Microbial Kinetics of Drug Action against Gram-Positive and Gram-Negative Organisms III: Effect of Lincomycin and Clindamycin Combinations on *Staphylococcus aureus* and *Escherichia coli*

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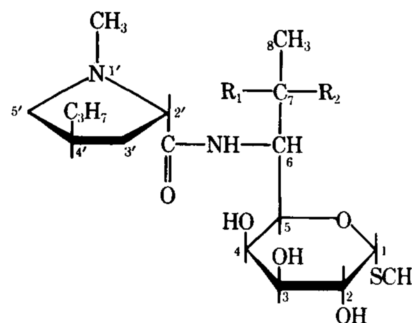
Abstract ☐ The functional dependencies of apparent first-order generation rate constants, k_{app}, of drug-affected cultures on drug concentrations indicate that lincomycin and clindamycin possess the same mechanism of action, which is bacteriostatic, against *Staphylococcus aureus*. Clindamycin also possesses another mechanism of action, which is bactericidal, at high concentration levels. However, clindamycin possesses only one of the two mechanisms of lincomycin action, which is bacteriostatic, against *Escherichia coli*. The relative potency of action of a clindamycin–lincomycin combination against *Staph. aureus* is variable, and the effective ratio ranges between 5:1 and 9:1; the effective ratio against *E. coli* is fixed at 6:1 over a wide concentration range. This difference is attributed to differences in bioavailability and/or binding characteristics of the drugs for bioreceptors, as a consequence of structural modifications in the drug molecules, and to differences in modes of action in the respective organisms. Mixtures containing equipotent fractions of clindamycin and lincomycin show "equivalence" or "indifference" of effects on *Staph. aureus*. The combined action of the mixtures can be quantitatively predicted from the separate dose–response curves of either component drug alone. Therefore, it is concluded that clindamycin and lincomycin may bind to the same receptor site that is engaged in microbial protein synthesis to inhibit the generation of *Staph. aureus*. However, combinations of clindamycin and lincomycin are less active than the *a priori* equipotent concentration of either drug alone in their action against *E. coli*, demonstrating unequivocally an antagonism of effects. Furthermore, the degree of antagonism is dependent on the order of addition of the drugs, which is attributed to the possibility that clindamycin and lincomycin bind differently on active and allosteric loci of the same receptor site functionally engaged in protein synthesis in *E. coli*. A rational approach to the quantification and prediction of combined antibiotic action must, therefore, be based not only on the kinetics and mechanisms of action as well as on the dose–response relationship over a wide concentration range for the separate antibiotics but also on the strain and species of the test organism.

Keyphrases ☐ Microbial kinetics—effect of lincomycin and clindamycin combinations on *Staph. aureus* and *E. coli*, drug–bacteria reactions, generation curves ☐ Lincomycin and clindamycin combinations—comparison of effect on *Staph. aureus* and *E. coli*, microbial kinetics, drug–bacteria reactions, generation curves ☐ Clindamycin and lincomycin combinations—comparison of effect on *Staph. aureus* and *E. coli*, microbial kinetics, drug–bacteria reactions, generation curves ☐ *Staphylococcus aureus*—effect of lincomycin and clindamycin combinations, compared to effects on *E. coli*, drug–bacteria reactions, generation curves, microbial kinetics ☐ *Escherichia coli*—effect of lincomycin and clindamycin combinations, compared to effects on *Staph. aureus*, drug–bacteria reactions, generation curves, microbial kinetics ☐ Antibiotic combinations—effect of lincomycin and clindamycin combinations on *Staph. aureus* and *E. coli*

Since the discovery of lincomycin (I), an antibiotic produced by *Streptomyces lincolnensis* (1, 2), different structurally modified analogs have been prepared with a view to understanding structure–activity relationships (3). Halogen substitution at the 7(*S*)-configuration of the lincomycin molecule is claimed to potentiate antibacterial effects. Clindamycin (II), which is the 7-deoxy-7(*S*)-chloro analog of lincomycin, is reported to be more than four times more active than the parent antibiotic against a variety of Gram-positive and Gram-negative organisms (4). The similarity in chemical structure would imply that clindamycin should have the same mechanism of action as lincomycin but possess different intrinsic biological activities, so the combined action of clindamycin with lincomycin should produce "additive" or "synergistic" effects (5) on microorganisms.

Comparative studies on the action of lincomycin and its 7(*S*)-chloro analogs, such as the 7-deoxy-7(*S*)-chloro-*N*-demethyl-4-pentyl analog (III) and clindamycin, against *Escherichia coli* by microbial kinetics (6) revealed that combinations of II and III yielded "indifference" of effects (5) but that combinations of I and II or I and III yielded "antagonism" of effects (5). The latter evidence indicates incompatibility in the use of combinations of lincomycin and clindamycin as recommended in current chemotherapy. However, these antibiotics are normally employed for the treatment of Gram-positive infections, which are highly sensitive to the action of the antibiotics (7), and not for Gram-negative infections, which show little or no sensitivity. Therefore, data obtained for *E. coli*, a typical Gram-negative organism, may not be readily extrapolated to Gram-positive organisms without experimental evidence.

Recently, microbial kinetic studies were applied to the action of lincomycin (8) and clindamycin (9) against *Staphylococcus aureus*, a representative Gram-positive coccus, and relative differences in their modes of action against *E. coli* were established. However, the effects of lincomycin and clindamycin combinations on *Staph. aureus* remain to be evaluated and quantified experimentally. These were the objectives of the study reported here.



I: R_1 = OH, R_2 = H
II: R_1 = H, R_2 = Cl

EXPERIMENTAL

Antibiotics—The antibiotics[1] were assayed samples of lincomycin hydrochloride (860 μg of base equivalent/mg) and clindamycin (860 μg of base equivalent/mg). Stock solutions of the antibiotics were prepared by membrane filtration and stored at 4°.

Bacteria and Growth Conditions—*Staph. aureus* (ATCC 6538) and *E. coli* (ATCC 12407) were cultivated in broth medium[2] and used for the experiments. An overnight (12–18 hr) culture of the respective organism was grown aerobically at 37.5° in a shaker water bath[3] to a cell density of 2.0×10^7 cells/ml. It was diluted 100-fold into 50-ml replicates of fresh broth medium and brought to the exponential phase of generation. Aliquots (0.5 ml) of the suitably diluted antibiotic solutions or mixtures were then added to the replicate exponential cultures at a cell density of about 10^6 cells/ml.

Total Count Method—Total counts of drug-free and drug-affected cultures were determined by a particle-size counter[4] as previously described (10). The suitability of the total count method for determining the generation rates of drug-affected cultures previously was established by a demonstrated correlation between the total count and viable (colony) count for lincomycin-affected *Staph. aureus* (8) and *E. coli* (11) and for clindamycin-affected *Staph. aureus* (9) and *E. coli* (9).

RESULTS

Effect of Drug Concentrations on Generation Rates—Semilogarithmic plots of the total count *versus* time yield generation curves of the drug-affected cultures as previously described (8). Lincomycin-affected or clindamycin-affected *Staph. aureus* cultures show two phases of steady-state generation. An initial (phase I) generation expressed as:

$$\ln N = \ln N_0 + k_{app_I} t \qquad \text{(Eq. 1)}$$

is followed by an ultimate (phase II) generation:

$$\ln N = \ln N_0 + k_{app_{II}} t \qquad \text{(Eq. 2)}$$

where k_{app_I} and $k_{app_{II}}$ are the apparent generation rate constants of the respective generation phases, N_0 is the number of organisms at some initial time (0), and N is the number of organisms at any time *(t)*. The $k_{app_{II}}$ is greater than k_{app_I} due to *Staph. aureus* adaptation or development of resistance to lincomycin (8) or clindamycin (9) action after a finite period of drug–bacteria contact.

Lincomycin-affected *E. coli* cultures also show two phases of steady-state generation whose characteristics are different from those of lincomycin-affected and clindamycin-affected *Staph. aureus* cultures. The k_{app_I} is greater than $k_{app_{II}}$, which is attributed (6) to dual mechanisms of the lincomycin action which become "synergistic" (5) in the subsequent generation phase of the culture. On the other hand, clindamycin-affected *E. coli* cultures show one phase of steady-state generation (6):

$$\ln N = \ln N_0 + k_{app} t \qquad \text{(Eq. 3)}$$

where k_{app} is the apparent generation rate constant.

Figure 1 shows the plots of k_{app} *versus* drug concentration for the lincomycin-affected *Staph. aureus* cultures (curve A), in which the phase I and phase II generation curves are superimposable by a potency factor. Likewise, curve B shows superimposable curves of the k_{app} dependence on drug concentration for phase I and phase II generations of clindamycin-affected *Staph. aureus.* The curve for lincomycin-affected *Staph. aureus* (curve A) is linear at low drug concentrations: 0–0.35 μg/ml (in the phase I action) or 0–0.55 μg/ml (in the phase II action). At concentrations greater than 0.35 μg/ml in the phase I action or greater than 0.55 μg/ml in the phase II action, the k_{app} is not a linear function of the increasing drug concentration. The plot for clindamycin-affected *Staph. aureus* (curve B) is a complex function of the drug concentration and yields a sigmoidal shape of curve for the phase I and phase II generations, which are also superimposable by a potency factor.

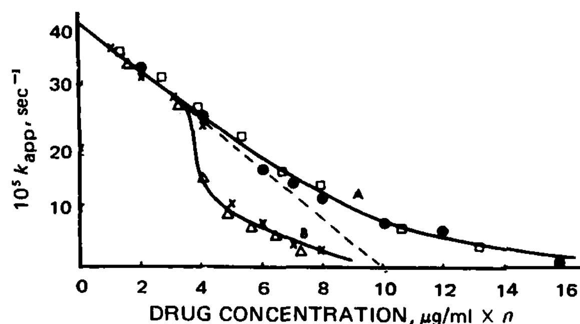


Figure 1—*Dependence of apparent generation rate constants,* k_{app} *in seconds*$^{-1}$, *for drug-affected* Staph. aureus *on drug concentrations (micrograms per milliliter) at pH 7.05 and 37.5°. Actual concentrations are multiplied by a factor,* n, *where* n = *20 for lincomycin phase I action (●) and* n = *13.20 for lincomycin phase II action (□) on* Staph. aureus *in curve A and* n = *100 for clindamycin phase I action (X) and* n = *80 for clindamycin phase II action (△) on* Staph. aureus *in curve B.*

Figure 2 shows plots of k_{app} *versus* drug concentration for the phase I lincomycin action on *E. coli* (curve A), which is coincident with that of clindamycin action on *E. coli* when normalized by a potency factor. The curve for the phase II lincomycin action on *E. coli* (curve B) is different from that of its phase I action (curve A) and is, therefore, not superimposable.

Effect of Order of Addition of Antibiotics on Microbial Generation—*Action of Equipotent Lincomycin Added to Clindamycin-Affected* Staph. aureus—The generation curves for the drug-free *Staph. aureus* cultures are shown as curves A in Figs. 3 and 4. In Fig. 3, the resultant generation curve for the action of 0.04 μg/ml of clindamycin on the *Staph. aureus* culture of curve A is shown as curve B; the generation curve for the culture affected with a mixture of equipotent concentrations of clindamycin (0.04 μg/ml) and lincomycin (0.3 μg/ml) is shown as curve E and is coincident with the curve for the culture affected with an *a priori* equipotent concentration of clindamycin (0.07 μg/ml).

Furthermore, Fig. 3 shows that equipotency of action is demonstrated by coincident or parallel generation curves of the drug-affected cultures having the same generation rate constant, k_{app}. The addition of an equipotent concentration of lincomycin (0.3 μg/ml) to the steady-state phase I generation of the clindamycin-affected culture of curve B produces an ultimate generation curve D, which shows two generation phases parallel to the respective phase I and phase II generations of the drug-affected cultures of curve E. Likewise, the addition of an equipotent concentration of

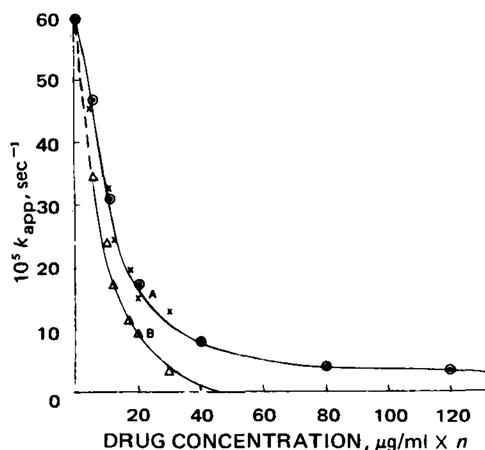


Figure 2—*Dependence of apparent generation rate constants,* k_{app} *in seconds*$^{-1}$, *for drug-affected* E. coli *on drug concentrations (micrograms per milliliter) at pH 7.05 and 37.5°. Actual concentrations are multiplied by a factor,* n, *where* n = *0.15 for lincomycin phase I action (X) and* n = *1.0 for clindamycin action (⊙) on* E. coli *in curve A and* n = *0.15 for lincomycin phase II action (△) on* E. coli *in curve B.*

[1] Courtesy of Dr. J. B. Whitfield, Jr., The Upjohn Co., Kalamazoo, Mich.
[2] Antibiotic Medium 3, Difco Laboratories, Detroit, Mich.
[3] Model RW 65, New Brunswick Scientific Co., New Brunswick, N.J.
[4] Coulter counter, model 2BI, Coulter Electronics Co., Hialeah, Fla.

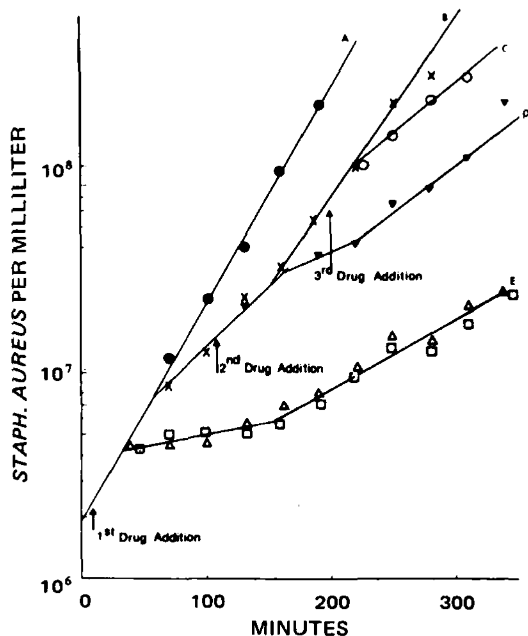


Figure 3—*Effects of addition of an equipotent concentration of lincomycin to clindamycin-affected* Staph. aureus. *Curve A is for the generation of drug-free culture (⊙). Curve B is for the generation of culture affected with 0.04 µg/ml of clindamycin (×). Curve E is for the generation of culture affected with either 0.07 µg/ml of clindamycin (△) or a mixture of equipotent 0.04 µg/ml of clindamycin and 0.3 µg/ml of lincomycin (□). Curve D is generated when an equipotent concentration of lincomycin (0.3 µg/ml) is added to the phase I clindamycin-affected culture of curve B (▼), and curve C is generated when an equipotent concentration of lincomycin (0.3 µg/ml) is added to the phase II clindamycin-affected culture of curve B (○).*

lincomycin (0.3 µg/ml) to the phase II generation of the clindamycin-affected culture of curve B yields a generation curve C, which is parallel only to the phase II generation of the drug-affected cultures of curve E.

Action of Equipotent Clindamycin Added to Lincomycin-Affected Staph. aureus—In Fig. 4, the generation curve produced by the action of 0.3 µg/ml of lincomycin on the *Staph. aureus* culture of curve A is shown as curve B; the generation curve for the culture of curve A affected with a mixture of equipotent concentrations of lincomycin (0.3 µg/ml) and clindamycin (0.04 µg/ml) is shown as curve E and is coincident with the curve obtained from the culture affected with an *a priori* equipotent concentration of lincomycin (0.6 µg/ml).

Furthermore, Fig. 4 shows that the addition of an equipotent concentration of clindamycin (0.04 µg/ml) to the steady-state phase I generation of the lincomycin-affected culture of curve B produces an ultimate generation curve D, which is parallel to that for the culture of curve E and shows typical phase I and phase II generations. The addition of an equipotent concentration of clindamycin (0.04 µg/ml) to the phase II generation of the lincomycin-affected culture of curve B results in a generation curve C, which is parallel only to the phase II generation curve of the drug-affected cultures of curve E.

These observations indicate that the order of addition of equipotent concentrations of lincomycin and clindamycin on *Staph. aureus* produces an ultimate generation inhibition that is not significantly different from that of the *a priori* equipotent concentration of either antibiotic alone.

Effect of Order of Addition of Clindamycin and Lincomycin on Generation of E. coli—In Fig. 5, the generation curve for the drug-free *E. coli* culture is shown as curve A; the generation curve for the action of clindamycin (15 µg/ml) on the *E. coli* culture of curve A is shown as curve B and is coincident with the phase I action of lincomycin (100 µg/ml) in curve C, which indicates equipotency of action. The generation curve produced by the action of clindamycin (30 µg/ml) in curve G is also coincident with the phase I action of lincomycin (200 µg/ml) in curve H.

The addition of a mixture of equipotent concentrations of clindamycin (15 µg/ml) and lincomycin (100 µg/ml) to the *E. coli* culture of curve A yields an ultimate generation phase (curve F), which has a slope higher than the curve for the action of the *a priori* equipotent concentration of clindamycin (30 µg/ml) in curve G or the phase I lincomycin (200 µg/ml) in curve H. This finding indicates decreased effectiveness or "antagonism" (5) of effects for the clindamycin and lincomycin mixture. The addition of an equipotent amount of lincomycin (100 µg/ml) to the steady-state phase generation of the clindamycin-affected *E. coli* culture of curve B results in an ultimate generation curve that is coincident with that of the phase II generation of the lincomycin-affected culture of curve C. This finding suggests that the added equipotent amount of lincomycin virtually nullifies the initial action of clindamycin on the culture.

However, the addition of an equipotent amount of clindamycin (15 µg/ml) to the steady-state phase I and phase II generations of the lincomycin-affected *E. coli* culture of curve C produces generation curves E and D, respectively, whose slopes are the same as that of the drug-affected culture of curve F. This finding indicates that the added equipotent amount of the clindamycin does not have an effect on the initial action of lincomycin on the culture but that clindamycin yields a relatively reduced effect on the ultimate generation inhibition.

These observations demonstrate that the order of addition of clindamycin and lincomycin to *E. coli* cultures produces significant effects on the ultimate generation inhibition and suggests antagonism of effects as observed previously (6).

Effect of Equipotent Mixtures of Different Fractions of Lincomycin and Clindamycin on Generation Rates—The gen-

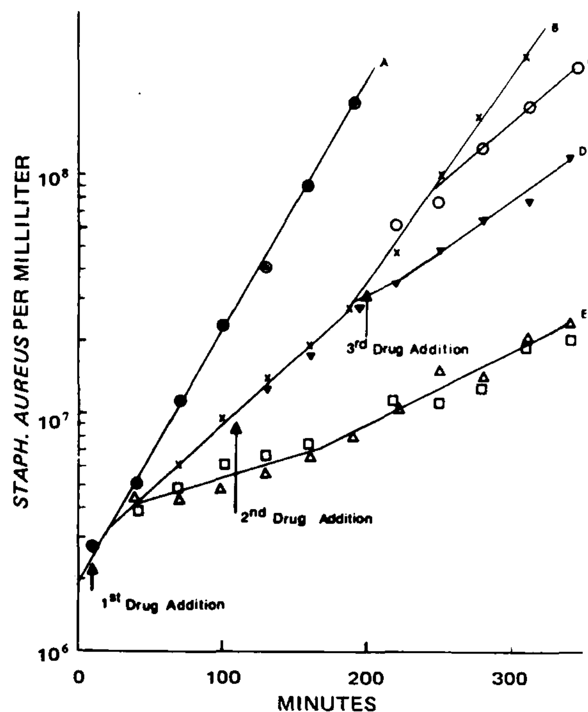


Figure 4—*Effects of addition of an equipotent concentration of clindamycin to lincomycin-affected* Staph. aureus. *Curve A is for the generation of drug-free culture (⊙). Curve B is for the generation of culture affected with 0.3 µg/ml of lincomycin (×). Curve E is for the generation of a culture affected with either 0.6 µg/ml of lincomycin (△) or a mixture of equipotent 0.3 µg/ml of lincomycin and 0.04 µg/ml of clindamycin (□). Curve D is generated when an equipotent concentration of clindamycin (0.04 µg/ml) is added to the phase I lincomycin-affected culture of curve B (▼), and curve C is generated when an equipotent concentration of clindamycin (0.04 µg/ml) is added to the phase II lincomycin-affected culture of curve B (○).*

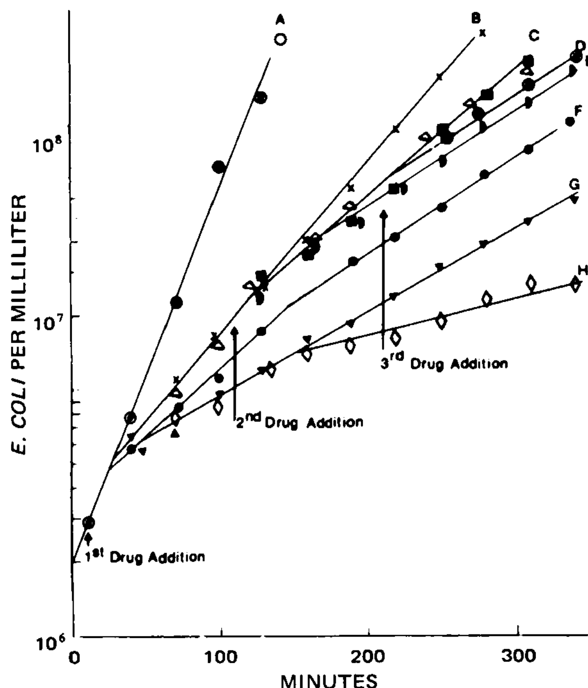


Figure 5—*Effects of the order of addition of equipotent concentrations of clindamycin and lincomycin on the generation rates of* E. coli. *Curve A is for the generation of drug-free culture. Curve B is for the generation of culture affected with 15.0 μg/ml of clindamycin (X). Curve C is generated by a culture affected with an equipotent 100-μg/ml concentration of lincomycin (Δ), and its coincident curve is generated when an equipotent 100-μg/ml concentration of lincomycin is added to the clindamycin-affected culture of curve B (■). Curve E is generated when an equipotent 15-μg/ml concentration of clindamycin is added to the phase I lincomycin-affected culture of curve C (◐). Curve D is generated when an equipotent 15-μg/ml concentration of clindamycin is added to the phase II lincomycin-affected culture of curve C (⊙). Curve F is generated when a mixture of equipotent concentrations of clindamycin (15 μg/ml) and lincomycin (100 μg/ml) is added to the culture of curve A (●). Curve G is generated when 30 μg/ml of clindamycin is added to the culture of curve A (▼), and curve H is generated when an equipotent 200-μg/ml concentration of lincomycin is added to the culture of curve A (◊).*

eration rate constant, k_{app}, for the steady-state phase I generation of *Staph. aureus* cultures affected with equipotent mixtures of clindamycin and lincomycin are shown in Fig. 6. The mixtures consisted of 100, 80, 60, 50, 40, 20, and 0% lincomycin and the residual percentage of an equipotent concentration of clindamycin. The mixtures were prepared so as to be *a priori* equipotent in their combined action on *Staph. aureus* generation in accordance with the dependence of k_{app} on drug concentration (Fig. 1). The null slopes for the plots of k_{app} for all the *a priori* equipotent mixtures at three different levels of activity demonstrate "indifference" or "equivalence" of effects (5).

The k_{app} values of *E. coli* cultures similarly affected with equipotent mixtures of clindamycin and lincomycin are shown in Fig. 7. The equipotent mixtures show lessened activity, as indicated by higher k_{app} values than when the *a priori* equipotent antibiotic was used alone at all levels of activity. These values demonstrate unequivocally an antagonism of effects for the combined action of clindamycin and lincomycin on *E. coli* cultures and confirm previous observations (6).

DISCUSSION

The data reported in this paper show (Fig. 1) that phase I and phase II generations of lincomycin-affected *Staph. aureus* (curve A) or clindamycin-affected *Staph. aureus* (curve B) have similar functional dependencies of generation rate constants, k_{app}, on drug

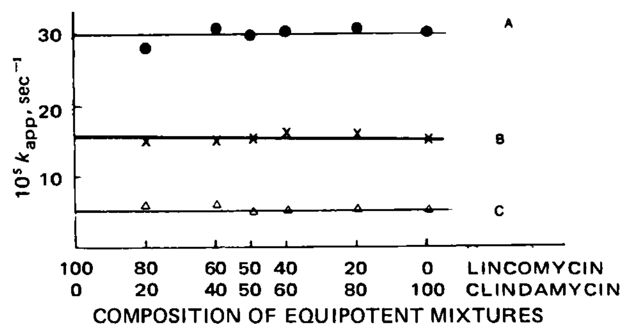


Figure 6—*Effect of varied clindamycin and lincomycin fractions in equipotent mixtures at three different levels of activity on the apparent generation rate constants,* k_{app} *in seconds*$^{-1}$, *of* Staph. aureus *at pH 7.05 and 37.5°. A, B, and C indicate the activities of* a priori *equipotent concentrations: 0.02 μg/ml of clindamycin = 0.11 μg/ml of lincomycin, 0.04 μg/ml of clindamycin = 0.36 μg/ml of lincomycin, and 0.05 μg/ml of clindamycin = 0.50 μg/ml of lincomycin, respectively.*

concentrations. This finding suggests the same mechanism of drug action for phase I and phase II generations of drug-affected cultures. The ratio of biological potency for the lincomycin action on phase I and phase II generations of *Staph. aureus* is 1:0.67. The corresponding ratio for the clindamycin action is 1:0.80. These apparent differences are attributable to adaptation or development of initial strains of *Staph. aureus* in phase I generation into resistant mutants in phase II generation whose ribosomal components have reduced binding affinity for the drugs (8).

The dependence of k_{app} on drug concentration for the lincomycin-affected *Staph. aureus* is similar to that of clindamycin-affected *Staph. aureus* in the low concentration range and indicates a common mechanism (9) of action. However, the dependencies are different at the high concentration levels, which may indicate variations in modes of action. It has been explained that both lincomycin (8) and clindamycin (9) inhibit microbial generation by competitive binding on a receptor site, which is also the binding site for a substrate (or metabolite) utilized in microbial protein synthesis. At low drug concentrations, when possibly few receptors are interacted with drug, there is a linear dependence of the k_{app} on drug concentration, as shown by the coincident portion of the regression for lincomycin-affected *Staph. aureus* and clindamycin-affected *Staph. aureus* (Fig. 1). At high drug concentrations, the already complexed receptor sites reduce the availability of remaining sites by steric effects, protective colloid action, or other mechanisms (11). Therefore, it takes progressively greater drug concentrations to bind the remaining sites, and the k_{app} is a nonlinear function of drug concentration but follows saturation kinetics (6, 8, 11).

In the case of clindamycin, it has been demonstrated (9) that a bactericidal phenomenon is also superimposed on generation inhibition by the action of clindamycin at the saturable level. Therefore, perturbations in the kinetics of generation inhibition result in a complex function for the dependence of the k_{app} on drug concentration. The overall effect yields a sigmoidal shape for curves of plots of k_{app} *versus* clindamycin concentration. The potency ratio for the action of lincomycin and clindamycin on *Staph. aureus* was not constant over the concentration range studied (Table I). Equipotent lincomycin and clindamycin concentrations are, therefore, estimated from the separate dose–response curves of either drug alone.

Equipotent mixtures comprised of different fractions of lincomycin and clindamycin are quantifiable as kinetically "equivalent" or "indifferent" (5) to the *a priori* equipotent concentration of either drug alone in the inhibition of *Staph. aureus* phase I generation at different levels of activity (Fig. 6). This is not inconsistent with the same mechanism (10) of action for the drugs. Furthermore, the order of addition of equipotent amounts of the drugs (Figs. 3 and 4) does not affect the ultimate generation inhibition of *Staph. aureus* cultures, which indicates a common locus of action (6).

There is an observable difference in the potency of action for

Table I—Potency Ratio for the Action of Clindamycin and Lincomycin against *Staph. aureus*[a]

Clindamycin Concentration, μg/ml	Lincomycin Concentration, μg/ml	k_{app} for Phase I of Drug-Affected Cultures, sec^{-1}	Potency Ratio of Clindamycin–Lincomycin
0.020	0.10	32.5	5.0
0.038	0.20	24.5	5.6
0.040	0.30	17.5	7.5
0.044	0.40	12.0	9.1
0.055	0.50	7.5	9.1
0.070	0.60	5.0	8.6

[a] Obtained from the data of Fig. 1.

lincomycin and clindamycin, but this difference can be attributed to relative differences in the drug partition through cell membranes and/or affinity for bioreceptors. Another factor is the bactericidal action of clindamycin, which causes a reduction in the number of survivor cells in the active state of growth and multiplication with a consequent decrease in the apparent generation rate of the drug-affected cultures. Of course, the high activity of clindamycin may be related to halogen substitution at the 7(*S*)-configuration. An increase in the lipophilicity of the molecule in the order I > Br > Cl, which correlates with enhanced antimicrobial activity, was reported for the 7(*S*)-halogen analogs of lincomycin (3). The enhanced activity may be the result of an increased drug availability in the biophase and/or modification of drug–receptor interactions. Additionally, halogen substitution in a homologous series of compounds was reported (12, 13) to alter antibacterial properties of compounds toward bactericidal activities.

The k_{app} dependence on drug concentration for clindamycin-affected *E. coli* is the same as for lincomycin-affected *E. coli* in phase I generation (Fig. 2) and suggests the same mechanism (10) of action, which is different from that for lincomycin-affected *E. coli* in phase II generation. On this presumption, the ratio of 1:6 for the biological potencies of lincomycin (phase I)–clindamycin confirms previous observations (6). However, equipotent mixtures of lincomycin (phase I) and clindamycin demonstrate unequivocally an antagonism (5) of effects (Fig. 7). The mixtures are less effective on the generation of *E. coli* cultures than the *a priori* equipotent concentrations of either drug alone at different levels of activity. This observation confirms a previous report (6) and suggests the possibility that lincomycin (phase I) and clindamycin do not have a common locus of action. Therefore, the similarity in the functional dependency of the k_{app} on drug concentration can be attributed to the binding of clindamycin and lincomycin at different loci of a receptor site that are functionally linked.

Steric configuration possibly plays an important role in the selective binding of the drugs at the sites, as was found for a number of compounds (14). In the case of lincomycin analogs, it was observed (3) that replacement of the 7(*R*)-hydroxy group by a 7(*R*)-halogen substituent has little or no effect on *in vitro* antibacterial activity. However, a 7(*S*)-halogen substituent increases the activity 4–32-fold. This type of evidence suggests that the 7(*R*)- and 7(*S*)-epimers bind differently on the same receptor site. On this premise, it was postulated (6) that two binding sites—a "catalytically active" site and an "allosterically modifying site" (14–16)—possibly exist on the same receptor surface in *E. coli* for the lincosaminide antibiotics. This conceivably may not be the case for *Staph. aureus*, which is different in gross morphology and physiology from *E. coli* (17).

The high activity of clindamycin relative to lincomycin phase I action against *E. coli* suggests that the former binds to the active site while the latter binds to the allosteric site. The binding of lincomycin (phase I) to the allosteric site possibly produces an extensive conformational change (15) which reduces the binding affinity of the active site for clindamycin (or the normal substrate) as a consequence of an unfavorable configuration. The effects of the order of addition of the drugs (Fig. 5) on the ultimate generation inhibition of *E. coli* cultures provide evidence in support of this hypothesis. Pretreatment of the cultures with lincomycin reduces the effect of an added equipotent concentration of clindamycin but not vice versa. It was also implied that, in this type of allosteric

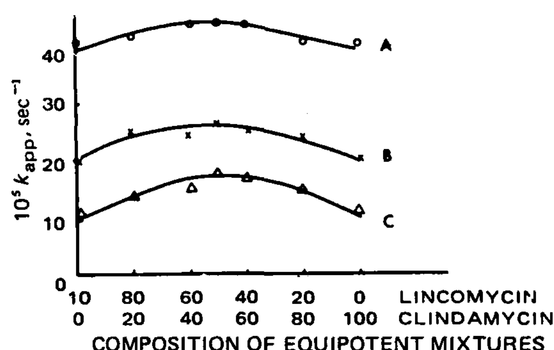


Figure 7—*Effect of varied clindamycin and lincomycin fractions in equipotent mixtures of three different levels of activity on the apparent generation rate constants,* k_{app} *in seconds*$^{-1}$, *of* E. coli *at pH 7.05 and 37.5°. A, B, and C indicate the activities of* a priori *equipotent concentrations: 10.0 μg/ml of clindamycin = 67.0 μg/ml of lincomycin, 20.0 μg/ml of clindamycin = 130.0 μg/ml of lincomycin, and 40.0 μg/ml of clindamycin = 260.0 μg/ml of lincomycin, respectively.*

interaction, only a noncompetitive type of antagonism (18), which is insurmountable by the addition of an excessive amount of one drug in the presence of the other, can result from the combined action of the drugs, as shown by the effects of mixtures of equipotent concentrations of lincomycin and clindamycin (Fig. 7).

The lincomycin phase II action on *E. coli* was attributed (6, 11, 19, 20) to a different mechanism, which is interdependent with the lincomycin phase I action but becomes rate limiting on microbial protein synthesis to enhance generation inhibition after a finite period of drug–bacteria contact. The lincomycin phase II action is the result of lincomycin binding at a receptor site that is different from the binding receptor site for the lincomycin phase I or clindamycin action. The separate sites are engaged in "sequential" metabolic pathways (21) leading to protein synthesis. Therefore, the sequential blocking (22) of the metabolic pathway by lincomycin results in "synergism" (5) of the phase I action by the onset of the phase II action, as evidenced by the effects of lincomycin alone or in combination with clindamycin on *E. coli* (Fig. 5). Although clindamycin action is antagonized by the lincomycin phase I action, it is kinetically equivalent (5) to the lincomycin phase II action, as was observed (20) for erythromycin and the lincomycin phase II action on *E. coli.*

The present work confirms earlier observations (6, 19, 20) that the kinetics and mechanisms of action, as well as the dose–response relationship, of the separate antibiotics must be considered in the quantification and prediction of combined antibiotic action. Moreover, the effect of antibiotic combinations is dependent on the strain and species of the test organism.

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Synthesis of Cyclopropyl Analogs of Stilbene and Stilbenediol as Possible Antiestrogens

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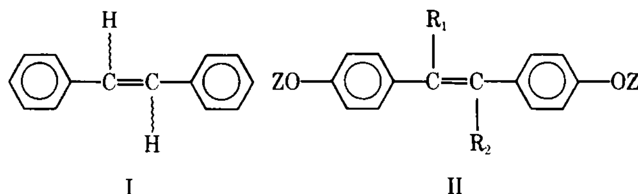
Abstract ☐ Conformationally rigid analogs of stilbene and stilbenediol were prepared *via gem*-dichlorocyclopropyl precursors utilizing two different synthetic methods: a two-phase catalytic method and an organomercurial method. These precursors were reduced to the corresponding cyclopropyl analogs using sodium and methanol. All compounds are being tested to discriminate between estrogenic and antiestrogenic activity, to determine estrogen binding ability, and to evaluate tissue culture anticancer activity.

Keyphrases ☐ Cyclopropyl analogs of stilbene and stilbenediol—synthesis as potential antiestrogens ☐ Stilbene and stilbenediol cyclopropyl analogs—synthesis as potential antiestrogens ☐ Antiestrogens, potential—synthesis of cyclopropyl analogs of stilbene and stilbenediol

A previous publication (1) discussed the cyclopropane moiety in connection with work on estrogen receptor elucidation. The preparation of *gem*-dichlorocyclopropyl analogs of the stilbene (I) and stilbenediol (II) series (Table I), using two different synthetic methods, and their subsequent reduction to the corresponding cyclopropyl analogs (Table II) are reported here.

Antiestrogens that arrest epithelial proliferation caused by estrogens in the uterus or vagina could have some utility in a particular uterine or vaginal cancer. Furthermore, certain antiestrogens may prove effective against other hormonal-dependent cancers such as breast cancer. Effective chemotherapeutic agents against such solid, hormone-sensitive tumors have not been fully developed and the duration of chemotherapeutic remissions is not as long lasting as those induced by endocrine manipulation.



Currently, the compounds reported in Tables I and II are being tested to discriminate between their estrogenic and antiestrogenic activities and for their estrogen binding ability, and they are being evaluated in a tissue culture anticancer assay[1].

DISCUSSION

A two-phase catalytic method (2) and an organomercurial method (3–7) have proven effective as applied to olefins of low reactivity (*e.g.*, *trans*-stilbene) toward dichlorocarbenes generated by other procedures. Of practical significance is the fact that yields are usually high by these two routes.

The *gem*-dichlorocyclopropyl analogs of stilbene were prepared according to both methods, while the stilbenediols were subjected only to the latter mercurial method since the phenolic groups were sensitive to strong base and some stilbenediols were poorly soluble in chloroform. The *gem*-dichlorocyclopropane precursors listed in Table I were subsequently reduced with sodium metal and methanol (8, 9).

The general reaction developed by Simmons and coworkers (10–12) for the stereospecific synthesis of cyclopropane derivatives involves the treatment of olefins with diiodomethane and zinc–copper couple. This convenient method could have allowed omission of the reductive step; however, in spite of various modifications (13–17), this method does not consistently produce respectable yields when the olefins are weak nucleophiles. Olefins in which the nucleophilic character of the double bond has been reduced by electron-withdrawing groups should react less readily with carbenes than those in which the nucleophilic character has been increased by electron-donating groups (18–20).

Method A—All reports of the syntheses of *gem*-dihalocyclopropane derivatives have stressed the necessity of operating under strictly anhydrous conditions because of the rapid hydrolysis of dichlorocarbene. On the other hand, the method established by Makosza and Wawrzyniewicz (2) and improved by Dehmlow and Schonefeld (21, 22) (Scheme I) generates dichlorocarbene in the reaction among chloroform, a concentrated solution (50%) of sodium hydroxide, and a catalytic amount of triethylbenzylammonium chloride, thus allowing the preparation of *gem*-dichlorocyclopro-

[1] Tests are being performed by the Cancer Section, Oklahoma Medical Research Foundation, Oklahoma City, and the College of Pharmacy, University of Oklahoma, Norman, Okla.