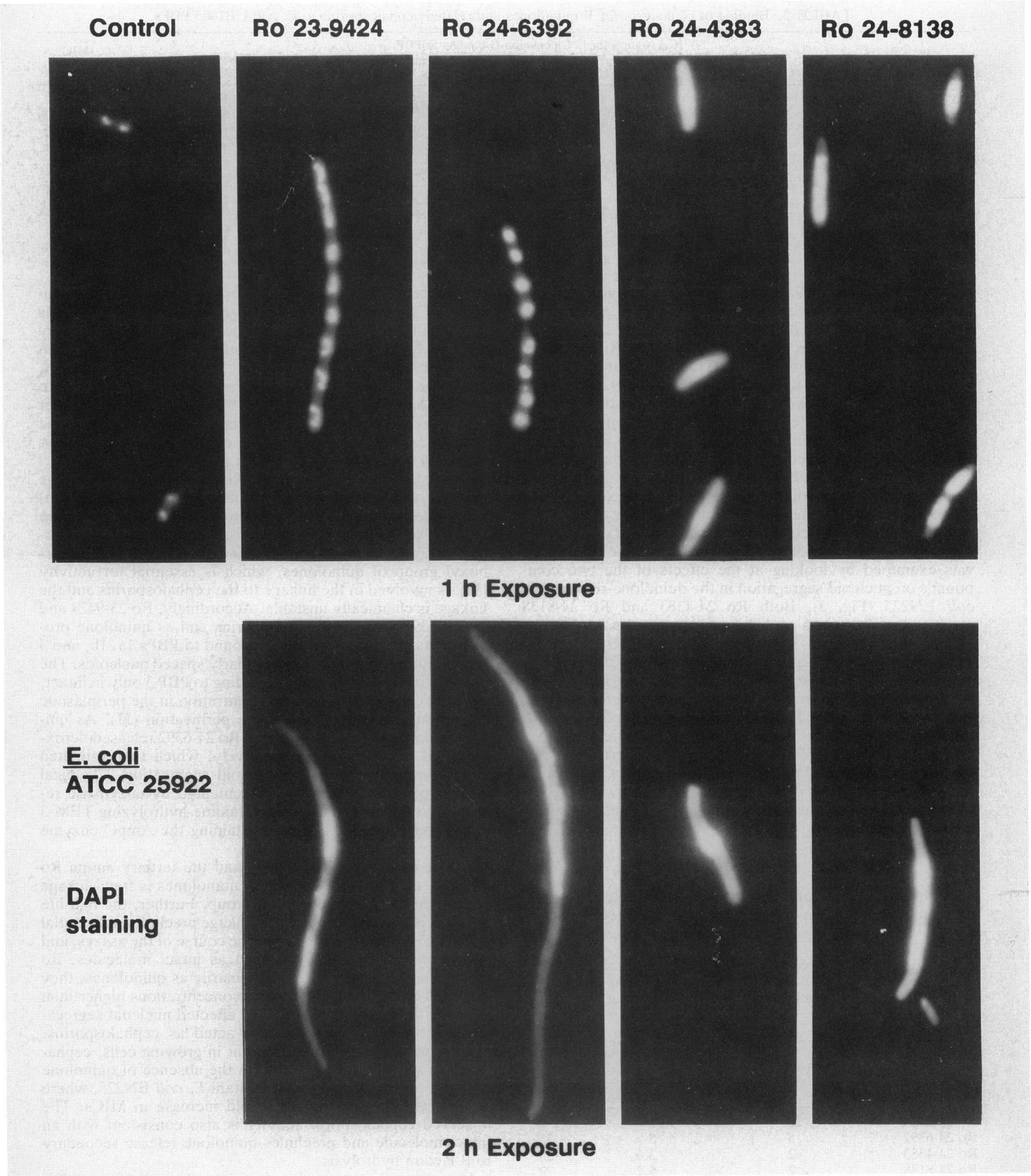


FIG. 2. Photomicrographs of *E. coli* ATCC 25922 grown for 1 or 2 h in concentrations equal to the MICs of cephalosporin 3′-quinolones. Cells were stained with DAPI as described in the text.

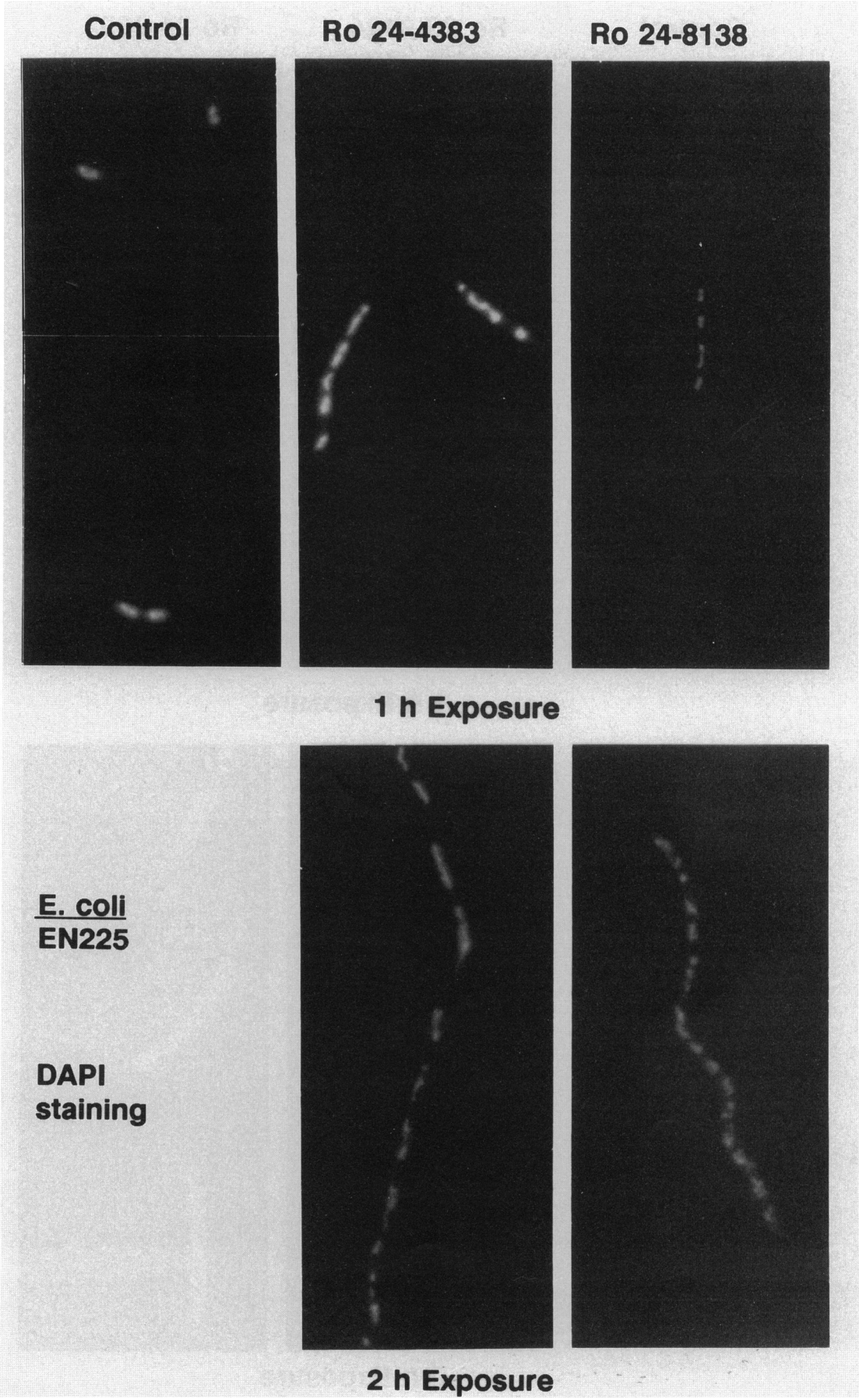


FIG. 3. Photomicrographs of *E. coli* EN225 grown for 1 or 2 h in concentrations equal to the MICs of Ro 24-4383 and Ro 24-8138. Cells were stained with DAPI as described in the text.

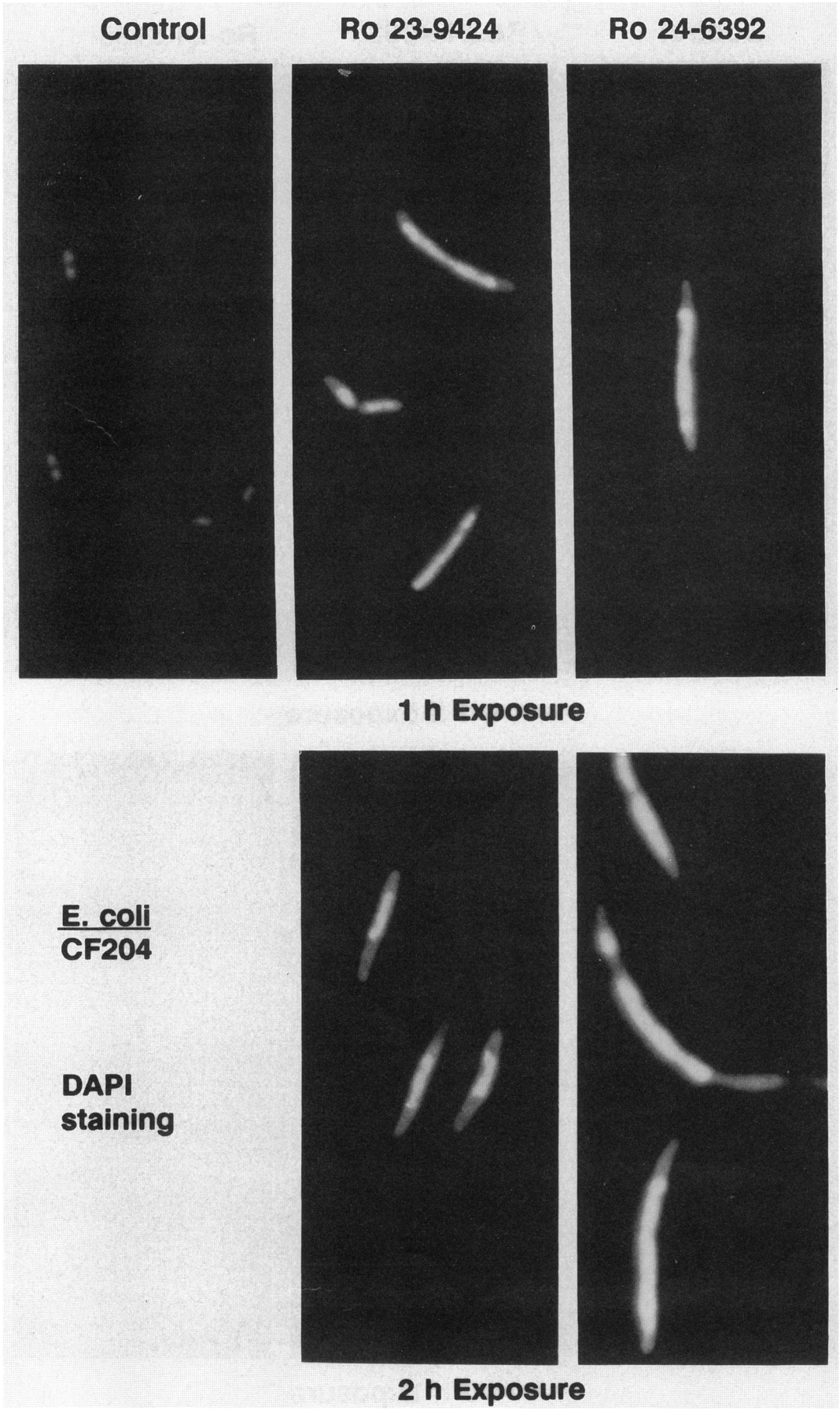


FIG. 4. Photomicrographs of *E. coli* CF204 grown for 1 or 2 h in concentrations equal to the MICs of Ro 23-9424 and Ro 24-6392. Cells were stained with DAPI as described in the text.

these compounds on nucleoid segregation, which permitted differentiation of cephalosporin and quinolone actions in growing cells.

REFERENCES

1. **Albrecht, H. A., G. Beskid, K.-K. Chan, J. G. Christenson, R. Cleeland, K. H. Deitcher, N. H. Georgopapadakou, D. D. Keith, D. L. Pruess, J. Sepinwall, A. C. Specian, Jr., R. L. Then, M. Weigele, K. F. West, and R. Yang.** 1990. Cephalosporin 3'-quinolone esters with a dual mode of action. J. Med. Chem. **33:**77–86.
2. **Albrecht, H. A., G. Beskid, J. G. Christenson, J. W. Durkin, V. Fallat, N. H. Georgopapadakou, D. D. Keith, F. M. Konzelmann, E. R. Lipschitz, D. H. McGarry, J. Siebelist, C. C. Wei, M. Weigele, and R. Yang.** 1991. Dual-action cephalosporins: cephalosporin 3'-quaternary ammonium quinolones. J. Med. Chem. **34:**669–675.
3. **Albrecht, H. A., G. Beskid, J. G. Christenson, N. H. Georgopapadakou, D. D. Keith, F. M. Konzelmann, D. L. Pruess, P. L. Rossman, and C.-C. Wei.** 1991. Dual-action cephalosporins: cephalosporin 3'-quinolone carbamates. J. Med. Chem. **34:** 2857–2864.
4. **Bliska, J. B., and N. R. Cozzarelli.** 1987. Use of site-specific recombination as a probe of DNA structure and metabolism *in vitro*. J. Mol. Biol. **194:**205–218.
5. **Corraz, A. J., S. L. Dax, N. K. Dunlap, N. H. Georgopapadakou, D. D. Keith, D. L. Pruess, P. L. Rossman, R. Then, J. Unowsky, and C.-C. Wei.** 1992. Dual-action penems and carbapenems. J. Med. Chem. **35:**1828–1839.
6. **Demuth, T. P., Jr., R. E. White, R. A. Tietjen, R. J. Storrin, J. R. Skuster, J. A. Andersen, C. C. McOsker, R. Freedman, and F. J. Rourke.** 1991. Synthesis and antibacterial activity of new C-10 quinolonyl-cephem esters. J. Antibiot. **44:**200–209.
7. **Domagala, J. M., L. D. Hanna, C. L. Heifetz, M. P. Hutt, T. F. Mich, J. P. Sanchez, and M. Solomon.** 1986. New structure-activity relationships of the quinolone antibacterials using the target enzyme. The development and application of a DNA gyrase assay. J. Med. Chem. **29:**394–404.
8. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50:**879–910.
9. **Georgopapadakou, N. H.** 1988. Penicillin-binding proteins, p. 409–431. *In* P. K. Peterson and J. Verhoef (ed.), Antimicrobial agents annual 3. Elsevier Science Publishers BV, Amsterdam.
10. **Georgopapadakou, N. H., and A. Bertasso.** 1991. Effects of quinolones on nucleoid segregation in *Escherichia coli*. Antimicrob. Agents Chemother. **35:**2645–2648.
11. **Georgopapadakou, N. H., A. Bertasso, K.-K. Chen, J. S. Chapman, R. Cleeland, L. M. Cummings, B. A. Dix, and D. D. Keith.** 1989. Mode of action of the dual-action cephalosporin Ro 23-9424. Antimicrob. Agents Chemother. **33:**1067–1071.
12. **Georgopapadakou, N. H., B. A. Dix, P. Angehrn, A. Wick, and G. L. Olson.** 1987. Monocyclic and tricyclic analogs of quinolones: mechanism of action. Antimicrob. Agents Chemother. **31:**614–616.
13. **Georgopapadakou, N. H., and F. Y. Liu.** 1980. Penicillin-binding proteins in bacteria. Antimicrob. Agents Chemother. **18:**148–157.
14. **Hiraga, S., H. Niki, T. Ogura, C. Ichinose, H. Mori, B. Ezaki, and A. Jaffe.** 1989. Chromosome partitioning in *Escherichia coli*: novel mutants producing anucleate cells. J. Bacteriol. **171:**1496–1505.
15. **Hooper, D. C., and J. S. Wolfson.** 1991. Fluoroquinolone antimicrobial agents. N. Engl. J. Med. **324:**384–394.
16. **Hooper, D. C., J. S. Wolfson, E. Y. Ng, and M. N. Swartz.** 1987. Mechanisms of action and resistance to ciprofloxacin. Am. J. Med. **82**(Suppl. 4A)**:**12–20.
17. **Ishino, F., and M. Matsuhashi.** 1981. Peptidoglycan synthetic enzyme activities of a highly purified penicillin-binding protein 3 in *Escherichia coli*: a septum-forming reaction sequence. Biochem. Biophys. Res. Commun. **101:**905–911.
18. **Kapuscinski, J., and W. Szer.** 1979. Interactions of 4'6-diamidino-2-phenylindole with synthetic polynucleotides. Nucleic Acids Res. **6:**3519–3534.
19. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29:**11–22.
20. **Manzini, G., M. L. Barcellona, M. Avitabile, and F. Quadrifoglio.** 1983. Interaction of diamidino-2-phenylindole (DAPI) with natural and synthetic nucleic acids. Nucleic Acids Res. **11:**8861–8876.
21. **McCaffrey, C., and N. H. Georgopapadakou.** Unpublished results.
22. **Mizuuchi, K., M. Mizuuchi, M. O'Dea, and M. Gellert.** 1984. Cloning and simplified purification of *Escherichia coli* DNA gyrase A and B proteins. J. Biol. Chem. **259:**9199–9201.
23. **Moses, R. E., and C. C. Richardson.** 1970. Replication and repair of DNA in cells of Escherichia coli treated with toluene. Proc. Natl. Acad. Sci. USA **67:**674–681.
24. **Nikaido, H., and S. Normark.** 1987. Sensitivity of *Escherichia coli* to various β-lactams is determined by the interplay of outer membrane permeability and degradation by periplasmic β-lactamases: a quantitative predictive treatment. Mol. Microbiol. **1:**29–36.
25. **Pace, J., A. Bertasso, and N. H. Georgopapadakou.** 1991. *Escherichia coli* resistant to cephalosporins and quinolones is still susceptible to the cephalosporin-quinolone ester Ro 23-9424. Antimicrob. Agents Chemother. **35:**910–915.
26. **Pedrini, A. M., D. Geroldi, A. Siccardi, and A. Falaschi.** 1972. Studies on the mode of action of nalidixic acid. Eur. J. Biochem. **25:**359–365.
27. **Perrone, E., D. Jabes, M. Alpegiani, B. P. Andreini, C. Della Bruna, S. Del Nero, R. Rossi, G. Visentin, F. Zarini, and G. Franceschi.** 1992. Dual-action penems. J. Antibiot. **45:**589–594.
28. **Richmond, M. H., D. C. Clark, and S. Wotton.** 1976. Indirect method for assessing the penetration of beta-lactamase-nonsusceptible penicillins and cephalosporins in *Escherichia coli* strains. Antimicrob. Agents Chemother. **10:**215–218.
29. **Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille-Michaud, R. Perroux, and R. Cluzel.** 1987. Transferable resistance to third generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. J. Antimicrob. Chemother. **20:**323–334.
30. **Spratt, B. G.** 1975. Distinct penicillin-binding proteins involved in the division, elongation, and shape of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **72:**2999–3005.
31. **Steck, T. R., and K. Drlica.** 1984. Bacterial chromosome segregation: evidence of DNA gyrase involvement in decatenation. Cell **36:**1081–1088.
32. **Wang, J. C.** 1985. DNA topoisomerases. Annu. Rev. Biochem. **54:**665–697.
33. **Wolfson, J. S., and D. C. Hooper.** 1989. Fluoroquinolone antimicrobial agents. Clin. Microbiol. Rev. **2:**378–424.