Kinetics and Mechanisms of Action of Drugs on Microorganisms XV: Effect of Erythromycin on the Action of Lincomycin (Phase II) and the 7(S)-Chloro Analogs of Lincomycin against *Escherichia coli*

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Abstract □ Lincomycin-affected *Escherichia coli* cultures show two phases of steady-state generation. The initial (Phase I) steady-state generation, expressed as $\ln N = k_{app.I}t$ + constant, is followed after several generations by an ultimate (Phase II) steady-state generation, $\ln N = k_{app.II}t$ + constant, at the same dose level, where $k_{app.I} > k_{app.II}$. Cultures affected with erythromycin or the 7(S)-chloro analogs of lincomycin show only one steady-state generation. A fixed potency ratio of erythromycin lactobionate–lincomycin hydrochloride (Phase I) as 1:5 (on weight basis) is operative over a wide drug concentration range. Combinations of erythromycin and lincomycin are quantifiable on the basis of this potency factor as kinetically equivalent in action to the equipotent lincomycin (in Phase I action) or erythromycin alone. However, the potency factor for erythromycin and lincomycin (Phase II) varies with the concentration level. Therefore, combinations of erythromycin and lincomycin, which *a priori* have the same potency as equivalent lincomycin alone (in Phase I action), are less potent in the Phase II action. This is attributed to an artifact of diluted lincomycin effect in Phase II action of the mixture and not to antagonism of effects. Combinations of erythromycin and the 7(S)-chloro analogs of lincomycin demonstrate *a priori* antagonism of effects because of possible allosteric interactions which decrease the effects of one drug in the presence of the other at their site of action. It is emphasized that the dose–response relationship over a wide concentration range, as well as the kinetics and mechanisms of the separate drug action, must be considered in the quantification and prediction of combined action of antibiotics.

Keyphrases □ Erythromycin—effect on lincomycin and lincomycin 7(S)-chloro analogs (Phase II) against *Escherichia coli* □ Lincomycin and lincomycin 7(S)-chloro analogs (Phase II)—against *Escherichia coli*, effect of erythromycin □ Antibiotics, combined—effect of erythromycin and lincomycin (Phase II) against *Escherichia coli*

We reported previously (1) on the similar functional dependencies of the generation rate constant, $k_{app.}$, on drug concentrations for erythromycin-affected cultures and for lincomycin-affected cultures in Phase I steady-state generation. This indicated that the same mechanism (2) and locus of action may exist for erythromycin and lincomycin (Phase I). The combined action of erythromycin and lincomycin (Phase I) on intact *Escherichia coli* cells could be quantified on a kinetically equivalent basis, and it demonstrated a lack of antagonism (2) between the erythromycin and lincomycin (Phase I). This, however, could not be reconciled with statements of other workers who observed antagonism for the combined action of erythromycin with lincomycin on the inhibition of protein synthesis in cell-free extracts (3–7) or from the "interference index" of microbial generation (8).

Lincomycin-affected cultures show two phases of steady-state generation: Phase I and Phase II (1, 9). The functional dependency of $k_{app.}$ on drug concentrations for lincomycin-affected cultures in Phase II generation is different from that of the Phase I and that of erythromycin-affected generation (1). This indicates that the lincomycin (Phase II) action may be due to a different mechanism and/or locus of action (2) than Phase I action. The lincomycin (Phase II) action (1, 9) limits the generation rate of the lincomycin-affected culture only after a finite time of drug–bacteria reaction. Consequently, an enhanced inhibition of the generation of the drug-affected culture is subsequently effected for the same drug concentration. On the basis of the potency ratio for equivalent erythromycin and lincomycin (Phase I) action, the effects of a combination of erythromycin and lincomycin in Phase I have been quantified (1) as equivalent to an equipotent amount of lincomycin alone in that phase of action. If the lincomycin Phase II action is due to a different locus and/or mechanism of action than lincomycin Phase I and erythromycin action, the combined action of the two antibiotics may possibly differ from expectation in the final steady-state effects on microbial generation.

This paper presents the results of such studies on the action of combinations of erythromycin and lincomycin in Phase II. Since our comparative studies (10) on the action of lincomycin (Phase I) and its 7(S)-chloro analogs (clindamycin and U24729A) revealed antagonism, we also examined the possible interaction between erythromycin and clindamycin (or U24729A) on the expectation that they will be antagonistic in their combined action against *E. coli*.

EXPERIMENTAL

Materials and Methods—The organism (*E. coli*, ATCC 12407), formerly referred to as strain B/r (1, 9–11), was cultivated in Bacto Antibiotic Medium 3[1] and used for determining antibiotic–bacteria reaction in the same manner as previously described (1, 10). The antibiotics (erythromycin lactobionate, lincomycin hydrochloride, clindamycin hydrochloride, and U24729A as the hydrochloride), were as previously described (1, 10) and were assayed samples[2]. The total count method, using Coulter counter[3] (11), was used in determining numbers of *E. coli* ml.$^{-1}$ in drug-free and in subcompletely inhibitory drug-affected cultures. The coincidence of plots of total (Coulter) count and viable (colony) count *versus* time for *E. coli* cells affected with erythromycin (1) or lincomycin (9) in the subcompletely inhibitory concentration ranges provided evidence that there was no significant kill superimposed on normal inhibition of generation in the presence of the drugs. It further indicated the suitability of the Coulter count method for determining the rates of generation of the drug-affected cultures.

Effects of Order of Addition on Erythromycin and Lincomycin [or Its 7(S)-Chloro Analogs] in Combination on Microbial Generation—Aliquots (0.5 ml.) of equipotent solutions of the antibiotics

[1] Difco Laboratories, Detroit, Mich.
[2] Obtained from The Upjohn Co., Kalamazoo, Mich.
[3] Coulter Electronics Co., Hialeah, Fla.

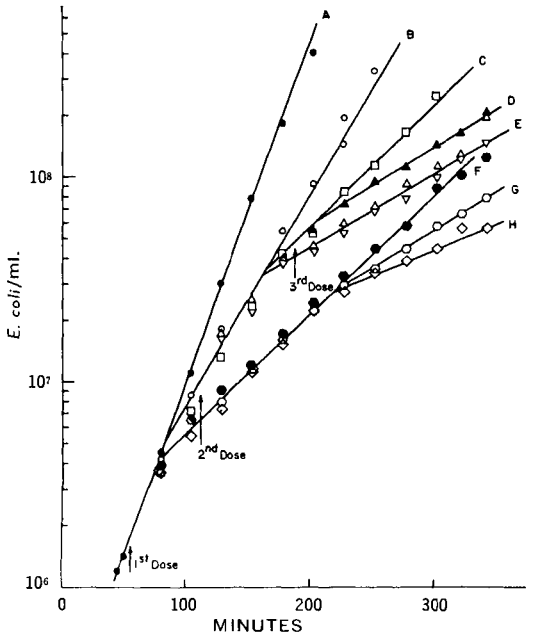


Figure 1—*Effects of order of addition of equipotent erythromycin and lincomycin on generation rates of* E. coli. *Curve A is for generation of* E. coli *in the absence of drug. Curve B is for generation of* E. coli *in the presence of 20 mcg. ml.$^{-1}$ erythromycin l, and curve C is for 100 mcg. ml.$^{-1}$ lincomycin hydrochloride. Curve E is when equipotent lincomycin is added to the erythromycin-affected culture of curve B, or when equipotent erythromycin is added to the lincomycin-affected culture of curve C in Phase I generation,* i.e., *60 min. after the initial drug addition. Curve D is when equipotent erythromycin is added to the lincomycin-affected culture of curve C in Phase II generation,* i.e., *140 min. after the initial drug addition. Curve G is when a mixture of 20 mcg. ml.$^{-1}$ erythromycin l and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride is added to the culture of curve B. Curves F and H are when 40 mcg. ml.$^{-1}$ erythromycin l or 200 mcg. ml.$^{-1}$ lincomycin hydrochloride, respectively, which have the same potency in Phase I action as the mixture of curve G, is added to the culture of curve A.*

were added to replicate 49.5-ml. samples of cultures containing 10^6 ml.$^{-1}$ *E. coli* in steady-state generation (curve A in Fig. 1). The resultant generation curves for the action of equipotent concentrations of 20 mcg. ml.$^{-1}$ erythromycin l and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride (Phase I) are given as curves B and C, respectively. Equipotency of action is shown by coincident or parallel generation curves of the drug-affected cultures having the same generation rate constant ($k_{app.}$). Replicate cultures of curve A were also treated with aliquots (0.5 ml.) of a mixture of equal parts of the equipotent concentrations of erythromycin and lincomycin (curve G), which was prepared to be equipotent to the erythromycin used alone in curve F or lincomycin (Phase I) used alone in curve H, *i.e.*, 40 mcg. ml.$^{-1}$ erythromycin l or 200 mcg. ml.$^{-1}$ lincomycin hydrochloride, respectively.

Sixty minutes after the erythromycin-affected culture of curve B had settled to a new steady-state generation, an equipotent amount of lincomycin was added. The resultant generation curve is given as curve E. A similar treatment of a lincomycin-affected culture of curve C in Phase I steady-state generation with an equipotent amount of erythromycin resulted in a coincident generation curve E.

One hundred and forty minutes after a replicate lincomycin-affected culture of curve C had entered into a new steady-state (Phase II) generation, an equipotent amount of erythromycin was added. The resultant generation curve appears as curve D.

The experiment was repeated for equipotent concentrations of 20 mcg. ml.$^{-1}$ erythromycin l and 16.67 mcg. ml.$^{-1}$ clindamycin hydrochloride (Fig. 2) or for equipotent concentrations of 20 mcg. ml.$^{-1}$ erythromycin l and 4 mcg. ml.$^{-1}$ U24729A as the hydrochloride (Fig. 3).

The resultant generation curves for cultures of curve A treated with equipotent concentrations of erythromycin or clindamycin are given as curve B in Fig. 2; the resultant generation curves for the cultures treated with equipotent concentrations of erythromycin or U24729A are given as curve B in Fig. 3. Curve D is for the effect of a mixture of equal parts of equipotent concentrations of erythromycin and clindamycin (Fig. 2) or for a mixture of equal parts of equipotent concentrations of erythromycin and U24729A (Fig. 3) which was prepared to be *a priori* as equipotent as the corresponding erythromycin or clindamycin or U24729A alone, whose effects are given as curve E.

Curve C (Fig. 2) is the resultant generation curve when an equipotent amount of clindamycin was added after 60 min. to the erythromycin-affected culture of curve B or when an equipotent amount of erythromycin was added after 60 min. to the clindamycin-affected culture of curve B. Curve C in Fig. 3 represents similar effects when an equipotent amount of U24729A was added to erythromycin-affected cultures or when erythromycin was added to U24729A-affected cultures in steady-state generation. However, since clindamycin and U24729A do not have the Phase II action of lincomycin (10), no further addition of erythromycin was made after 140 min. to replicates of clindamycin or to U24729A-affected cultures of curves B in Figs. 2 and 3. Coulter counts were obtained on samples of cultures withdrawn every 25 min.

Effects of Combinations of Equipotent Amounts of Erythromycin and Lincomycin (Phase I) on Lincomycin (Phase II) Action at Various Levels of Activity—Aliquots (0.5 ml.) of equipotent solutions of erythromycin and lincomycin (Phase I) were added to replicate 49.5-ml. samples of cultures containing 10^6 ml.$^{-1}$ *E. coli* in steady-state generation (curve A in Fig. 4*a*). The separate effects for 10 mcg. ml.$^{-1}$ erythromycin l and 50 mcg. ml.$^{-1}$ lincomycin hydrochloride on the generation of cultures are shown, respectively, as curves B and C. Curve E is for the effect of a mixture of equal parts of the equipotent concentrations of erythromycin and lincomycin which was prepared so as to be *a priori* as equipotent as the corresponding erythromycin (curve D) or lincomycin (curve F) alone. The experiment was repeated in like manner for equipotent concentrations of 15 mcg. ml.$^{-1}$ erythromycin l and 75 mcg. ml.$^{-1}$ lincomycin hydrochloride (Fig. 4*b*), 20 mcg. ml.$^{-1}$ erythromycin l and

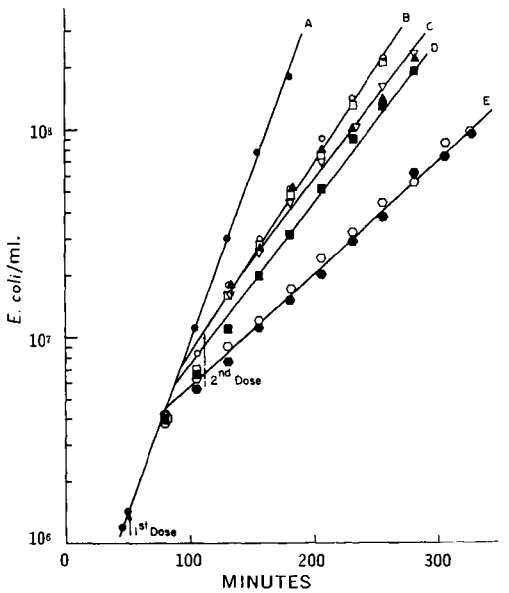


Figure 2—*Effects of order of addition of equipotent erythromycin and clindamycin on generation rates of* E. coli. *Curve A is for generation of* E. coli *in the absence of drug. Curve B is for generation of* E. coli *in the presence of 20 mcg. ml.$^{-1}$ erythromycin l or of 16.67 mcg. ml.$^{-1}$ clindamycin hydrochloride. Curve C is when equipotent clindamycin is added to the erythromycin-affected culture of curve B, or when equipotent erythromycin is added to the clindamycin-affected culture of curve B,* i.e., *60 min. after the initial drug addition. Curve D is when a mixture of 20 mcg. ml.$^{-1}$ erythromycin l and 16.67 mcg. ml.$^{-1}$ clindamycin hydrochloride is added to the culture of curve B. Curve E is when 40 mcg. ml.$^{-1}$ erythromycin l or 33.34 mcg. ml.$^{-1}$ clindamycin hydrochloride, which should have the same potency as the mixture of curve D, is added to the culture of curve B.*

100 mcg. ml.$^{-1}$ lincomycin hydrochloride (Fig. 4*c*), 30 mcg. ml.$^{-1}$ erythromycin I and 150 mcg. ml.$^{-1}$ lincomycin hydrochloride (Fig. 4*d*), or 40 mcg. ml.$^{-1}$ erythromycin I and 200 mcg. ml.$^{-1}$ lincomycin hydrochloride (Fig. 4*e*).

Coulter counts were obtained from samples of cultures withdrawn every 25 min.

Effects of Graded Concentrations of Erythromycin on Lincomycin-Affected Cultures in Phase I and Phase II Generation—Aliquots (0.5 ml.) of solutions of erythromycin and lincomycin and combinations thereof were added to replicate 49.5-ml. samples of cultures containing 10^6 ml.$^{-1}$ *E. coli* in steady-state generation (curve A in Fig. 5). The separate effects for equipotent concentrations of 20 mcg. ml.$^{-1}$ erythromycin I and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride (Phase I) on the generation of cultures are shown, respectively, as curves B and C in Fig. 5. Curve H is for the effect of a mixture of equal parts of equipotent concentrations of erythromycin and lincomycin (Phase I) which was prepared to be *a priori* as equipotent as the corresponding erythromycin (curve G) or lincomycin (curve I) alone, *i.e.*, 40 mcg. ml.$^{-1}$ erythromycin I or 200 mcg. ml.$^{-1}$ lincomycin hydrochloride.

When the lincomycin-affected culture of curve C had settled to a new steady-state Phase I generation, *i.e.*, 65 min. after addition of lincomycin, aliquots (0.5 ml.) of graded concentrations of erythromycin were added to replicates of the lincomycin-affected cultures so that the final erythromycin I concentrations maintained were 20, 30, and 40 mcg. ml.$^{-1}$, respectively. The resultant generation curves are given as J, K, and L, respectively.

Again, when the lincomycin-affected cultures of curve C had entered into steady-state generation Phase II, *i.e.*, 165 min. after the addition of lincomycin, three other replicates were treated with similar concentrations of the erythromycin (curves D, E, and F).

Coulter counts were obtained on samples of the cultures withdrawn every 20–25 min.

RESULTS

Growth Curves for Equipotent Concentrations of Antibiotics—The addition of a concentration of antibiotic to growing balanced cultures of *E. coli* demonstrates a linear semilogarithmic plot (Figs. 1–5) shortly after the addition of the antibiotic in accordance with:

$$\ln N_t = k_{app.}t + \text{intercept} \qquad \text{(Eq. 1)}$$

where N_t is the number of organisms at time t, and $k_{app.}$ is the apparent generation rate constant obtained from the slope of the appropriate plots in the steady-state generation. The intercept is the natural logarithm of the inoculum size ln N_0 at a time t_0, which is the apparent time at which the drug manifests steady-state action on microbial generation. If t_0' is the time at which drug was added, then:

$$t_0 - t_0' = t_e + t_i \qquad \text{(Eq. 2)}$$

where t_e is time for equilibration of drug between the broth medium and the biophase, and t_i is the induction time for the receptor sites to show response to action of the drug.

Therefore:

$$\ln N_0 = k_0(t_0 - t_0') + \ln N_0' \qquad \text{(Eq. 3)}$$

where N_0' is the number of organisms at the time t_0' of drug addition, and k_0 is the apparent generation rate constant for the drug-free culture.

When Eqs. 1–3 are combined:

$$\ln N_t = k_{app.}t + \{k_0(t_e + t_i) + \ln N_0'\} \qquad \text{(Eq. 4)}$$

This implies that the generation curves for cultures affected with equipotent amounts of two different antibiotics may be coincident or parallel, depending on whether $t_e + t_i$ is the same or different for the two antibiotics, even if the $k_{app.}$ remains the same.

Coincident or parallel generation curves are obtained for cultures affected with 20 mcg. ml.$^{-1}$ erythromycin I (curve F) and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride (initial portion of curve H in Fig. 1), 20 mcg. ml.$^{-1}$ erythromycin I and 16.67 mcg. ml.$^{-1}$ clindamycin hydrochloride (curve E in Fig. 2), 20 mcg. ml.$^{-1}$ erythromycin I and 4 mcg. ml.$^{-1}$ U24729A as the hydrochloride (curve E in Fig. 3), and corresponding ratios at other levels of activity. The potency ratio of

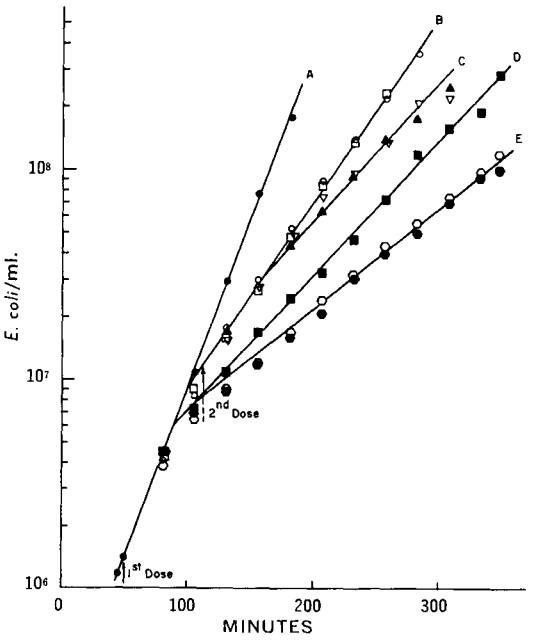


Figure 3—*Effects of order of addition of equipotent erythromycin and U24729A on generation rates of* E. coli. *Curve A is for generation of* E. coli *in the absence of drug. Curve B is for generation of* E.coli *in the presence of 20 mcg. ml.$^{-1}$ erythromycin I or of 4 mcg. ml.$^{-1}$ U24729A as the hydrochloride. Curve C is when equipotent U24729A is added to the erythromycin-affected culture of curve B or when equipotent erythromycin is added to the U24729A-affected culture of curve B,* i.e., *60 min. after the initial drug addition. Curve D is when a mixture of 20 mcg. ml.$^{-1}$ erythromycin I and 4 mcg. ml.$^{-1}$ U24729A as the hydrochloride is added to the culture of curve B. Curve E is when 40 mcg. ml.$^{-1}$ erythromycin I or 8 mcg. ml.$^{-1}$ U24729A, which should have the same potency as the mixture of curve D, is added to the culture of curve B.*

lincomycin hydrochloride–erythromycin I–clindamycin hydrochloride–U24729A as the hydrochloride is 1:5:6:25 on a weight basis. This confirms previous potency estimates for the action of the drugs (1, 10) derived in accordance with the expression:

$$k_{app.} = k_0 - k_eC \qquad \text{(Eq. 5)}$$

which describes their action in the low concentration C range, where k_0 is the generation rate constant of drug-free culture, $k_{app.}$ is the generation rate constant of drug-affected culture, and k_e is the inhibitory rate constant for the drug.

Effects of Order of Addition of Erythromycin and Lincomycin on Generation of *E. coli* Cultures—There are no significant differences among the generation inhibition produced by the action of 20 mcg. ml.$^{-1}$ erythromycin I (curve B) and the equipotent concentration 100 mcg. ml.$^{-1}$ lincomycin hydrochloride in phase I (curve C) in Fig. 1, where curve B has the same slope as the initial portion of curve C. A mixture of 20 mcg. ml.$^{-1}$ erythromycin I and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride produces an initial (Phase I) action (curve G) which is not significantly different from that of the *a priori* equipotent concentration of either drug alone, *i.e.*, 40 mcg. ml.$^{-1}$ erythromycin I (curve F) or 200 mcg. ml.$^{-1}$ lincomycin hydrochloride (curve H), where curve F and the initial portions of curves G and H have the same slopes. However, the mixture of curve G shows a Phase II action, where the slope is greater than that of the Phase II lincomycin action in curve H. This demonstrates an apparent dilution of the lincomycin (Phase II) action by erythromycin, which does not possess such a phase.

An equipotent amount of lincomycin (Phase I) added after 55 min. to the erythromycin-affected culture of curve B or an equipotent amount of erythromycin added after 55 min. to the lincomycin (Phase I)-affected culture of curve C produces the same steady-state generation rate (curve E), which is not significantly different from that produced by the Phase II action of the equipotent mixture of Curve G. Similarly, an amount of erythromycin, equi-

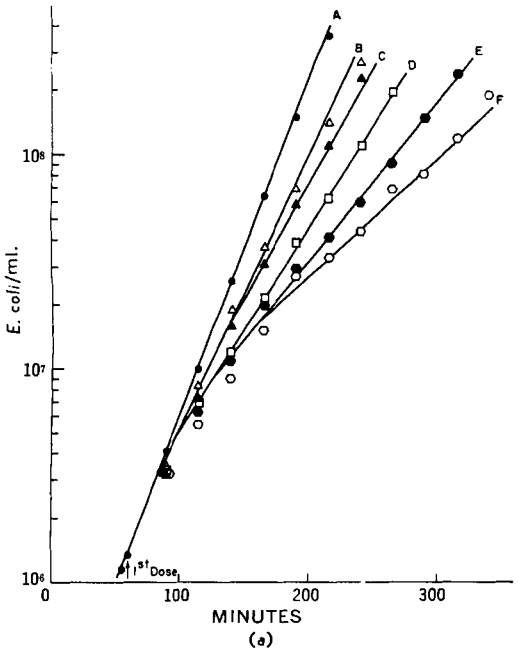
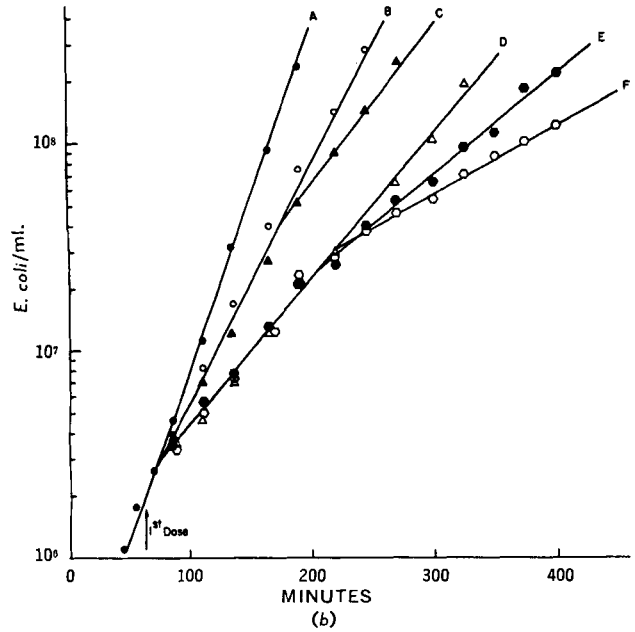
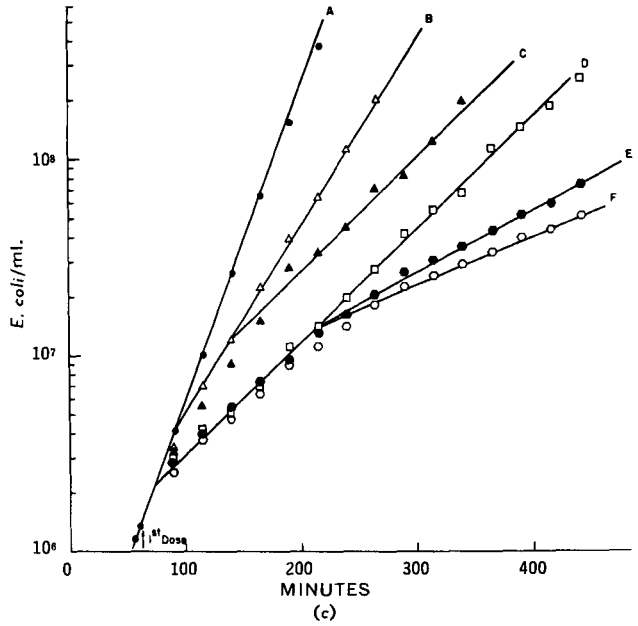
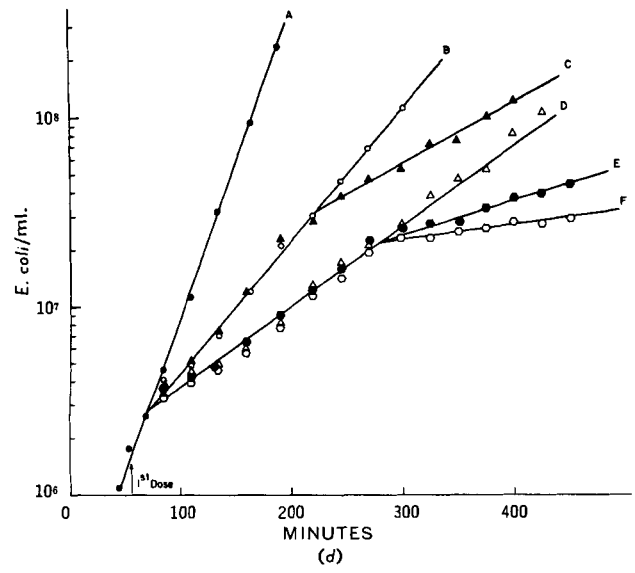
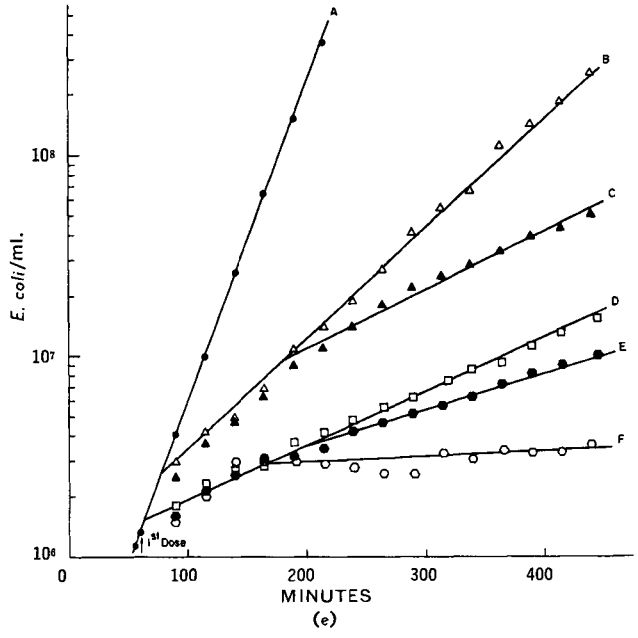


Figure 4—*Phase II action of mixtures of equal equipotent parts of erythromycin and lincomycin. Curve A is for generation of* E. coli *in the absence of drug. Curve B is for generation of* E. coli *in the presence of erythromycin, and curve C is in the presence of lincomycin which is equipotent in Phase I of its action. Curve E is when the mixture of equal equipotent parts of the erythromycin and lincomycin is added to the culture of curve A. Curve D is for the action of a concentration of erythromycin and curve F is for the action of a concentration of lincomycin, which have the same potency in Phase I action as the mixture of curve E. The particular concentrations of antibiotics for the various curves in* 4a *are: (B) 10 mcg. ml.$^{-1}$ erythromycin l, (C) 50 mcg. ml.$^{-1}$ lincomycin hydrochloride, (D) 20 mcg. ml.$^{-1}$ erythromycin l, (E) 10 mcg. ml.$^{-1}$ erythromycin l + 50 mcg. ml.$^{-1}$ lincomycin hydrochloride, and (F) 100 mcg. ml.$^{-1}$ lincomycin hydrochloride. The particular concentrations of antibiotics for the various curves in* 4b *are: (B) 15 mcg. ml.$^{-1}$ erythromycin l, (C) 75 mcg. ml.$^{-1}$ lincomycin hydrochloride, (D) 30 mcg. ml.$^{-1}$ erythromycin l, (E) 15 mcg. ml.$^{-1}$ erythromycin l + 75 mcg. ml.$^{-1}$ lincomycin hydrochloride, and (F) 150 mcg. ml.$^{-1}$ lincomycin hydrochloride. The particular concentrations of antibiotics for the various curves in* 4c *are: (B) 20 mcg. ml.$^{-1}$ erythromycin l, (C) 100 mcg. ml.$^{-1}$ lincomycin hydrochloride, (D) 40 mcg. ml.$^{-1}$ erythromycin l, (E) 20 mcg. ml.$^{-1}$ erythromycin l + 100 mcg. ml.$^{-1}$ lincomycin hydrochloride, and (F) 200 mcg. ml.$^{-1}$ lincomycin hydrochloride. The particular concentrations of antibiotics for the various curves in* 4d *are: (B) 30 mcg. ml.$^{-1}$ erythromycin l, (C) 150 mcg. ml.$^{-1}$ lincomycin hydrochloride, (D) 60 mcg. ml.$^{-1}$ erythromycin l, (E) 30 mcg. ml.$^{-1}$ erythromycin l + 150 mcg. ml.$^{-1}$ lincomycin hydrochloride, and (F) 300 mcg. ml.$^{-1}$ lincomycin hydrochloride. The particular concentrations of antibiotics for the various curves in* 4e *are: (B) 40 mcg. ml.$^{-1}$ erythromycin l, (C) 200 mcg. ml.$^{-1}$ lincomycin hydrochloride, (D) 80 mcg. ml.$^{-1}$ erythromycin l, (E) 40 mcg. ml.$^{-1}$ erythromycin l + 200 mcg. ml.$^{-1}$ lincomycin hydrochloride, and (F) 400 mcg. ml.$^{-1}$ lincomycin hydrochloride.*









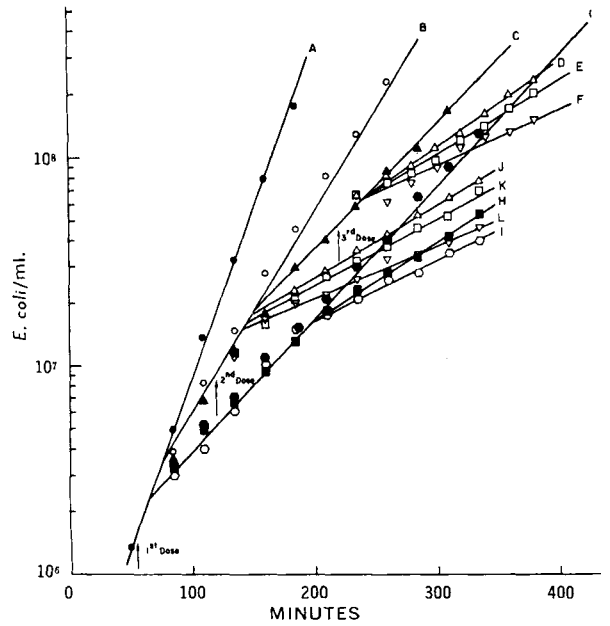


Figure 5—*Demonstration of nonantagonistic action of erythromycin on Phase I and Phase II generation of lincomycin-affected cultures. Curve A is for generation of culture in the absence of drug. Curve B is for generation of culture in the presence of 20 mcg. ml.$^{-1}$ erythromycin I; curve C is for generation of culture in the presence of equipotent 100 mcg. ml.$^{-1}$ lincomycin hydrochloride (Phase I). Curves D, E, and F are for generations of Phase II lincomycin-affected cultures of curve C when 20, 30, and 40 mcg. ml.$^{-1}$, respectively, of erythromycin I are added 165 min. after the initial addition of the lincomycin. Curves J, K, and L are for generations of Phase I lincomycin-affected cultures of curve C when 20, 30, and 40 mcg. ml.$^{-1}$, respectively, of erythromycin I are added 65 min. after the initial addition of the lincomycin. Curve H is for a mixture of equal parts of 20 mcg. ml.$^{-1}$ erythromycin I and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride, which are equipotent in Phase I action, when added to the culture of curve A. Curves G and I result when 40 mcg. ml.$^{-1}$ erythromycin I and 200 mcg. ml.$^{-1}$ lincomycin hydrochloride, respectively, both of which have the same potency in Phase I action as the mixture of curve H, are added to the culture of curve A.*

potent to lincomycin Phase I action, added after 140 min. to the lincomycin (Phase II)-affected culture of curve C produces an ultimate steady-state generation rate (curve D) that is the same as that of curve E or G. Thus, the order of addition of these two antibiotics produces no significant change on the ultimate generation inhibition. Also, the addition of erythromycin, equipotent to lincomycin Phase I action, to the lincomycin-affected cultures at the Phase I or Phase II generation does not make any significant change on the ultimate generation inhibition. Thus, there may not be any antagonism of effects between erythromycin and lincomycin, but the Phase II lincomycin effect in the mixture of curve G is one-half the effect of the doubled concentration of lincomycin in curve H, even though the mixture of curves G and H was *a priori* and in fact equipotent during the Phase I action.

Effects of Order of Addition of Erythromycin and Clindamycin (or U24729A) in Combination on Generation of *E. coli* Cultures—There are no significant differences in the generation inhibition produced by the action of 20 mcg. ml.$^{-1}$ erythromycin I and an equipotent concentration of 16.67 mcg. ml.$^{-1}$ clindamycin hydrochloride (curve B in Fig. 2) as shown by coincidence of generation curves. There are, however, significant differences in the effective inhibition produced by the action of a mixture of 20 mcg. ml.$^{-1}$ erythromycin I and 16.67 mcg. ml.$^{-1}$ clindamycin hydrochloride in curve D and that of the predicted equipotent concentration of either drug alone, *i.e.*, 40 mcg. ml.$^{-1}$ erythromycin I or 33.34 mcg. ml.$^{-1}$ clindamycin hydrochloride in curve E (Fig. 2). The slope of curve D is higher than that of curve E and is almost the same as that of curve B which, herefore, demonstrates that the erythromycin and clindamycin combination has less effect on the inhibition of microbial generation than an equipotent amount of erythromycin or clindamycin used separately. Also, an equipotent amount of clindamycin added after 60 min. to erythromycin-affected cultures of curve B or an equipotent amount of erythromycin added after 60 min. to clindamycin-affected cultures of curve B produces the same generation rate (curve C) which, however, has the same slope as that of the mixture of curve D. This indicates that the order of addition of these two antibiotics does not contribute significantly to the antagonism of effects between erythromycin and clindamycin.

This pattern of response is likewise observed for combinations of 20 mcg. ml.$^{-1}$ erythromycin I with an equipotent concentration of 4 mcg. ml.$^{-1}$ U24729A as the hydrochloride (Fig. 3).

Effects of Lincomycin (Phase II) Action in Mixtures of Equipotent Concentrations of Erythromycin and Lincomycin at Different Levels of Activity—The generation curves for cultures affected with equipotent concentrations of erythromycin and lincomycin and combinations thereof at five levels of activity are given in Figs. 4*a*–4*e*. Table I shows the $k_{app.}$ for the drug-affected cultures derived as the slopes of the linear portions of plots of ln *N versus t* in accordance with Eq. 1 from the generation curves in Figs. 4*a*–4*e*. The $k_{app.}$ for Phase II action of the mixture is higher than the $k_{app.}$ for Phase II action of the lincomycin alone, which *a priori* has the same potency in Phase I of its action. This is consistently shown at all levels of activity and demonstrates an apparent dilution of the lincomycin (Phase II) action by erythromycin.

Effects of Addition of Graded Concentrations of Erythromycin to Lincomycin-Affected Cultures in Phase I and Phase II Generation—The generation curves for the action of 20 mcg. ml.$^{-1}$ erythromycin I (curve B) and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride (curve C) on drug-free culture (curve A) are given in Fig. 5. The effect for a mixture of 20 mcg. ml.$^{-1}$ erythromycin I and 100 mcg. ml.$^{-1}$ lincomycin hydrochloride is given as curve H; that of the *a priori* equipotent concentration, *i.e.*, 40 mcg. ml.$^{-1}$ erythromycin I or 200 mcg. ml.$^{-1}$ lincomycin hydrochloride (Phase I) is given as curve G or curve I, respectively.

The addition of graded concentrations of erythromycin I (20, 30, and 40 mcg. ml.$^{-1}$) to the lincomycin-affected cultures of curve C in steady-state (Phase I) generation results in graded responses, as shown by decreasing slopes of the Phase II generation curves with increasing concentrations of the erythromycin (curves J, K, and L, respectively).

This pattern of response is likewise observed for the same graded concentrations of erythromycin added to the lincomycin-affected cultures of curve C in Phase II steady-state generation (curves D, E, and F). Curves D, J, and H, which are generation curves for cultures affected with the same combinations of the antibiotics, have the

Table I—Generation Rate Constants ($k_{app.}$ in sec.$^{-1}$) for *E. coli* Cultures Affected with Mixtures of Equipotent Concentrations of Erythromycin and Lincomycin

Antibiotics	$10^5\ k_{app.I}$[a]	$10^5\ k_{app.II}$[b] (Experimental Values)	$10^5\ k_{app.II}$[c] (Predicted Values)
(10)*E*[d]	48.99	—	
(50)*L*[e]	48.99	41.57	
(10)*E* + (50)*L*	36.74	28.61	31.17
(20)*E*	36.74	—	
(100)*L*	36.74	20.61	
(15)*E*	44.09	—	
(75)*L*	44.09	29.27	
(15)*E* + (75)*L*	26.57	18.50	17.63
(30)*E*	26.57	—	
(150)*L*	26.57	12.52	
(20)*E*	36.74	—	
(100)*L*	36.74	20.61	
(20)*E* + (100)*L*	21.88	12.16	12.27
(40)*E*	21.88	—	
(200)*L*	21.88	9.80	
(30)*E*	26.57	—	
(150)*L*	26.57	12.44	
(30)*E* + (150)*L*	16.42	6.81	7.67
(60)*E*	16.42	—	
(300)*L*	16.42	2.34	
(40)*E*	21.88	—	
(200)*L*	21.88	9.80	
(40)*E* + (200)*L*	10.28	5.88	4.60
(80)*E*	10.28	—	
(400)*L*	10.28	1.03	

[a] $k_{app.}$ for drug-affected culture in Phase I generation. [b] $k_{app.}$ for drug-affected culture in Phase II generation. [c] Predicted in accordance with Eq. 15. [d] Erythromycin I. [e] Lincomycin hydrochloride. Figures in parentheses are in mcg. ml.$^{-1}$.

Table II—Composition of Equipotent Mixtures of Erythromycin and Lincomycin (Phase II)

Concentration Level	Experimentally Determined Potency Ratio, *E*:*L*, at the Given Concentration Level[a]	Equipotent Antibiotic Solutions: Experimentally Determined Equipotent Concentrations, mcg. ml.$^{-1}$		Composition of Antibiotic Mixtures that Should Be Equipotent at This Concentration Level for Experimentally Determined Potency Ratio		Composition of Antibiotic Mixtures that Should Be Equipotent at This Concentration Level if Potency Ratio, *E*:*L*, Were 3.0 at All Concentration Levels		Conclusion of Combined Action if the Expectation Were Based on the Fixed Potency Ratio, *E*:*L*, of 3.0 Rather than the Experimental
		E	*L*	*E*	*L*	*E*	*L*	
1	2.5	10	25	5	12.5	5	15	Synergism
2	3.0	30	90	15	45	15	45	Indifference
3	3.3	60	200	30	100	30	90	Antagonism

[a] *E* = erythromycin 1; *L* = lincomycin hydrochloride.

same slopes. Curve E has correspondingly the same slope as curve K, but these curves are not parallel to curve F which is parallel to curve L. This indicates that the ultimate Phase II generation inhibition produced by combinations of erythromycin with lincomycin-affected cultures in Phase I generation does not differ significantly from that produced by combinations of eythromycin with lincomycin-affected cultures in Phase II generation.

DISCUSSION

The generation rate constants, $k_{app.}$ for erythromycin-affected and lincomycin (Phase I)-affected cultures have the same functional dependency on drug concentrations (1). Equipotent mixtures, composed of different fractions of erythromycin and lincomycin (Phase I), are quantifiable as kinetically equivalent in their inhibitory action on microbial generation for the resultant Phase I action of the combination, which is not inconsistent with the same mechanism and locus of action (2) for the two antibiotics. The only observable difference between the two antibiotics is the potency of action that can be assigned to the inverse ratio of the product of the drug partition constant K_1 and the drug affinity constant K_2 (13) for the respective drugs.

Figure 1 and Table I show that mixtures composed of equipotent concentrations of erythromycin and lincomycin (Phase I) produce the same degree of inhibition on generation of *E. coli* as the corresponding *a priori* equipotent concentration of either drug alone at the different levels of activity. This confirms unequivocally that erythromycin and lincomycin (Phase I) are not antagonistic in the resultant Phase I action of the combination.

The functional dependency of $k_{app.}$ for lincomycin (Phase II)-affected cultures is different from that of erythromycin-affected and lincomycin (Phase I)-affected cultures (1). Therefore, the $k_{app.}$ *versus* concentration curve for lincomycin (Phase II)-affected cultures is not coincident with that of erythromycin-affected and lincomycin (Phase I)-affected cultures when the actual concentrations of erythromycin and lincomycin are multiplied by a potency factor over the entire range of concentrations studied. Thus, the action of combinations of erythromycin, based on a potency factor for erythromycin and lincomycin (Phase II) at one dose level, may indicate antagonism or synergism (2) at other dose levels. For instance, potency ratios derived from the plot of $k_{app.}$ *versus* concentration for erythromycin and lincomycin (Phase II) in Fig. 6 of the Garrett *et al.* (1) study at levels of activity corresponding to 10, 30, and 60 mcg. ml.$^{-1}$ erythromycin 1 are 2.5, 3, and 3.3, respectively. Table II shows that when a fixed potency factor of 3 is assumed to be operative at all levels of activity, the mixtures of erythromycin and lincomycin formulated so as to be *a priori* as equipotent as the erythromycin or lincomycin (Phase II) alone contain lincomycin in quantities that are either less, the same, or more than would be prepared on the basis of the experimentally determined potency factor which varies with drug concentration levels. Similarly, the prediction of action of combinations of erythromycin and lincomycin in all phases, based on the fixed potency factor for erythromycin and lincomycin (Phase I) action, may indicate apparent antagonism in Phase II, unless the mechanisms and kinetics of action of the separate drugs are taken into consideration.

It has been proposed (10) that the two-phase lincomycin action may be explained by sequential blocking of metabolic pathways in protein synthesis as given in the equation:

$$S + E_1 \overset{K_{E_1}}{\rightleftharpoons} (E_1S) \overset{k_1}{\rightarrow} M_1 + E_2 \overset{K_{E_2}}{\rightleftharpoons} (E_2M_1) \overset{k_2}{\rightarrow} P \quad \text{(Eq. 6)}$$

The erythromycin or lincomycin (Phase I) action may be due to inhibition of the receptor site E_2 involved in the utilization of an essential metabolite M in the synthesis of protein P. The lincomycin (Phase II) action may be due to an inhibition of receptor site E_1 engaged in the cellular synthesis of the metabolite from normal substrate S available in excess in broth medium. The metabolite may be available in stored form at the initial logarithmic phase of organism generation. Therefore, the inhibition of its synthesis does not initially kinetically perturb the overall rate of protein synthesis. When the metabolite becomes depleted in successive generations (9), the inhibition of its synthesis imposes another rate-limiting factor, which enhances the initial (Phase I) lincomycin action and results in a Phase II lincomycin action at the same dose level.

On the assumption that the rate of microbial generation dN/dt is proportional to the overall rate of protein synthesis, dP/dt (14), the generation rate constant k_0 for the drug-free culture may be expressed as:

$$k_0N = dN/dt = q(dP/dt)N \quad \text{(Eq. 7)}$$

where q is a constant of proportionality. The rate of formation of P in the sequential process represented in Eq. 6 for a constant supply of substrate S may be expressed (13) as:

$$dP/dt = k_mE_1E_2 \quad \text{(Eq. 8)}$$

where k_m is a constant of proportionality related to the affinities K_{E_1} and K_{E_2} of the substrate S and the metabolite M to their respec-

Table III—Kinetic Expressions for the $k_{app.}$ of Cultures Affected with Equipotent Concentrations of Erythromycin and Lincomycin

Equipotent Antibiotics	$k_{app.I}$[a]	$k_{app.II}$[b]
E or *L*[c]	$(k_{app.I})_E = (k_{app.I})_L = k(1 - \theta_1)$	$(k_{app.II})_L = k\{(1 - \theta_1)(1 - \theta_2)\}$[d]
2*E* or 2*L*	$(k_{app.I})_{2E} = (k_{app.I})_{2L} = k\{(1 - \theta_1)/(1 + \theta_1)\}$	$(k_{app.II})_{2L} = k\{[(1 - \theta_1)(1 - \theta_2)]/[(1 + \theta_1)(1 + \theta_2)]\}$[d]
E + *L*	$(k_{app.I})_{E+L} = k\{(1 - \theta_1)/(1 + \theta_1)\}$	$(k_{app.II})_{E+L} = k\{[(1 - \theta_1)(1 - \theta_2)]/(1 + \theta_1)\}$

[a] $k_{app.}$ for drug-affected cultures in Phase I steady-state generation. [b] $k_{app.}$ for drug-affected cultures in Phase II steady-state generation. [c] Equipotent concentrations of erythromycin (*E*) or lincomyin (*L*). [d] $k_{app.II}$ for lincomycin alone since no such Phase II action exists for erythromycin.

tive sites and to the rate constants k_1 and k_2 of product formation from receptor site complexes E_1S and E_2M, respectively. Combination of Eqs. 7 and 8 yields:

$$k_0 = qk_mE_1E_2 = k'E_1E_2 \quad \text{(Eq. 9)}$$

where $k' = qk_m$.

Suppose a concentration of erythromycin E or an equipotent concentration of lincomycin L binds reversibly a fraction θ_1 of receptor site E_1 and, therefore, leaves a fraction $(1 - \theta_1)$ to exercise its metabolic activity. The generation rate constant $k_{app.I}$ for either equipotent drug-affected culture may be expressed as:

$$k_{app.I} = k'(1 - \theta_1)E_1E_2 = k(1 - \theta_1) \quad \text{(Eq. 10)}$$

where $k = k'E_1E_2$.

If, however, the lincomycin also binds a fraction θ_2 of receptor site E_2, then:

$$k_{app.II} = k(1 - \theta_1)(1 - \theta_2) \quad \text{(Eq. 11)}$$

where $k_{app.II}$ is the generation rate constant for lincomycin (Phase II)-affected cultures. It has been shown (13) that if a concentration C of drug reversibly binds a fraction θ of total receptors, then increasing the concentration of drug $(n + 1)$-fold results in a fraction:

$$\theta' = (n + 1)\theta/(1 + n\theta) \quad \text{(Eq. 12)}$$

This leaves a fraction of free or unbound receptors:

$$1 - \theta' = (1 - \theta)/(1 + n\theta) \quad \text{(Eq. 13)}$$

Table III lists the kinetic expressions for the $k_{app.}$ of cultures affected with equipotent concentrations of erythromycin and lincomycin deduced from Eqs. 10–13. The potency factor is based on erythromycin action as compared with the lincomycin in Phase I action on microbial generation. The ratio:

$$(k_{app.II})_{E+L}/(k_{app.II})_{2L} = k\{[(1 - \theta_1)(1 - \theta_2)]/(1 + \theta_1)\} \times \{[(1 - \theta_1)(1 + \theta_2)]/k[(1 - \theta_1)(1 - \theta_2)]\} = 1 + \theta_2 > 1 \quad \text{(Eq. 14)}$$

where $(k_{app.II})_{E+L}$ is $k_{app.}$ for Phase II generation of a culture affected with a mixture of equal equipotent parts of erythromycin and lincomycin, and $(k_{app.II})_{2L}$ is $k_{app.}$ for Phase II generation of a culture affected with a concentration of lincomycin which *a priori* has the same potency as the mixture in the Phase I generation of the drug-affected culture. Equation 14 implies that the Phase II action of the mixture must be less than the Phase II action of the *a priori* equipotent lincomycin (Phase I) alone, which is the experimental observation. However, this is not antagonism (2) but an artifact of diluted lincomycin effect in the Phase II action of the mixture. Since erythromycin has no Phase II action, the formulation of equipotent mixtures on the basis of a potency factor for erythromycin and lincomycin (Phase I) action results in less Phase II action than would have been effected by the *a priori* equipotent lincomycin alone.

The functional dependency of the $k_{app.}$ for erythromycin-affected cultures on drug concentration is different from that of lincomycin-affected cultures in Phase II generation. Therefore, the subsequent Phase II action for the combinations of erythromycin and lincomycin cannot be simply predicted on the basis of equivalency (or additivity) of effects (2). However, it can be derived from the kinetic expressions in Table III that:

$$(k_{app.II})_{E+L} = (k_{app.I})_{2E \text{ (or } 2L)}\{(k_{app.II})_L/(k_{app.I})_{L \text{ (or } E)}\} \quad \text{(Eq. 15)}$$

where the various $k_{app.}$ values are as defined in Table III. Table I shows good agreement between the experimentally determined values of $(k_{app.II})_{L+E}$ and those theoretically predicted in accordance with Eq. 15. This demonstrates unequivocally that both the kinetics and mechanisms of the separate drug action must be considered in the quantification of the Phase II action for combinations of erythromycin and lincomycin.

The $k_{app.}$ values of erythromycin-, lincomycin (Phase I)-, clindamycin-, and U24729A-affected cultures have the same functional dependencies on drug concentrations. The actions of equipotent mixtures composed of different fractions of lincomycin (Phase I) and erythromycin (1) or of clindamycin and U24729A (9) are quantifiable as kinetically equivalent, yet those of lincomycin (Phase I) and clindamycin (or U24729A) demonstrate antagonism (9). The possibility that clindamycin (or U24729A) binds differently from lincomycin (Phase I) on an allosteric receptor site has been discussed (9). Since erythromycin has the same locus of action as lincomycin (Phase I), it may be concluded that the observed antagonism for the combined action of erythromycin and clindamycin (Fig. 2) or erythromycin and U24729A (Fig. 3) is likewise due to the effects of allosteric interactions, which decrease the binding affinity of one drug in the presence of the other. Erythromycin-, clindamycin-, and U24729A-affected cultures, however, show one steady-state phase of generation. These facts indicate that the drugs possess only the Phase I action of lincomycin and none of the Phase II lincomycin action.

Thus, the effects of combinations of erythromycin with lincomycin (Phase I or Phase II action) on *E. coli* generation may not be concluded as being antagonistic on the basis of these microbial kinetics. The present work showed the importance of considering not only the dose–response relationship over a wide concentration range but also the kinetics and mechanisms of action of the separate drugs in the quantification and prediction of action of drug combinations.

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