Increased sensitivity to erythromycin in *Escherichia coli* associated with the presence of the ColV,I-K94 virulence plasmid

C. E. ALFA, C. F. L. REAKES and R. J. ROWBURY*

Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT

Summary. Introduction of the virulence plasmid, ColV,I-K94, into *Escherichia coli* strains led to increased sensitivity to erythromycin. This was the result of increased passage of antibiotic into ColV,I-K94⁺ organisms because the plasmid effect was abolished in bacteria which had been made permeable by chemical treatment. Full sensitivity in ColV⁺ strains appears to depend on the simultaneous presence of transfer and colicin components. Increased erythromycin sensitivity associated with the plasmid was demonstrated in organisms grown at 37°C; the sensitivity of ColV,I-K94⁺ organisms grown at 25°C was similar to that of the parent strain. Added Mg⁺⁺ or Ca⁺⁺ ions reversed erythromycin inhibition in strains with the basal level of sensitivity (i.e., the Col⁻ parent grown at 25°C or 37°C or the ColV,I-K94⁺ derivative grown at 25°C) and in those with the plasmid-associated increase in sensitivity. Addition of phosphate or EDTA to broth increased erythromycin sensitivity in Col⁻ and ColV,I-K94⁺ strains although the latter was affected most. Erythromycin was more inhibitory at pH 8.5 than at pH 7.4. This enhanced activity was more marked against the ColV,I-K94⁺ strain than against the Col⁻ strain. The effects of growth in phosphate-containing medium and at alkaline pH were partially additive. We suggest that ColV,I-K94⁺ strains may be sensitive to erythromycin because ColV-specified proteins are extruded by a process of “self-promoted transfer” and that the effects of these proteins on the lipopolysaccharide component of the outer membrane facilitates antibiotic influx.

Introduction

Plasmids of the ColV group are of considerable medical and veterinary importance because they are believed to increase virulence of pathogenic strains of enterobacteria including *Escherichia coli*. Originally, Williams-Smith (1974) and Williams-Smith and Huggins (1976) established that ColV⁺ strains of *E. coli* are more pathogenic than isogenic Col⁻ strains for poultry and calves and that ColV⁺ organisms are invasive. Subsequently, Binns *et al.* (1979) and Williams (1979) confirmed that ColV⁺ organisms are more virulent for animals than are Col⁻ ones. The following ColV-associated properties may contribute to increased pathogenicity: (1) a ColV-encoded iron chelation mechanism, found in some ColV⁺ strains (Williams, 1979); (2) ColV-associated serum resistance (Binns *et al.*, 1979); and (3) ColV-associated attachment properties

(Clancy and Savage, 1981) that may result from increased hydrophobicity (Tewari *et al.*, 1985).

The plasmid ColV,I-K94 is a large (mol. wt. 93 × 10⁶) F-like element that encodes derepressed transfer properties and the production of colicins V and Ia (and their immunity components). ColV,I-K94 (and several other ColV elements) also encodes the major outer membrane VmpA protein (Moores and Rowbury, 1982; Rowbury *et al.*, 1985). Like other ColV plasmids, ColV,I-K94 is a virulence plasmid (Binns *et al.*, 1979) that confers two of the potential virulence characters listed above (serum resistance and attachment) and two others—autoagglutination and, for some isolates, a mucoid colonial phenotype (Rowbury *et al.*, 1985).

The macrolide antibiotic erythromycin, although active against most gram-positive bacteria, is less effective against many gram-negative bacteria. Gram-negative bacteria that show marked erythromycin sensitivity, such as *Legionella pneumophila* and *Neisseria gonorrhoeae*, have outer membranes permeable to hydrophobic agents (Maness and

Received 19 Mar. 1986; accepted 1 Oct. 1986.

* Correspondence should be sent to R. J. Rowbury.

Sparling, 1973; Wolf-Watz *et al.*, 1975; Miller, 1983). The hypothesis that erythromycin resistance in enterobacteria is due to outer membrane impenetrability is supported by several findings. First, spheroplasts are much more erythromycin-sensitive than are intact gram-negative bacteria (Guze and Kalmanson, 1964). Second, erythromycin sensitivity increases in deep rough mutants that allow hydrophobic molecules to cross their outer membranes (Nikaido and Nakae, 1979). Third, enterobacteria such as *E. coli* have a very sensitive cell site for erythromycin inhibition (Tanaka and Teraoka, 1968) and their growth would be inhibited if erythromycin reached the ribosomes. Erythromycin is more effective at alkaline pH. The uncharged form of the antibiotic, which predominates at alkaline pH, probably permeates through the bacterial envelope better (Sabath *et al.*, 1968) and is more inhibitory to the ribosome (Mao and Wiegand, 1968). Therefore, we studied the effect of the ColV,I-K94 plasmid on the sensitivity to erythromycin of *E. coli* strains at neutral and alkaline pH values.

Materials and methods

Bacterial strains and plasmids

All strains were derivatives of *Escherichia coli* K12. Strains 1829, C600, P678-54 and D21e7 have been described previously (Moores and Rowbury, 1982; Rossouw and Rowbury, 1984). Strain 414 was derived from *E. coli* KH262 (a *thr leu* ColV,I-K94⁺ strain kindly provided by Dr K. G. Hardy) by curing with an SDS plus high temperature growth regimen (Tewari *et al.*, 1985). Subsequently, plasmid ColV,I-K94 was re-transferred into strain 414 by conjugation as described by Rossouw and Rowbury (1984). In one case, plasmid ColV,I-K94 was cured from *E. coli* 1829 ColV,I-K94⁺ (Tewari *et al.*, 1985). Other plasmid-bearing derivatives of the strains were prepared by conjugation or transformation (Rossouw and Rowbury, 1984; Tewari *et al.*, 1985). The plasmids used and their characteristics have been described by Rossouw and Rowbury (1984) and Rowbury *et al.* (1985).

Growth media and methods

The liquid medium used throughout was Nutrient Broth No. 2 (Oxoid; 25 g/L) (NB), solidified, where necessary, with Bacto Agar (Difco) 2% w/v to give nutrient agar (NA). Except where otherwise stated, NB and NA were at pH 7.35–7.4; in some experiments, however, NB or NA were adjusted to acid or alkaline pH with N HCl or 2N NaOH.

Soft agar (Moores and Rowbury, 1982) was used for colicin and disk sensitivity tests (Multodisc; Oxoid).

Erythromycin sensitivity was assessed by overlaying NA plates with 4 ml of soft agar containing the sensitive organisms (10⁸ cfu/ml) placing a Multodisc on the surface and incubating at 37°C. Zone sizes around Multodiscs were more reproducible on overlaid than on spread plates.

Protein synthesis in permeable organisms

Organisms were made permeable by plasmolysis with 2M sucrose (Wickner and Hurwitz, 1972) and treated organisms were tested immediately or after storage at –20°C. Protein synthesis was assayed by the method of Staudenbauer (1975) except that the amino-acid supplement was replaced by a ¹⁴C-protein hydrolysate (supplied by Amersham International and used at 5 µCi/ml; 56 µCi/mg Atom) supplemented with L-cysteine-HCl, L-tryptophan, L-methionine, L-glutamic acid and L-aspartic acid, each 0.5 mM. Protein synthesis was tested at 37°C in the presence of appropriate levels of erythromycin. Samples were processed and counted as described by Staudenbauer (1975).

Results

Erythromycin sensitivity of ColV,I-K94⁺ strains

At an erythromycin concentration of 20 µg/ml in NA, many cells from *E. coli* strains 1829, P678–54 and C600 formed colonies at 37°C; with strain D21e7, 5–10% of organisms formed colonies. The ColV,I-K94 plasmid, however, rendered the strains more sensitive to the drug (table I). This plasmid had a similar effect on *E. coli* 414 (data not shown) although the strain was already very sensitive to erythromycin. The increased erythromycin sensi-

Table I. ColV, I-K94-associated erythromycin sensitivity in *E. coli*

Strains	Percentage of organisms forming colonies on NA + erythromycin 20 µg/ml
1829	100
1829 ColV,I-K94 ⁺	3
P678-54	66
P678-54 ColV,I-K94 ⁺	1
C600	35
C600 ColV, I-K94 ⁺	1
D21e7	7
D21e7 ColV,I-94 ⁺	0*

Organisms grown with shaking in broth at 37°C were plated (after dilution in 0.85% saline) on to NA + erythromycin 20 µg/ml and colonies were counted after incubation at 37°C for 48 h.

* Actual value for colony formation was 0.07%.

tivity was also apparent when antibiotic-impregnated disks were used; e.g., a 50- μ g disk (see *Methods*) produced a 6-mm zone of inhibition with strain C600 and an 8.5-mm zone with strain C600 ColV,I-K94⁺.

Derivatives carrying plasmid ColV,I-K94 were also more sensitive to erythromycin than the parent strains when grown in NB. Table II shows that *E. coli* 1829 ColV,I-K94⁺ was markedly affected at 37°C by erythromycin 2.5, 5 and 10 μ g/ml whereas the effect on the parent strain 1829 was much less.

Table II. Erythromycin inhibition in liquid media; effect of ColV,I-K94

Strain	Percentage growth inhibition* with erythromycin		
	2.5 μ g/ml	5 μ g/ml	10 μ g/ml
1829	14	42	49
1829 ColV,I-K94 ⁺	48	75	88
1829 cured†	15	42	55
1829	NT	42	53
1829 ColV,I-K94 ⁺	NT	64	79
1829 ColV,I-K94 ⁺ transformed	NT	63	81

NT = not tested

* Organisms grown with shaking at 37°C were diluted to c. 3×10^7 cfu/ml and optical density followed in shaken cultures at 37°C. Growth (cell mass increase) at 5 h is given as a percentage of that in the control without erythromycin.

† This strain was derived by curing 1829 ColV,I-K94⁺ as described by Tewari *et al.* (1985).

Erythromycin sensitivity of permeable organisms

The ribosomes of *E. coli* K12 derivatives are very sensitive to inhibition by erythromycin (Tanaka and Teraoka, 1968; Tanaka *et al.*, 1971); it seems likely, therefore, that the ColV,I-94 plasmid rendered the bacterial envelope more permeable to the drug, permitting it to reach the sensitive ribosomes. Studies with permeable cells confirmed this interpretation. Organisms made permeable by plasmolysis in concentrated sucrose behave as cell-free systems and allow free entry of hydrophobic inhibitors (Staudenbauer, 1975). Such preparations from *E. coli* strains 1829 and 1829 ColV,I-K94⁺ incorporated labelled amino acids into TCA-insoluble material actively for at least 1 h. ColV,I-K94 did not increase the erythromycin sensitivity of such permeable organisms (table III).

Table III. Erythromycin effect on protein synthesis in permeable cells

Erythromycin concentration (μ g/ml)	Incorporation of ¹⁴ C (percentage of control*)	
	<i>E. coli</i> 1829	<i>E. coli</i> 1829 ColV,I-K94 ⁺
0	100	100
0.25	64	68
1.0	50	48
5.0	38	34

* Incorporation of ¹⁴C amino acids into TCA-insoluble material in permeable organisms was measured for 40 min at 37°C (see *Methods*) and recorded as a percentage of the incorporation into a control culture without erythromycin.

Erythromycin sensitivity associated with plasmid ColV,I-K94

Erythromycin sensitivity was thought to be due to the presence of the plasmid. An alternative possibility, that plasmid ColV,I-K94 had been preferentially transferred into erythromycin-sensitive mutant cells, was excluded by two findings: (1) a ColV,I-K94⁺ strain produced by transformation with plasmid DNA also showed increased erythromycin sensitivity (table II); and (2) a Col⁻ isolate derived from *E. coli* 1829 ColV,I-K94⁺ by curing with heat and SDS (Rowbury *et al.*, 1985) had a level of erythromycin sensitivity very similar to that of the parent *E. coli* strain (table II).

Organisms are more sensitive to erythromycin at alkaline pH (Sabath *et al.*, 1968). The pH of cultures of ColV⁺ strains may, therefore, have increased more than the pH of the cultures of Col⁻ strains during growth in NB, rendering the ColV⁺ strains more sensitive, but this proved not to be so. In a typical experiment, a culture of *E. coli* strain 1829 growing with erythromycin 5 μ g/ml showed an increase in pH from 7.35 to 7.99 during the 5-h incubation period at 37°C whereas the pH of an equivalent culture of *E. coli* strain 1829 ColV,I-K94⁺ increased from 7.35 to 7.79 during the same period.

ColV component(s) responsible for erythromycin sensitivity

The ColV,I-K94 plasmid confers several properties on *E. coli* that could lead to outer-membrane permeability changes (see *Introduction*). Tables IV and V show that the plasmid-associated erythro-

Table IV. Plasmid components and erythromycin sensitivity

Strain	Colicin and immunity components	Transfer components	Percentage growth inhibition by erythromycin	
			5 μ g/ml	10 μ g/ml
1829	None	None	40	53
1829 ColV,I-K94 ⁺	V & Ia	Derepressed	72	87
1829 ColV-M40(5) ⁺	None	Derepressed	49	63
1829 ColV-M50(1) ⁺	V & Ia	None	20	38
1829 <i>Flac</i> ⁺	None	Derepressed	50	59
1829	None	None	48	56
1829 ColV,I-K94 ⁺	V & Ia	Derepressed	67	85
1829 R483 Col Ia ⁺	Ia	Repressed	37	53
1829 ColV-41 ⁺	V	Repressed	43	61

Organisms were grown at 37°C and percentage growth inhibition assessed as for table II.

mycin sensitivity is totally dependent on the presence of transfer components. A mutant form of ColV,I-K94 —ColV-M50(1)—which does not encode production of transfer components, did not cause increased erythromycin sensitivity (table IV). Indeed, *E. coli* 1829 ColV-M50(1)⁺ appeared to be more resistant than the parent strain 1829 to erythromycin. The ColV,I-K94 effect was also reduced by the *fin*⁺ plasmid R124 and abolished by the ColB-K98 plasmid (table V). Colicin components are also needed for sensitivity because *E. coli* 1829 ColV-M40(5)⁺ was less sensitive than strain 1829 ColV,I-K94⁺ (table IV).

Table V. Transfer components and erythromycin sensitivity

Strain	Transfer components	Percentage growth inhibition by erythromycin	
		5 μ g/ml	10 μ g/ml
1829	None	35	50
1829 ColV,I-K94 ⁺	Derepressed	70	89
1829 R124 ⁺	Repressed	47	62
1829 ColV,I-K94, R124 ⁺	Repressed	52	71
1829	None	35	48
1829 ColV,I-K94 ⁺	Derepressed	64	85
1829 ColB-K98 ⁺	Repressed	32	47
1829 ColV,I-K94, ColB-K98 ⁺	Repressed	30	51

Growth and assessment of percentage growth inhibition were as for table II.

The presence of ColV-41 (a ColV plasmid from a sewage isolate of *E. coli*) did not confer sensitivity (table IV) although it encoded production of colicin V and its immunity component. Similarly, strain 1829 R483 Col Ia⁺, which produced colicin Ia and its immunity component, was erythromycin resistant (table IV).

The effect of growth temperature on erythromycin sensitivity

Growth of strain 1829 ColV,I-K94⁺ was much less sensitive to inhibition by erythromycin when the cells had been previously grown at 25 or 30°C than when they had been grown at 37°C. Strain 1829 showed similar sensitivity after growth at the three temperatures, whereas with strain 1829 ColV,I-K94⁺, the culture that had been grown at 37°C was much more sensitive to erythromycin than were the other two (table VI).

Reversal of erythromycin inhibition by increased Mg⁺⁺ or Ca⁺⁺ concentration

Increasing the concentration of Mg⁺⁺ ions in growth media reduces the antibiotic sensitivity characteristic of certain classes of envelope mutants (Hancock, 1984); accordingly, the effect on erythromycin sensitivity of adding Mg⁺⁺ or Ca⁺⁺ salts to media was tested. Table VII shows that MgSO₄ reduced the sensitivity to erythromycin of strain 1829 and strain 1829 ColV,I-K94⁺. At 10 mM, MgSO₄ produced substantial reversal of inhibition (table VII) and MgCl₂ had the same effect (results not shown). Calcium salts had a similar reversing

Table VI. Effect of prior growth temperature on erythromycin sensitivity

Strain	Prior growth temperature (°C)	Percentage growth inhibition* by erythromycin 10 µg/ml
1829	25	36
	30	44
	37	42
1829 ColV,I-K94 ⁺	25	54
	30	58
	37	81

Organisms were grown in NB with shaking at the stated temperature and then diluted to $c. 3 \times 10^7$ cfu/ml in fresh NB with erythromycin and incubation continued with shaking at 37°C.

* Percentage growth inhibition defined as for table II.

effect (table VII); a 3 mM concentration caused substantial reversal of erythromycin inhibition of strain 1829 ColV,I-K94⁺. Both Ca⁺⁺ and Mg⁺⁺ salts (20 mM) also reversed the limited erythromycin effect on organisms of both strains that had been grown at 25°C.

Table VII. Reversal of erythromycin inhibition by Mg⁺⁺ or Ca⁺⁺ salts

Strain	Divalent cations added (mM)		Percentage growth inhibition with erythromycin	
	Mg ⁺⁺	Ca ⁺⁺	5 µg/ml	10 µg/ml
1829	0	0	NT	54
	10	0	NT	33
	20	0	NT	20
	50	0	NT	13
1829 ColV,I-K94 ⁺	0	0	64	NT
	10	0	41	NT
	20	0	32	NT
	50	0	18	NT
1829	0	0	NT	42
	0	10	NT	21
	0	20	NT	9
1829 ColV, I-K94 ⁺	0	0	65	NT
	0	3	32	NT
	0	10	20	NT
	0	20	12	NT

NT = not tested.

Organisms were grown with shaking in NB with MgSO₄ or CaCl₂ at 37°C and percentage growth inhibition assessed as for table II.

The NB used in the above experiments contained low but detectable concentrations of Mg⁺⁺ and Ca⁺⁺ ions ($c. 0.1$ mM Mg⁺⁺ salts and 0.08 mM Ca⁺⁺ salts) that might have interfered with erythromycin inhibition. The broth was treated, therefore, to remove or chelate divalent cations. Organisms were more sensitive to inhibition by the antibiotic in NB to which phosphate had been added to precipitate Mg⁺⁺ and Ca⁺⁺ ions. Strain 1829 and its ColV,I-K94⁺ derivative were more sensitive in this medium but the effect seemed to be greater for the ColV⁺ strain (table VIII). EDTA had a similar effect; at 0.5 mM it increased growth inhibition of strain 1829 in NB pH 7.4 by erythromycin 10 µg/ml from 42% to 91% and of strain 1829 ColV,I-K94⁺ by erythromycin 5 µg/ml from 38% to 96%.

Table VIII. Erythromycin sensitivity; effects of alkaline pH and of added phosphate

Strain	Initial pH of NB	Phosphate (65 mM) added to NB	Percentage growth inhibition with erythromycin	
			2.5 µg/ml	5.0 µg/ml
1829	7.35	No	NT	33
	7.35	Yes	NT	52
	8.4	No	NT	63
	8.4	Yes	NT	76
1829 ColV,I-K94 ⁺	7.35	No	34	NT
	7.35	Yes	67	NT
	8.4	No	75	NT
	8.4	Yes	95	NT

NT = not tested.

Organisms were grown with shaking at 37°C in NB, with or without sodium phosphate, at pH 7.35 or 8.4, and with erythromycin.

Enhanced effect of erythromycin on strain 1829 ColV,I-K94⁺ at alkaline pH

Erythromycin acts more effectively on gram-negative bacteria at alkaline pH (Sabath *et al.*, 1968). All the experiments described above were with organisms growing in NB at pH 7.35–7.4. Strains 1829 and 1829 ColV,I-K94⁺ tested with erythromycin at alkaline pH showed greater sensitivity (table VIII). Strain 1829 ColV,I-K94⁺ remained more sensitive than strain 1829 at pH 8.4 and this alkaline pH appeared to enhance sensitivity of the ColV⁺ strain more than that of the Col[−]

parent (table VIII). Increased sensitivity to erythromycin of strain C600 and its ColV,I-K94⁺ derivative at alkaline pH in NB was also observed (results not shown). The effects of alkaline pH and of added phosphate were partially additive with strains 1829 and 1829 ColV,-K94⁺ (table VIII).

Erythromycin sensitivity at alkaline pH was, like that at pH 7.35, reversed by Mg⁺⁺ ions; for organisms growing in NB at pH 8.4, 20 mM MgSO₄ reduced the growth inhibition produced by erythromycin 10 µg/ml from 73% to 50% with strain 1829 and from 93% to 45% with strain 1829 ColV,I-K94⁺. CaCl₂ (20 mM) had a similar effect.

Discussion

The erythromycin sensitivity of *E. coli* strain 1829 ColV,I-K94⁺ resulted from the presence of the plasmid (table II) which rendered the organisms permeable to the antibiotic (table III). This erythromycin sensitivity was totally dependent on plasmid ColV,I-K94 transfer components (tables IV and V) and somewhat enhanced by colicin components (table IV). The finding that growth at 25 or 30°C abolished the plasmid effect is in keeping with the above, because synthesis of transfer and colicin components is reduced at 25 and 30°C (Tewari *et al.*, 1985).

Hancock (1984) proposed that certain agents (e.g., polymyxins and aminoglycosides) enter gram-negative bacteria by a process which may be termed

"self-promoted transfer", whereby cationic groups on the agents cause the dissociation and separation of groups of lipopolysaccharide (LPS) molecules. Hydrophobic agents may also enter such cells in association with these cationic agents. Proteins extruded from cells might also traverse the LPS leaflet by such "self-promoted transfer" and, if the colicins, immunity components or transfer components encoded by the ColV plasmids used such an extrusion mechanism, this might allow concomitant entry of erythromycin.

If erythromycin entered *E. coli* ColV,I-K94⁺ strains by such a mechanism, agents which stabilise LPS-LPS bonds might reduce antibiotic influx and sensitivity; this was observed with Mg⁺⁺ and Ca⁺⁺ ions. In contrast, treatments that weaken LPS-LPS bonds should have sensitised cells to the antibiotic; the effects of phosphate and EDTA were in keeping with this hypothesis.

Erythromycin has been used, in conjunction with alkalisation, to treat urinary infections (Sabath *et al.*, 1968). The availability of other active agents led to its replacement but the present findings suggest that urinary infections with strains of *E. coli* carrying virulence plasmids may be susceptible to erythromycin.

We are grateful to the Department of Medical Microbiology, University College Hospital; Imperial Chemical Industries plc; and the Central Research Fund of the University of London for financial assistance, and to the Medical Research Council for a partnership award to C.F.L.R.

REFERENCES

- Binns, M M, Davies, D L, Hardy K G 1979. Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance. *Nature* **279**: 778-781.
- Clancy J, Savage D C 1981 Another colicin V phenotype: *in vitro* adhesion of *Escherichia coli* to mouse intestinal epithelium. *Infection and Immunity* **32**: 343-352.
- Guze L B, Kalmanson G M 1964 Action of erythromycin on "protoplasts" *in vivo*. *Science* **146**: 1299-1300.
- Hancock R E W 1984 Alterations in outer membrane permeability. *Annual Review of Microbiology* **38**: 237-264.
- Mao J C-H, Wiegand R G 1968 Mode of action of macrolides. *Biochimica et Biophysica Acta* **157**: 404-413.
- Maness M J, Sparling P F 1973 Multiple antibiotic resistance due to a single mutation in *Neisseria gonorrhoeae*. *Journal of Infectious Diseases* **128**: 321-330.
- Miller R D 1983 *Legionella pneumophila* cell envelope: permeability to hydrophobic molecules. *Current Microbiology* **9**: 349-354.
- Moore J C, Rowbury R J 1982 A new major outer membrane protein in derivatives of *Escherichia coli* carrying the virulence plasmid ColV,-K94. *Zeitschrift für Allgemeine Mikrobiologie* **22**: 465-475.
- Nikaido H, Nakae T 1979 The outer membrane of Gram-negative bacteria. *Advances in Microbial Physiology* **20**: 163-250.
- Rossouw F T, Rowbury R J 1984 Effects of the resistance plasmid R124 on the level of the OmpF outer membrane protein and on the response of *Escherichia coli* to environmental agents. *Journal of Applied Bacteriology* **56**: 63-79.
- Rowbury R J, Deeney C M, Reakes C, Rossouw F T, Smith D G, Tewari R 1985 Envelope protein changes, autoagglutination, sensitivity to hydrophobic agents and a conditional division lesion in *Escherichia coli* strains carrying ColV virulence plasmids. *Annales de l'Institut Pasteur Microbiologie* **136A**: 147-157.
- Sabath L D, Lorian V, Gerstein D, Loder P B, Finland M 1968 Enhancing effect of alkalization of the medium on the activity of erythromycin against gram-negative bacteria. *Applied Microbiology* **16**: 1288-1292.
- Staudenbauer W L 1975 Novobiocin—a specific inhibitor of semiconservative DNA replication in permeabilized *Escherichia coli* cells. *Journal of Molecular Biology* **96**: 201-205.
- Tanaka K, Teraoka H 1968 Effect of erythromycin on polylysine synthesis directed by polyadenylic acid in an *Escherichia coli* cell-free system. *Journal of Biochemistry (Tokyo)* **64**: 635-648.
- Tanaka K, Teraoka H, Tamaki M, Takata R, Osawa S 1971 Phenotypes represented by a mutational change in a 50S

- ribosomal protein component, 50–8, in *Escherichia coli*. *Molecular and General Genetics* **114**: 9–13.
- Tewari R, Smith D G, Rowbury R J 1985 Effect of ColV plasmids on the hydrophobicity of *Escherichia coli*. *FEMS Microbiology Letters* **29**: 245–249.
- Wickner R B, Hurwitz J 1972 DNA replication in *Escherichia coli* made permeable by treatment with high sucrose, *Biochemical and Biophysical Research Communications* **47**: 202–211.
- Williams, P H 1979 Novel iron uptake system specified by ColV plasmids: an important component in the virulence of invasive strains of *Escherichia coli*. *Infection and Immunity* **26**: 925–932.
- Williams Smith H 1974 A search for transmissible pathogenic characters in invasive strains of *Escherichia coli*: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicine V. *Journal of General Microbiology* **83**: 95–111.
- Williams Smith H, Huggins M B 1976 Further observations on the association of the colicine V plasmid of *Escherichia coli* with pathogenicity and with survival in the alimentary tract. *Journal of General Microbiology* **92**: 335–350.
- Wolf-Watz H, Elmros T, Normark S, Bloom G D 1975 Cell envelope of *Neisseria gonorrhoeae*: outer membrane and peptidoglycan composition of penicillin-sensitive and -resistant strains. *Infection and Immunity* **11**: 1332–1341.