(13) C.-J. Lee and R. Dubos, *J. Exp. Med.*, **135**, 220(1972).
(14) S. G. McKenzie and H. P. Bar, *Can. J. Physiol. Pharmacol.*, **51**, 190(1973).
(15) E. Miyamoto, J. F. Kuo, and P. Greengard, *J. Biol. Chem.*, **244**, 6395(1969).
(16) J. F. Kuo, B. K. Krueger, J. R. Sanes, and P. Greengard, *Biochim. Biophys. Acta*, **212**, 79(1970).
(17) G. Melnykovych and C. F. Bishop, *J. Nat. Cancer Inst.*, **51**, 353(1973).
(18) J. R. Ortiz, T. Yamada, and A. W. Hsie, *Proc. Nat. Acad. Sci. USA*, **70**, 2286(1973).
(19) R. Ebert and U. Schwabe, *Arch. Pharmacol.*, **278**, 247(1973).
(20) J. N. Fain, *Mol. Pharmacol.*, **9**, 595(1973).
(21) G. Lindblad, G. Jonsson, and J. Falk, *Acta Pharmacol. Toxicol.*, **32**, 246(1973).
(22) A. Bendich, C. B. Brown, F. S. Phillips, and J. B. Thiersch, *J. Biol. Chem.*, **183**, 267(1950).
(23) F. S. Phillips, J. B. Thiersch, and A. Bendich, *J. Pharmacol. Exp. Ther.*, **104**, 20(1952).
(24) I. Merits and D. J. Anderson, *Xenobiotica*, **3**, 381(1973).
(25) I. Merits, *ibid.*, **3**, 541(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 7, 1974, from the *Department of Drug Metabolism, Squibb Institute for Medical Research, New Brunswick, NJ 08903*

Accepted for publication February 19, 1975.

The authors thank Mr. Peter Egli for synthesizing the ^{35}S- and ^{14}C-labeled I; Mr. Carmen Mondi, Mr. Michael Palfey, and Mr. John J. Ross, Jr., for technical assistance; Dr. Chandra Parekh for the determination of glucose concentrations in plasma; Dr. Don Harris for the determination of insulin concentrations in plasma; and Mr. Sal Meloni for preparing the illustrations. Supplies of nonradioactive I were provided by the International Chemical and Nuclear Corp.

[x] To whom inquiries should be directed.

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

SAMUEL M. HEMAN-ACKAH

Abstract □ Clindamycin-affected *Staphylococcus aureus* cultures show biphasic steady-state generation curves. An initial (phase I) generation of the clindamycin-affected *Staph. aureus* is followed by an ultimate (phase II) generation at the same dose level. The phase I apparent generation rate constant is greater than the phase II apparent generation rate constant and suggests the development of resistant *Staph. aureus* mutants to clindamycin action after a finite period of drug–bacteria contact at any subcompletely inhibitory concentration level. It is rationalized that the increased resistance to drug action in mutant strains is due to a comparatively reduced ribosomal binding affinity for clindamycin. In contrast, clindamycin-affected *Escherichia coli* cultures show monophasic steady-state generation curves at all concentration levels; *E. coli* cultures do not develop resistance to clindamycin action. The dependence of the apparent generation rate constant on drug concentration yields a sigmoidal curve, which is coincident by a potency factor for the phase I and phase II generations of clindamycin-affected *Staph. aureus* and suggests a common mechanism of action for both generation phases. That of clindamycin-affected *E. coli* yields an asymptote curve, which indicates a different mechanism of action. Clindamycin possesses both a bacteriostatic and a bactericidal action on initial and mutant resistant strains of *Staph. aureus*, whereas its action on *E. coli* is only bacteriostatic. Consequently, clindamycin has a minimum inhibitory concentration (MIC) against *E. coli* that is about 1000 times the MIC value against *Staph. aureus* at 37.5°. The effect of pH changes in broth media on generation inhibition of both *Staph. aureus* and *E. coli* by clindamycin action indicates that the unprotonated fraction of drug concentration contributes to the activity, possibly because of its ready penetration through cell membranes.

Keyphrases □ Microbial kinetics—drug action on Gram-positive and Gram-negative organisms, comparison of clindamycin action on *Staphylococcus aureus* and *Escherichia coli* □ Clindamycin—comparison of effects on *Staphylococcus aureus* and *Escherichia coli*, microbial kinetics □ *Staphylococcus aureus*—effects of clindamycin, compared to *Escherichia coli* □ *Escherichia coli*—effects of clindamycin, compared to *Staphylococcus aureus* □ Antimicrobial activity—comparison of clindamycin on *Staphylococcus aureus* and *Escherichia coli*

Clindamycin is one of the 7*(S)*-chloro analogs of lincomycin (1, 2) that is claimed to be more than four times as active as the parent antibiotic against a variety of Gram-positive and Gram-negative organisms (3). Like lincomycin, it interferes with ribosomal functioning in the synthesis of cell proteins (4, 5) to inhibit microbial growth and generation.

Comparative studies on the action of lincomycin and its 7*(S)*-chloro analogs against *Escherichia coli* by microbial kinetics (6) confirmed the enhanced potency of clindamycin action relative to that of lincomycin. These studies also revealed that clindamycin has only one of two mechanisms of lincomycin action against *E. coli*. Recently, it was shown (7) that lincomycin also possesses one mechanism of action against *Staphylococcus aureus*. However, *Staph. aureus* cultures, unlike *E. coli*. cultures, develop resistence to lincomycin after a finite period of organism contact with subcompletely inhibitory concentrations of the drug.

It was of interest to study the kinetics and dependencies related to modes of clindamycin action

against *Staph. aureus* and to compare the data with those obtained for *E. coli*. The results of such studies are presented in this paper.

EXPERIMENTAL

Materials and Methods—*Staph. aureus* (ATCC 6538) and *E. coli* (ATCC 12407) were used in all of the experiments. They were cultivated in broth medium[1] and used for determining antibiotic–bacteria reaction in the same manner as previously described (7). The antibiotic was an assayed sample of clindamycin hydrochloride[2] (860 μg base equivalent/mg). A particle-size counter[3] and the poured plate method, using sandwiched agar plates, were employed in the determination of total and viable counts, respectively, on drug-free and drug-affected *Staph. aureus* cultures as previously described (7).

Effect of Antibiotic Concentrations on Generation Rates—Aliquots (0.5 ml) of aqueous solutions of clindamycin were aseptically added to replicate 50-ml samples of cultures to yield the desired antibiotic concentrations. The solutions were added to the cultures in an exponential phase of generation at 37.5°. The cell concentration at the time of drug addition was 2.0×10^6/ml. Culture samples were withdrawn every 30 min, and the organism population was determined by the appropriate method.

Generation curves were obtained by the total count method for *Staph. aureus* and *E. coli* cultures affected with a range of clindamycin concentrations (0–0.1 and 0–120 μg/ml, respectively). Generation curves for *Staph. aureus* cultures affected with selected clindamycin concentrations (0, 0.04, 0.05, 0.07, 0.08, 0.16, and 0.32 μg/ml) and for *E. coli* cultures affected with selected clindamycin concentrations (0, 25, 50, 100, and 200 μg/ml) were also obtained by both total and viable count methods.

Effect of Broth Composition on Drug-Affected Generation Rates—Three different types of broth, containing half, single, and double concentrations of the ingredients as specified by the manufacturer, were prepared and adjusted to pH 7.05 ± 0.05 where necessary. They were used for determining the generation curves at 37.5° of replicate *Staph. aureus* cultures affected with clindamycin concentrations of 0, 0.03, 0.05, 0.07, and 0.09 μg/ml. The organism concentration at the time of drug addition was 2×10^6 cells/ml. Total counts were obtained on samples withdrawn every 30 min.

Effect of Organism Population on Drug-Affected Generation Rates—Three sets of single strength broth were prepared and used for determining the generation curves of replicate *Staph. aureus* cultures affected with clindamycin concentrations of 0, 0.03, 0.05, 0.07, and 0.09 μg/ml. The organism concentrations at the time of drug addition were 5.0×10^5, 2.0×10^6, and 2.0×10^7 cells/ml in the respective sets of broth. Total counts were obtained on samples withdrawn every 30 min.

Reversibility of Drug Action—An aliquot (0.5 ml) of an aqueous solution of clindamycin was aseptically added to a 50-ml sample of *Staph. aureus* culture in the exponential phase of generation at 37.5° to yield a final concentration of 0.03 μg/ml of clindamycin. The cell concentration at the time of drug addition was 2.0×10^6 cells/ml. When the drug-affected culture settled to a steady-state generation phase I, an aliquot (0.5 ml) was added to 49.5 ml of fresh broth so that both the organism and drug concentrations were diluted 100-fold. At the same time, an aliquot (0.5 ml) of the culture was diluted 100-fold in broth containing enough clindamycin so that the drug concentration was restored to 0.03 μg/ml.

A drug-free culture was likewise diluted 100-fold as a control. When the sample of the drug-affected culture, which was diluted in fresh broth, emerged from a lag phase into steady-state generation, an aliquot (0.5 ml) of clindamycin solution was added to its replicate culture to reestablish the drug concentration at 0.03 μg/ml.

The entire dilution steps were repeated on the initial drug-affected culture when it entered into a steady-state generation phase II. Total counts were obtained on samples withdrawn every 30 min.

The experiment then was performed on another 50-ml sample of *Staph. aureus* culture affected with a higher concentration of the drug, *i.e.*, 0.06 μg/ml of clindamycin.

Effect of pH on Drug-Affected Generation Rates—Sufficient amounts of 2.0 *N* HCl and 2.0 *N* NaOH were added to the broth media to obtain pH values of 5.9, 6.3, 6.5, 7.0, 7.3, 7.6, 7.8, and 8.2. Six replicate 50-ml volumes of each broth, inoculated with *Staph. aureus*, were maintained at 37.5° until the organism population reached 2.0×10^6 cells/ml. Aliquots (0.5 ml) of clindamycin solutions were added to five replicates to achieve the desired concentrations of antibiotic. The sixth replicate in each set contained no drug. Total counts were obtained from samples withdrawn every 30 min.

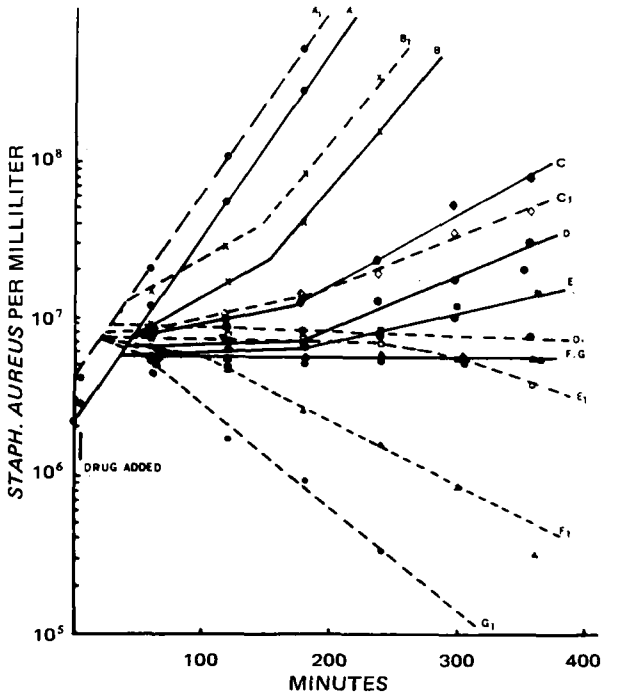


Figure 1—*Generation curves of* Staph. aureus *at pH 7.05 and 37.5° in the absence and presence of clindamycin obtained by total (——) and viable (- - -) counts. The curves and respective drug concentrations (micrograms per milliliter) are: A and A_1, 0.0; B and B_1, 0.04; C and C_1, 0.05; D and D_1, 0.07; E and E_1, 0.08; F and F_1, 0.16; and G and G_1, 0.32.*

RESULTS

Shape of Generation Curves for Drug-Affected Cultures—Semilogarithmic plots of total and viable counts *versus* time are shown in Fig. 1 for the action of selected clindamycin concentrations of 0, 0.04, 0.05, 0.07, 0.08, 0.16, and 0.32 μg/ml on *Staph. aureus*. Figure 2 shows these plots for the action of clindamycin concentrations of 0, 25, 50, 100, and 200 μg/ml on *E. coli*. The addition of graded concentrations of clindamycin to balanced cultures of *Staph. aureus* (Fig. 1) and *E. coli.* (Fig. 2) in the exponential phase of generation at 37.5° and pH 7.05 decreased the generation rates after a lag period approximating 20 min. With the *Staph. aureus* cultures, a newly established steady-state phase of generation (phase 1) was followed after one to two generations by an ultimate steady-state phase of generation (phase II). Clindamycin-affected *E. coli* cultures exhibited one steady-state phase of generation.

Apparent generation rate constants of the drug-affected cultures are obtained from the slopes of the linear portions of semilogarithmic plots of the counts *versus* time in accordance with the expression:

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

where N is the number of organisms per unit volume of broth medium of any time, t; N_0 is the number of organisms per unit volume of broth medium at some initial time, 0, in the generation phase; and k_{app} (in seconds^{-1}) is the apparent generation rate con-

[1] Antibiotic Medium 3, Difco Laboratories, Detroit, Mich.
[2] Courtesy of Dr. J. B. Whitfield, Jr., The Upjohn Co., Kalamazoo, Mich.
[3] Model ZB1, Coulter Electronics Co., Hialeah, Fla.

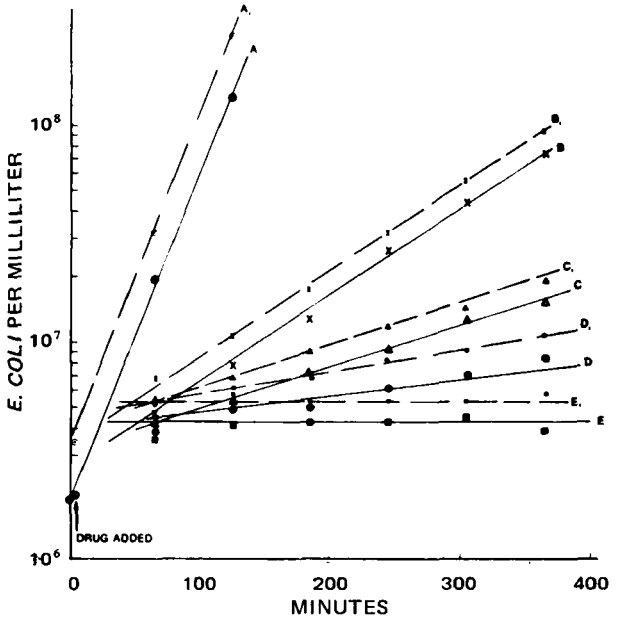


Figure 2—*Generation curves of* E. coli *at pH 7.05 and 37.5° in the absence and presence of clindamycin obtained by total (——) and viable (- - -) counts. The curves and respective drug concentrations (micrograms per milliliter) are: A and A_1, 0.0; B and B_1, 25.0; C and C_1, 50.0; D and D_1, 100.0; and E and E_1, 200.0.*

stant. In the absence of drug, $k_{app} = k_0$, which is the generation rate constant for the drug-free or control culture.

The k_{app} for phase I and phase II generations of clindamycin-affected *Staph. aureus* is designated k_{app_I} and $k_{app_{II}}$, respectively. The k_{app_I} value is less than the $k_{app_{II}}$ value for the action of subcompletely inhibitory concentrations of clindamycin determined by the total count method.

Generation Curves of Drug-Affected Cultures Determined by Total and Viable Count Methods—Parallel generation curves are obtained from plots of the total and viable counts for *Staph. aureus* cultures (Fig. 1) affected with clindamycin in the 0–0.04-μg/ml concentration range. The total count was 50% of the viable count at each concentration level (curves A_1, A and B_1, B). The cultures affected with 0.05–0.08 μg/ml of clindamycin showed

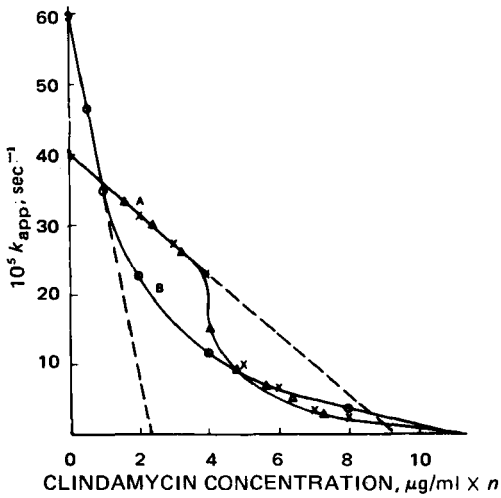


Figure 3—*Functional dependency of apparent generation rate constants,* k_{app} *in seconds*$^{-1}$*, for drug-affected* Staph. aureus *and drug-affected* E. coli *on concentrations of clindamycin (micrograms per milliliter) at pH 7.05 and 37.5°. Actual concentrations are multiplied by a factor,* n, *where* n = *100 in curve A for* Staph. aureus *phase I generation (×),* n = *80 in coincident curve for* Staph. aureus *phase II generation (▲), and* n = *0.1 in curve B for* E. coli *(○).*

complete generation inhibition for 200 min when the total count also remained 80–90% of the viable count. Thereafter, there was a drop in the viable count whereas there was a progressively small increase in the total count with time (curves C_1, C, D_1, D, and E_1, E).

Cultures affected with concentrations of clindamycin greater than 0.08 μg/ml showed coincidence of plots for the total counts, which remained constant for the entire experiment (curves F and G). The corresponding plots for the viables showed a rapid drop of the count with time as a function of the drug concentration (curves F_1 and G_1). This finding clearly indicates that a bactericidal or lytic phenomenon is superimposed on generation inhibition by the action of clindamycin at concentrations of 0.04 μg/ml.

Parallel generation curves are obtained from plots of the total and viable counts for *E. coli* cultures (Fig. 2) affected with clindamycin in the subcompletely inhibitory concentration range (curves A_1, A, B_1, B, C_1, C, and D_1, D) and also above the bacteriostatic concentration (curves E_1 and E). The total count was 50–80% of the viable count at each concentration level. There was no evidence of a bactericidal or lytic phenomenon superimposed on generation inhibition by the action of clindamycin in the concentration range studied.

Functional Dependency of Generation Rates on Drug Concentrations—Figure 3 shows the plot of k_{app} *versus* clindamycin concentration for *Staph. aureus*-affected cultures (curve A) and for *E. coli*-affected cultures (curve B). Coincidence of plots was obtained when the actual concentrations for the phase I and phase II clindamycin action on *Staph. aureus* were multiplied by a factor of 1.0 and 0.80, respectively (curve A). The extent of generation inhibition was directly proportional to the clindamycin concentration, C, in the 0–0.04-μg/ml range for the phase I action and in the 0–0.05-μg/ml range for the phase II action on *Staph. aureus* or in the 0–10.0-μg/ml range for the action on *E. coli*, in accordance with the expression:

$$k_{app} = k_0 - k_c C \qquad \text{(Eq. 2)}$$

where k_c is the specific inhibitory rate constant.

At higher drug concentrations, the k_{app} was not a linear function of increasing drug concentrations, but it asymptotically approached zero. There was a striking difference between the k_{app} dependency on drug concentration for *Staph. aureus* cultures affected with clindamycin at concentrations greater than 0.04 μg/ml and that for *E. coli* cultures affected with clindamycin at concentrations greater than 10.0 μg/ml. The rate of decrease of k_{app} with an increase in drug concentration was greater than would be obtained by extrapolation of the initial linear portion of the regression for the *Staph. aureus* cultures (curve A) but was less than would be predicted by such an extrapolation for *E. coli* cultures (curve B). Consequently, sigmoidal curves were obtained from plots of the data for *Staph. aureus* cultures. This finding may indicate that there is a change in the bioavailability or binding affinity of the clindamycin for bioreceptors which permits enhanced inhibition and/or kill of the *Staph. aureus* cultures but reduces the extent of generation inhibition of *E. coli* cultures.

Applicability of Saturation Kinetics to Action of Clindamycin on *Staph. aureus* and *E. coli*—The plot of $C/(k_0 - k_{app})$ *versus* C is given in Fig. 4. The plot is in accordance with a previously (8) derived, saturable receptor site model:

$$C/(k_0 - k_{app}) = C(k_b/k_a) + 1/k_a \qquad \text{(Eq. 3)}$$

where $k_b = K_1K_2$ and $k_a = qK_1K_2$. The term K_1 is the drug partition constant through cell membranes, K_2 is the drug affinity constant for bioreceptors, and q is a constant of proportionality related to the metabolic activity of the microbial cell.

Adherence to Eq. 3 is observed from linear plots obtained for concentrations of clindamycin greater than 0.04 μg/ml in phase I action (curve A) and greater than 0.05 μg/ml in phase II action (curve B) against *Staph. aureus* or greater than 10.00 μg/ml (curve C) against *E. coli*. Deviations from linearity of the plot occurred at concentrations of clindamycin less than 10.00 μg/ml against *E. coli* or less than 0.04 μg/ml in phase I and less than 0.05 μg/ml in phase II against *Staph. aureus*. These findings demonstrate a nonadherence to a saturable process at the lower concentration ranges. Additionally, the plots for *Staph. aureus* show a complete break and displacement of the saturable from the nonsaturable level, which may indicate a change in drug partitioning and/or binding properties in the biophase.

Table I—Kinetic Parameters of Clindamycin Action on *Staph. aureus* and *E. coli*

Kinetic Parameter[a]	*Staph. aureus* Phase I	*Staph. aureus* Phase II	*E. coli*
$10^5 k_c$, ml/μg sec[b]	416.67	322.50	2.56
$10^5 k_a$, ml/μg sec[c]	1333.33	1111.11	3.70
$10^5 k_a/k_b$, sec^{-1}[c]	54.17	54.17	70.00
$(k_a/k_b)/k_0$[c]	1.35	1.35	1.11
$10^2 k_b$, ml/μg[c]	2461.53	2051.27	5.28
MIC, μg/ml[d]	0.11	0.14	113.20

[a] Derived from data of Fig. 3. [b] Calculated from the slope of the plot of k_{app} *versus* concentration from 0 to 0.04 μg/ml of clindamycin in phase I action and from 0 to 0.25 μg/ml of clindamycin in phase II action on *Staph. aureus* or from 0 to 10 μg/ml of clindamycin action on *E. coli*, in accordance with Eq. 2. [c] The k_a, k_a/k_b, $(k_a/k_b)/k_0$, and k_b values are estimated from the slopes and intercepts of plots of C $(k_0 - k_{app})$ *versus* C from Eq. 3 for clindamycin concentrations greater than 0.04 μg/ml in phase I action and greater than 0.05 μg/ml in phase II action on *Staph. aureus* or greater than 10 μg/ml on *E. coli*. [d] Calculated from Eq. 4, where clindamycin is at the MIC.

The values of k_a and k_b calculated from the slopes and intercepts of such plots are given in Table I. The minimum inhibitory concentration (MIC) of clindamycin in Table I is calculated from the expression (7):

$$C_m = k_0/(k_a - k_0 k_b) \qquad \text{(Eq. 4)}$$

where C_m is the MIC. It is estimated that clindamycin has an MIC value against *E. coli* that is about 1000 times the MIC value against *Staph. aureus*.

Effect of Broth Media of Different Compositions—Table II gives the apparent generation rate constants of drug-free and drug-affected *Staph. aureus* cultures in three broth media of different compositions. Variations in generation rate constants among the different media at each concentration level of clindamycin were not significantly different from those observed in the daily variations observed in organism generation rates, as previously was found for *E. coli* (6).

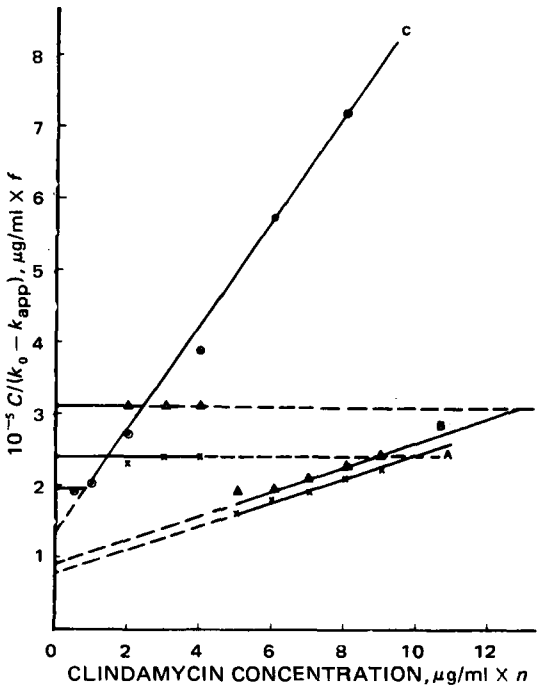


Figure 4—*Applicability of saturation kinetics to the action of clindamycin on* Staph. aureus *and* E. coli *at pH 7.05 and 37.5°. Curve A is for clindamycin-affected* Staph. aureus *in phase I generation (×), curve B is for clindamycin-affected* Staph. aureus *in phase II (▲), and curve C is for clindamycin-affected* E. coli *(⊙). Curves are plotted in accordance with Eq. 3. Actual values of* $C/(k_0 - k_{app})$ *are multiplied by a factor,* f, *where* f = *1000 for curves A and B and* f = *5 for curve C. Actual clindamycin concentrations are also multiplied by a factor,* n, *where* n = *100 for curves A and B and* n = *0.1 for curve C.*

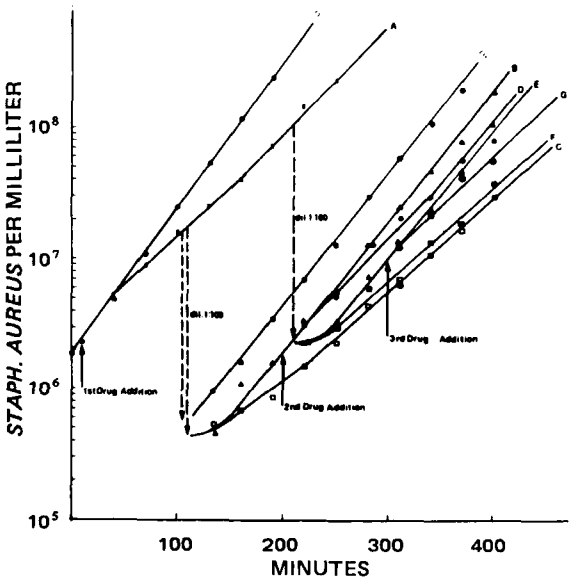


Figure 5—*Semilogarithmic plots of reversibility studies of* Staph. aureus *with time of addition of clindamycin and dilution of the cultures with broth. Curve O is without drug. Curve A is after addition of clindamycin to the culture of curve O to a final concentration of 0.03 μg/ml; curve O_1 is for the drug-free culture of curve O diluted 100-fold with broth. Curves B and E are for the drug-affected culture of curve A diluted 100-fold with broth during phase I and phase II generations, respectively, so that the final clindamycin concentration is reduced to 0.0003 μg/ml. Curves C and F are for the drug-affected culture of curve A diluted 100-fold with broth containing a sufficient amount of clindamycin during phase I and phase II generations, respectively, so that the final clindamycin concentration is reestablished at 0.03 μg/ml. Curve D is for the diluted culture of curve B that has emerged from lag phase and clindamycin has been added to restore the concentration to 0.03 μg/ml. Curve G is for the diluted culture of curve E that has similarly emerged from lag phase and clindamycin has been added to restore the concentration to 0.03 μg/ml.*

Effect of Inoculum Size at Time of Drug Addition—The apparent generation rate constants of drug-free and drug-affected *Staph. aureus* cultures, which varied in organism population at the time of drug addition, are also given in Table II.

There were no significant variations among the generation rate constants for the different organism populations at each concentration level of the clindamycin studied, as previously was found for *E. coli* (6).

Reversibility of Clindamycin Action—The results obtained for reversibility of clindamycin action on *Staph. aureus* at concentration levels of 0.03 and 0.06 μg/ml are shown in Figs. 5 and 6, respectively.

The equilibration of clindamycin between the broth medium and the biophase in *Staph. aureus* was readily achieved. It took 20–25 min after drug addition for cultures affected with 0.03 μg/ml (curve A in Fig. 5) and 0.06 μg/ml (curve A in Fig. 6) to attain a new steady-state phase of generation.

The steady-state generation of drug-free culture (curve O in Figs. 5 and 6), when diluted 100-fold, reverted to a new steady state with a predictable rate constant (curve O_1 in Figs. 5 and 6). The diluted culture showed an apparent initial lag phase period of 5–10 min before reverting to the new steady state. This lag may be attributed to a possible need for cell rejuvenation before reestablishment of the new steady state or to a consequence of the shock of dilution.

Table II—Effect of Variations in Broth Composition and Inoculum Size on Phase I and Phase II Generation Rates of Clindamycin-Affected *Staph. aureus*

Clindamycin Concentration, μg/ml	$k_{app} \times 10^{-5}$ sec^{-1}											
	Phase I			Phase II			Phase I			Phase II		
	B_1[a]	B_2[b]	B_3[c]	B_1	B_2	B_3	I_2[d]	I_2[e]	I_3[f]	I_1	I_2	I_3
0	37.96	41.00	42.90	—	—	—	41.98	40.13	38.50	—	—	—
0.03	28.94	27.64	25.90	28.67	31.33	29.03	28.44	27.53	27.08	30.17	30.40	29.61
0.05	8.60	8.26	7.52	14.98	12.67	15.75	7.07	8.78	8.79	18.21	14.73	12.35
0.07	3.20	3.49	2.76	6.66	7.45	7.96	3.40	3.61	2.97	6.70	6.68	7.77
0.09	0	0	0	2.30	2.24	3.62	0	0	0	2.89	2.65	3.18

[a] Half strength broth (B_1). [b] Normal strength broth (B_2). [c] Double strength broth (B_3). [d] Inoculum size of 5.0×10^5 cells/ml (I_1). [e] Inoculum size of 2.0×10^6 cells/ml (I_2). [f] Inoculum size of 2.0×10^7 cells/ml (I_3).

The generation pattern of drug-affected *Staph. aureus*, when diluted 100-fold, depended on the concentration level of the clindamycin employed. For the culture affected with the 0.03-μg/ml clindamycin concentration, dilution of the culture in phase I (curve B in Fig. 5) or phase II (curve E in Fig. 5) generation resulted in a short lag phase of approximately 20–25 min. Thereafter, a new steady generation was established at a rate similar to that of the control culture (curve O in Fig. 5). This finding shows that the drug action is nullified by high dilution in broth. It also indicates that the rate of dissociation of the drug–receptor complex in the biophase is the same as the rate of formation of complex and/or the rate of diffusion of drug from the broth medium into the biophase through cell membranes.

The addition of clindamycin to the diluted cultures of curves B and E (Fig. 5), which had emerged from the lag phase, to restore the concentration to 0.03 μg/ml resulted in a short period of equilibration, *i.e.*, 5–10 min, followed by a new steady-state generation (curves D and G, respectively) with predictable rate constants. Likewise, a 1:100 dilution of the drug-affected culture of curve A (Fig. 5) during phase I (curve C in Fig. 5) or phase II (curve F in Fig. 5) so as to reestablish the concentration to 0.03 μg/ml resulted in a new steady-state generation with predictable rate constants. The new steady-state generations were preceded by a short lag period of 20–25 min. These observations indicate that clindamycin action on *Staph. aureus* at a concentration level of 0.03 μg/ml is rapidly reversible and is similar to that reported (6) for *E. coli* at a

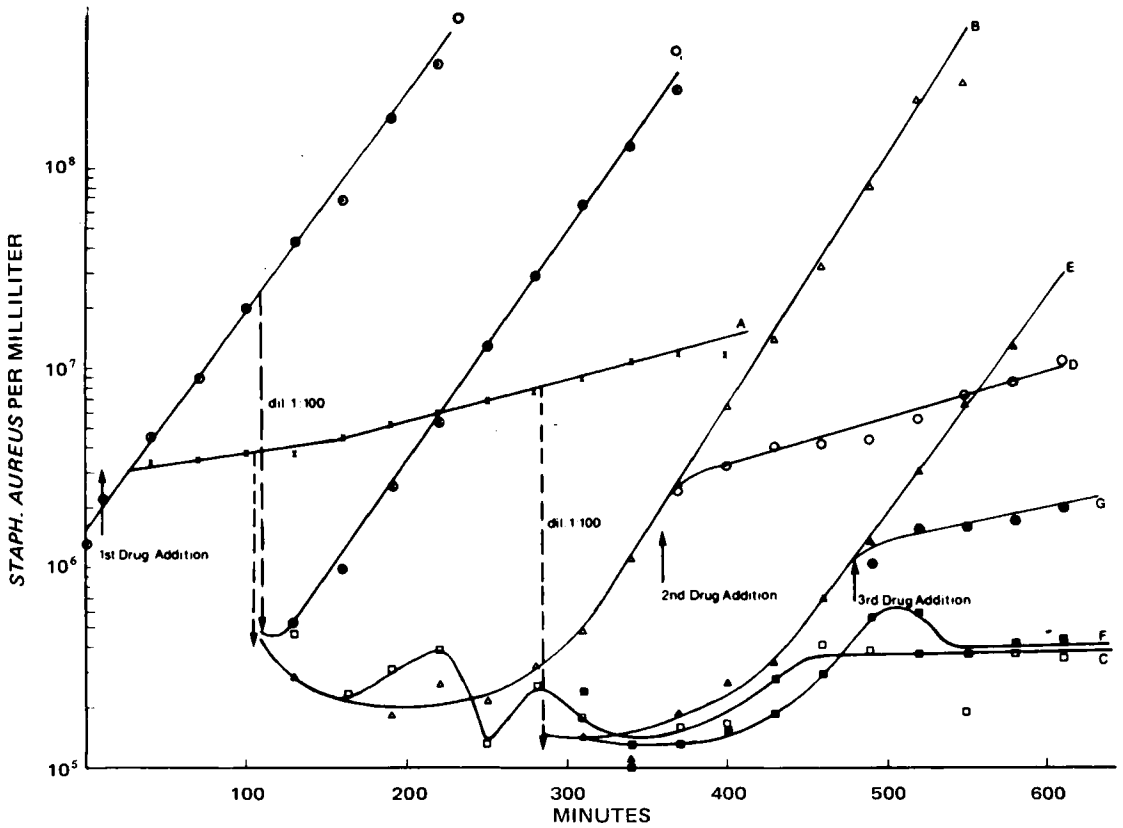


Figure 6—*Semilogarithmic plots of reversibility studies of* Staph. aureus *with time of addition of clindamycin and dilution of the cultures with broth. Curve O is without drug. Curve A is after addition of clindamycin to the culture of curve O to a final concentration of 0.06 μg/ml; curve O_1 is for the drug-free culture of curve O diluted 100-fold with broth. Curves B and E are for the drug-affected culture of curve A diluted 100-fold with broth during phase I and phase II generations, respectively, so that the final clindamycin concentration is reduced to 0.0006 μg/ml. Curves C and F are for the drug-affected culture of curve A diluted 100-fold with broth containing a sufficient amount of clindamycin during phase I and phase II generations, respectively, so that the final clindamycin concentration is reestablished at 0.06 μg/ml. Curve D is for the diluted culture of curve B that has emerged from lag phase and clindamycin has been added to restore the concentration to 0.06 μg/ml. Curve G is for the diluted culture of curve E that has similarly emerged from lag phase and clindamycin has been added to restore the concentration to 0.06 μg/ml.*

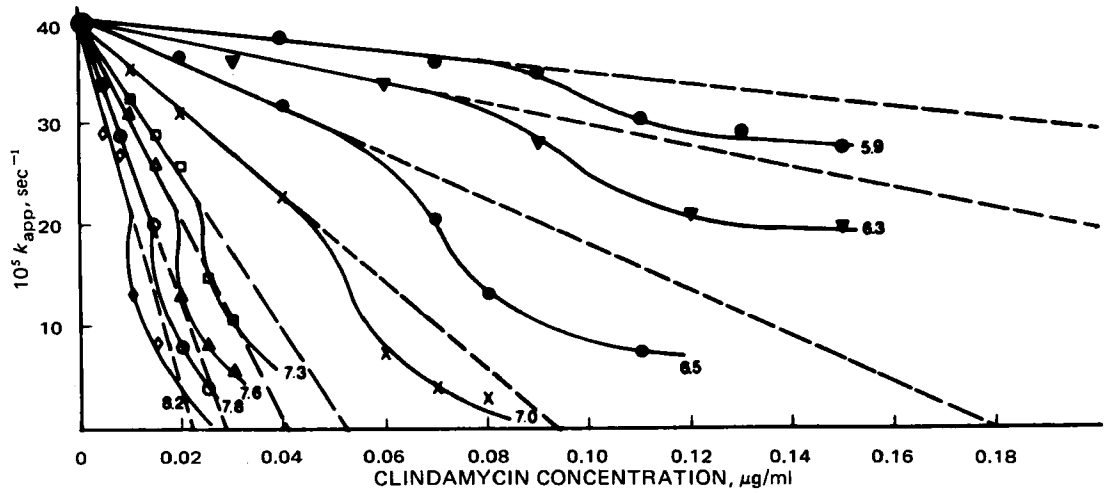


Figure 7—*Dependence of apparent generation rate constant,* k_{app} *in seconds*$^{-1}$*, of clindamycin-affected* Staph. aureus *cultures in phase I generation on the pH of the broth medium. Curves are labeled with the pH values.*

concentration level of 33.34 μg/ml (6).

On the other hand, a 1:100 dilution of the culture affected with clindamycin at a concentration of 0.06 μg/ml during phase I (curve B in Fig. 6) or phase II (curve E in Fig. 6) generation resulted in a prolonged lag period of approximately 150–200 min. Subsequently, a new steady-state generation was established at a rate similar to that of the control culture (curve O in Fig. 6). This finding indicates that the drug action is nullified by the high dilution of the culture. However, even if the time for rejuvenation of the cells or the consequence of shock on dilution is considered, the prolonged lag period observed for the diluted culture to attain a new steady-state generation suggests that the rate of dissociation of the drug–receptor complex in the biophase is less than the rate of drug diffusion from the broth medium into the biophase through cell membranes. Alternatively, it may suggest that there is a kill of the greater proportion of cells by a bactericidal or lytic phenomenon, which leaves relatively few survivors whose lag phase becomes unduly prolonged.

The addition of clindamycin to the diluted cultures of curves B and E (Fig. 6), which had emerged from the lag phase, to restore the concentration to 0.06 μg/ml resulted in a short period of equilibration, *i.e.*, 20–25 min. Subsequently, a new steady-state generation was established with predictable rate constants (curves D and G in Fig. 6). However, a 1:100 dilution of the drug-affected culture of curve A (Fig. 6) during phase I (curve C in Fig. 6) or phase II (curve F in Fig. 6) generation to reestablish the concentration of clindamycin in the broth to 0.06 μg/ml produced an initial phase of erratic generation curves followed by a stationary phase. This finding may indicate a type of generation inhibition in which an increase in the number of survivor cells is offset by a decrease in the number of cells killed by a bactericidal or lytic phenomenon. Since the dilution did not result in changes in thermal or osmotic conditions of the environment, it is concluded that lysis of the cells could occur from permanent damage or injury of cell membranes and/or cell walls resulting in leakage of cell contents or as a consequence of autolysis of cells killed by bactericidal action of clindamycin at a concentration level of 0.06 μg/ml.

The action of clindamycin at the high concentration level (0.06 μg/ml) clearly was not as readily and rapidly reversible as at the low concentration level (0.03 μg/ml). Furthermore, a bactericidal or lytic phenomenon could be superimposed on normal generation inhibition at the high concentration level.

Effect of pH on Drug-Affected Generation Rates—Apparent generation rate constants obtained for *Staph. aureus* in broth at pH 5.9–8.2, in the absence and presence of graded concentrations of clindamycin, are plotted in Fig. 7. The generation rate constants for drug-free cultures were invariant with pH, but those for drug-affected cultures were significantly decreased as the pH was increased at the same clindamycin concentration. The sigmoidal shape of the curve obtained from plots of the generation rate constant *versus* drug concentration at pH 7.05 (Fig. 3) was reproduced at all other pH values studied.

The calculated values of k_c, obtained from slopes of plots of k_{app_I} *versus* clindamycin concentration, *C*, in accordance with Eq. 2, are plotted as a function of pH (curve C) in Fig. 8. The calculated values k_a, obtained from intercepts of plots of $C/(k_0 - k_{app_I})$ *versus C* in accordance with Eq. 3, are also plotted as a function of pH (curve B in Fig. 8). The values for k_c and k_a increased about 10-fold per unit increase in pH over the 5.9–7.0 range, but the slopes of the plots tended to lessen, in both cases, as the pH approached the pKa value of clindamycin. The plots of k_a *versus* pH obtained from the data (6) of clindamycin action on *E. coli* (curve A in Fig. 8) show similar evidence.

DISCUSSION

The suitability of a particle-size counter for monitoring the organism population in an estimate of apparent generation rate constants of drug-affected *E. coli* cultures has been well established (8). Its applicability for similar determinations on drug-affected *Staph. aureus* cultures recently was reported (7). The present work also confirms that the method is applicable for determining

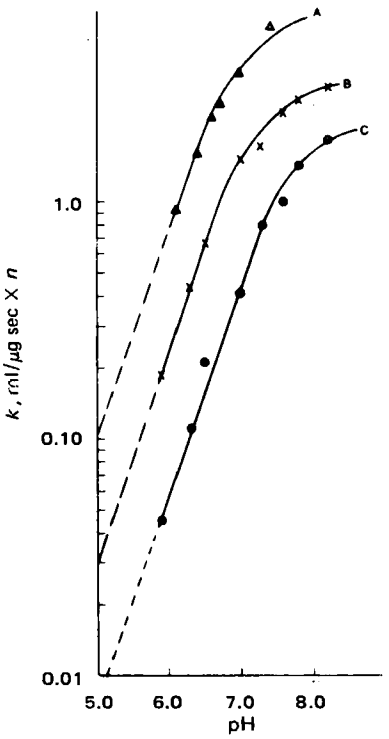


Figure 8—*Semilogarithmic plots of apparent inhibitory rate constant,* k *(in milliliters per microgram seconds), for the action of clindamycin on* E. coli *(curve A) and* Staph. aureus *(curves B and C). In curves A and B,* k *values are the* k_a *values derived from the intercepts of plots of* $C/(k_0 - k_{app})$ versus C *in accordance with Eq. 3; k values in curve C are the* k_c *values derived from the slopes of plots of* k_{app} versus C *in accordance with Eq. 2.*

the generation rates of *Staph. aureus* and *E. coli* cultures affected with subcompletely inhibitory concentrations of clindamycin.

At the low clindamycin concentration level (0–0.04 μg/ml), the total count on *Staph. aureus*-affected cultures was 50% of the viable count (Fig. 1). This finding is attributed (7) to the dissociation of cell aggregates by the high dilution steps and/or subsequent generation of the culture samples withdrawn for the viable count procedure, which result in relatively high colony counts. However, the generation curves obtained from the total count remained parallel to those of the viable count. Reversibility studies (Fig. 5) demonstrated a rapidly reversible action of the clindamycin on *Staph. aureus* cells. The conclusion is that there is no kill or death of the organisms superimposed on normal generation inhibition by the action of clindamycin in this low concentration range.

At the high clindamycin concentration level (> 0.04 < 0.1 μg/ml), the total count on the drug-affected *Staph. aureus* in phase I generation was increased to 80–90% of the viable count (Fig. 1). This finding suggests a condition of "dynamic bacteriostasis" (9), in which the rate of kill of the organisms is balanced by the rate of generation of survivor cells in the culture. Consequently, the total count remains virtually the same as the viable count. During the phase II generation of the cultures, however, there was an apparent increase in the rate of generation obtained by the total count. This result may be attributed to an increased rate of generation of mutant-resistant survivor cells that are counted indiscriminately with dead cells by the particle counter. The corresponding viable counts, on the other hand, showed that the rate of generation was: *(a)* increased to a relatively less extent than the increase obtained by total count for the culture affected with a subcompletely inhibitory clindamycin concentration of 0.05 μg/ml, *(b)* unchanged for the culture affected with an inhibitory clindamycin concentration of 0.07 μg/ml, or *(c)* slightly decreased for the culture affected with the clindamycin concentration of 0.08 μg/ml.

These observations indicate that there is a kill and/or poor recovery of a large percentage of cells damaged or injured by a bactericidal or lytic phenomenon as a function of drug concentration and drug–bacteria contact time. Furthermore, the clindamycin action in this subcompletely inhibitory concentration range is not readily and rapidly reversible (Fig. 6). The inference is that a bactericidal or lytic phenomenon may be superimposed on generation inhibition of the *Staph. aureus* cultures by the action of clindamycin. At very high clindamycin concentrations (> 0.1 μg/ml), there was a rapid drop in the viable count with time as a function of drug concentration whereas the total count remained constant with time and was invariant with drug concentration.

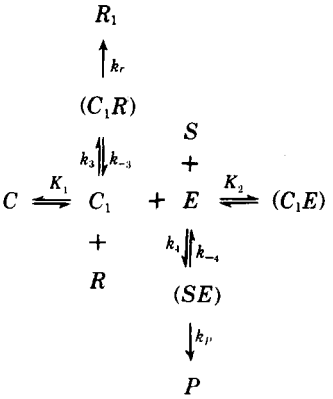
These findings demonstrate unequivocally that clindamycin exerts a bactericidal effect on the cultures in this concentration range. In contrast, the viable count of clindamycin-affected *E. coli* was 50–80% of the total count (Fig. 2), and the generation curves obtained from the viable count remained parallel to those obtained from the total count at any concentration level. There was no evidence of a bactericidal or lytic phenomenon superimposed on generation inhibition by the action of clindamycin on *E. coli* in the subcompletely inhibitory concentration range of 0–100 μg/ml or above the inhibitory concentration levels, *e.g.*, 200 μg/ml. It is, therefore, quite evident that clindamycin exerts a "true" bacteriostatic action (9) on *E. coli*.

Staph. aureus cultures affected with subcompletely inhibitory concentrations of clindamycin exhibited biphasic steady-state generation curves (Fig. 1). The apparent generation rate constant (k_{app_I}) of the initial (phase I) generation was less than the apparent generation rate constant ($k_{app_{II}}$) of the subsequent (phase II) generation of the clindamycin-affected *Staph. aureus*, suggesting organism adaptation or development of resistance to drug action after a finite period of drug–bacteria contact. On the other hand, *E. coli* cultures affected with subcompletely inhibitory concentrations of clindamycin exhibited monophasic steady-state generation curves (Fig. 2). There was no evidence of organism adaptation or development of resistance to drug action.

The functional dependency of the apparent generation rate constant, k_{app}, on drug concentration for clindamycin-affected *Staph. aureus* in phase I was the same as that of the phase II generation (Fig. 3), suggesting a common mechanism of action in both generation phases. Therefore, the plots of k_{app} *versus* drug concentration for phase I and phase II generations are superimposable by a potency factor of 0.80 over the entire concentration range studied. This finding indicates that only 80% of the available drug concentration in broth is effective against the resistant mutants of *Staph. aureus* in phase II generation.

The curve for the dependence of the k_{app} on drug concentration for the clindamycin-affected *Staph. aureus* has a sigmoidal shape similar to that recently reported for the action of fusidate sodium (10) and quinacrine (11) on *E. coli*. It has been shown from theoretical considerations (10) that this shape can be attributed to either: *(a)* a saturable enzymatic degradation of the drug in the biophase of the microorganism or *(b)* drug binding to saturable binding sites in the medium. Table II shows no evidence of interference in clindamycin action on *Staph. aureus* to suggest that degradation of the drug occurs from microbial metabolic activities as a function of organism numbers or that a depletion of free or unbound drug in the broth medium is produced by binding, absorption, or inactivation processes as a result of variations in broth composition. On the other hand, there is demonstrated evidence of a bactericidal phenomenon which operates concurrently with generation inhibition at subcompletely inhibitory concentrations of clindamycin (>0.04 μg/ml). It is possible, therefore, that perturbations in the kinetics of the drug inhibitory action produced by the onset of a bactericidal action may account for the sigmoidal shape of the curve for the dependence of k_{app} on drug concentration.

A model consistent with the dual mechanism of clindamycin action on *Staph. aureus* is shown in Scheme I.



Scheme I

In this model, C is the clindamycin concentration in the broth medium, which is in equilibrium with C_1, the clindamycin concentration within the cell or biophase that reversibly binds with enzyme, E, to form the drug–enzyme complex, C_1E; S is the normal substrate (or metabolite) synthesized by the enzyme into the protein product, P, from the substrate–enzyme complex, SE, and is utilized in cell development and generation. The clindamycin concentration in the biophase also reacts irreversibly with a "target" site (or vital structure), R, to form a damaged (or injured) site or a toxic product, R_1, from a reaction complex, C_1R. The target site may be a sensitive compound of the cytoplasmic membrane, protoplasmic or nuclear material, or a metabolic or transport agent, which is essential for organism survival (12–16). The following equations apply if it is postulated that:

1. The rate of increase in microbial numbers, dN/dt, is proportional to the net rate of microbial generation, dG/dt, above microbial death, dD/dt, and to the number, N, of organisms present.
2. The rate of microbial generation, dG/dt, is proportional to the net rate of protein synthesis, dP_1/dt, above a certain minimum, Δp, required for life-sustaining processes and to the number, N, of organisms present.
3. The rate of microbial death, dD/dt, is proportional to the rate of formation of the damaged (or injured) site or the toxic product and to the number, N, of organisms present.

The equations are:

$$dN/dt = k_n(dG/dt - dD/dt)N = k_{app}N \qquad \text{(Eq. 5)}$$

where k_n is a constant of proportionality and k_{app} is the apparent generation rate constant of the drug-affected culture, and:

$$dG/dt = k_g(dP_1/dt)N = k_g(dP/dt - \Delta p)N \qquad \text{(Eq. 6)}$$

where k_g is a constant of proportionality and dP/dt is the overall

rate of protein synthesis. Moreover:

$$dD/dt = k_d(dR_1/dt)N \quad \text{(Eq. 7)}$$

Combination of Eqs. 5–7 yields:

$$k_{app} = k_n\{k_g(dP/dt - \Delta p) - k_d(dR_1/dt)\} \quad \text{(Eq. 8)}$$

On the basis of Scheme I, it can be stated that:

$$dP/dt = (k_pk_4/k_p + k_{-4})(S)(E) = k_p{}^1E = k_p{}^1(1 - \theta)E_t \quad \text{(Eq. 9)}$$

where $k_p{}^1 = (k_pk_4/k_p + k_{-4})S$, S is assumed invariant, and $\theta = C_1E/E_T$ is a fraction of total enzyme, E_t, bound to the drug.

The value of θ is explicitly defined by a previously derived equation (1, 6) as follows:

$$\theta = K_1K_2C/(1 + K_1K_2C) \quad \text{(Eq. 10)}$$

where K_1 is the drug partition constant through cell membranes and K_2 is the drug affinity constant for the enzyme, E.

Combination of Eqs. 9 and 10 yields:

$$dP/dt = k_m\{1 - [K_1K_2C/(1 + K_1K_2C)]\} \quad \text{(Eq. 11)}$$

where $k_m = k_p{}^1E_t$.

Also, under steady-state conditions:

$$dR_1/dt = (k_rk_3/k_r + k_{-3})(C_1)(R) \quad \text{(Eq. 12)}$$

and:

$$C_1 = K_1C \quad \text{(Eq. 13)}$$

where K_1 is as defined in Eq. 10.

Combination of Eqs. 12 and 13 yields:

$$dR_1/dt = (k_rk_3/k_r + k_{-3})(K_1)(C)(R) \quad \text{(Eq. 14}a\text{)}$$

$$dR_1/dt = k_eC \quad \text{(Eq. 14}b\text{)}$$

where $k_e = (k_rk_3/k_r + k_{-3})(K_1)(R)$, and R is assumed to be comprised of an infinitely large number of molecules (12) so that the rate of formation of R_1 becomes only dependent on drug concentration. Substitution of Eq. 14*b* into Eq. 7 yields:

$$dD/dt = (k_dk_eC)N = (k_uC)N = -dN/dt \quad \text{(Eq. 15)}$$

where $k_u = k_dk_e$ is the death rate constant, and $-dN/dt$ is the rate of decrease in microbial numbers. Equation 15 is the typical equation of the "mechanistic theory" that attempts to explain the bactericidal process of many compounds (12–20).

Combining Eqs. 8, 11, and 14*b* and simplifying give:

$$k_{app} = k_nk_g(k_m - \Delta p) - [k_nk_gk_mK_1K_2C/(1 + K_1K_2C) + k_dk_eC] \quad \text{(Eq. 16}a\text{)}$$

$$k_{app} = k_0 - \{[k_{a_1}C/(1 + k_bC)] + k_uC\} \quad \text{(Eq. 16}b\text{)}$$

where k_0 is the generation rate constant of the drug-free culture, $k_b = K_1K_2$, $k_{a_1} = k_nk_gk_mK_1K_2$, and k_u is as defined in Eq. 15. Therefore:

$$k_a/k_b = k_nk_gk_m > k_0 \quad \text{(Eq. 17)}$$

Equation 16*b* describes the functional dependencies of k_{app} on the drug concentrations for clindamycin-affected *Staph. aureus* in phase I and phase II generations (Fig. 3). At low clindamycin concentrations in phase I action (0–0.04 μg/ml) or in phase II action (0–0.05 μg/ml), when the death rate is exceedingly small so that $k_uC \rightarrow 0$ and when few enzyme sites are possibly interacted with drug to inhibit protein synthesis so that $k_bC \ll 1$, Eq. 16 simplifies to Eq. 2, which is the expression for the observed linear dependence of k_{app} on drug concentration in these concentration ranges (Fig. 3).

At high drug concentrations, it is possible that the extent of generation inhibition is decreased with an increase in drug concentration because of the saturation of enzyme sites by the drug, *i.e.*, $k_bC > 1$. Under such conditions, the k_{app} should decrease asymptotically to zero with an increase in drug concentration similar to that observed for lincomycin action on *Staph. aureus* (7). However, an onset of a bactericidal action, when $k_uC \gg 0 < 1$, causes a reduction in the numbers of viable cells in the active phase of multiplication to alter the functional dependency of the k_{app}. The overall interaction of bacterial death from a bactericidal action, with generation inhibition as a consequence of decreased rate of protein synthesis by the clindamycin action, results ultimately in a sigmoidal shape of the curve for the dependence of k_{app} on drug concentrations, which is described by Eq. 16*b*.

Transformation of Eq. 16*b* yields:

$$C/(k_0 - k_{app}) = Ck_b/[(k_{a_1} + k_u) + k_bk_uC] + 1/[(k_{a_1} + k_u) + k_bk_uC] \quad \text{(Eq. 18)}$$

If k_uk_bC values are extremely small compared to exceedingly large $k_{a_1} + k_u$ values, then Eq. 18 simplifies to Eq. 3 as follows:

$$C/(k_0 - k_{app}) = k_bC/(k_{a_1} + k_u) + 1/(k_{a_1} + k_u) = k_bC/k_a + 1/k_a \quad \text{(Eq. 19)}$$

where $k_a = k_{a_1} + k_u$.

Equation 19 is adhered to by the data of Fig. 3 from linear plots of $C/(k_0 - k_{app})$ *versus* C for clindamycin concentrations greater than 0.04 μg/ml in phase I action (curve A in Fig. 4) or greater than 0.05 μg/ml in phase II action (curve B in Fig. 4) and may indicate a saturable mechanism of the clindamycin action at high concentration levels. The plots are, however, completely displaced from those of clindamycin action in the low concentration range, *i.e.*, 0–0.04 μg/ml in phase I or 0–0.05 μg/ml in phase II action, which do not adhere to the saturable receptor site model. This finding confirms that a different mechanism of action (*i.e.*, the bactericidal action) is superimposed on generation inhibition at the saturable level.

Table II shows kinetic parameters derived from plots of the data of Fig. 3 in accordance with Eqs. 12 and 19. The k_a/k_b value for *Staph. aureus* is 1.35 times the k_0 value, which is in agreement with theoretical predictions of Eq. 17. The calculated value of k_b in Eq. 19 for the phase I generation is 1.20 times the value for the phase II generation. Since k_b is derived from K_1 and K_2 as defined in Eq. 10, it can be rationalized for clindamycin–*Staph. aureus* interactions, as in the case of lincomycin–*Staph. aureus* interactions (7), that K_2 is decreased in the phase II generation of the drug-affected cultures. The resistant mutant strains in the phase II generation may have ribosomal components whose binding affinity for clindamycin is less than that of the initial strains in the phase I generation. Therefore, the observation that 80% of the total drug concentration in broth is kinetically active against the drug-affected organism in phase II generation is attributed to this phenomenon.

The dependence of the generation rate constant, k_{app}, on drug concentration, C, for clindamycin-affected *E. coli* is linear at low drug concentrations (0–10 μg/ml) but is curvilinear at high drug concentrations (>10 μg/ml) when it also decreases asymptotically to zero with an increase in drug concentration (Fig. 3). An operative kinetic model (6), which defined the action of lincomycin and its 7(*S*)-chloro analogs, is applicable. Clindamycin action on *E. coli* is explained as competitive binding of a saturable receptor site, which is also the binding locus for a metabolite normally utilized by the receptor site in the synthesis of protein for microbial generation. The functional dependency of k_{app} on clindamycin (curve B in Fig. 3) is explicitly defined by the equation:

$$k_{app} = k_0 - k_aC/(1 + k_bC) \quad \text{(Eq. 20)}$$

where k_0, k_a, and k_b are as defined in Eq. 10. Adherence to a saturable receptor site model is observed from linear plots of $C/(k_0 - k_{app})$ *versus* C (Fig. 4), obtained from the data of Fig. 3 in accordance with the expression in Eq. 3 for clindamycin concentrations greater than 10 μg/ml. Deviations occur at low concentrations (0–10 μg/ml), when only few receptors can possibly be interacted with drug.

The calculated value of k_b (Table II) for clindamycin-affected *E. coli* is 0.21% of the value for clindamycin-affected *Staph. aureus* in phase I or 0.25% of the value for clindamycin-affected *Staph. aureus* in phase II generation. Since $k_b = K_1K_2$ as defined in Eq. 10, it is inferred that clindamycin is far more readily partitioned through cell membranes into the biophase and/or has exceedingly higher affinity for receptors in *Staph. aureus* than in *E. coli*, due possibly to differences in gross morphology and physiology of the organisms.

At the MIC, C_m, when $k_{app} = 0$, Eq. 3 simplifies to Eq. 7 and the calculated values of C_m (Table II) for clindamycin action against

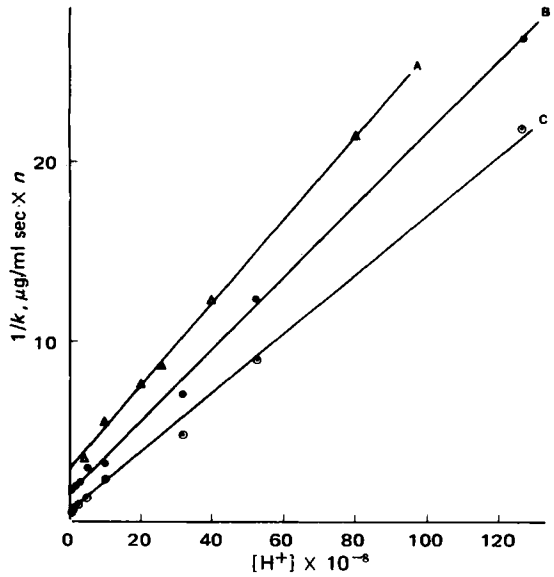


Figure 9—*Linearity of plots for 1/k (micrograms per milliliter second)* versus *[H+] in accordance with Eq. 22 for the action of clindamycin on* E. coli *(curve A) and* Staph. aureus *(curves B and C). In curves A and B,* k *values are the* k_a *values derived from the intercepts of plots of* $C/(k_0 - k_{app})$ versus C *in accordance with Eq. 3;* k *values in curve C are the* k_c *values derived from the slopes of plots of* k_{app} versus C *in accordance with Eq. 2.*

E. coli are 1000 times that against *Staph. aureus*, which is attributed to differences in the modes of clindamycin action and the nature of the respective organisms.

The pH of the broth medium influences the activity of clindamycin on *Staph. aureus* (Fig. 7) in a manner similar to what was observed for its action on *E. coli* (6). The k_{app} values of drug-affected cultures decrease with an increase in the pH at any specified concentration. Therefore, progressively larger quantities of the drug are needed to produce the same generation inhibition as the pH is decreased. The sigmoidal shape of curve for the k_{app} dependence on drug concentration is observed at all other pH values studied. The constant k_c (milliliters per microgram second), as defined in Eq. 2, and the constant k_a (milliliters per microgram second), as defined in Eq. 3, for various pH values from the data of drug-affected *Staph. aureus* in Fig. 7, or the constant k_a (milliliters per microgram second), as defined in Eq. 3, for various pH values obtained from previous data (6) of drug-affected *E. coli* adhere to the expression:

$$k = k^* f K_a/(K_a + [H^+]) \qquad \text{(Eq. 21)}$$

where k is either k_c or k_a, k^* is the intrinsic value of k for the unprotonated drug molecule, f is the fraction of the unprotonated drug, K_a is the dissociation constant of the unprotonated base, and $[H^+]$ is the hydrogen-ion concentration. The plots of log k *versus* pH (Fig. 8) approach a slope of unity when $[H^+] > K_a$ but have a null slope when $K_a > [H^+]$, suggesting that the unprotonated drug molecule is the active species. The linear plots of $1/k$ *versus* $[H^+]$ in Fig. 9 are obtained from the data of Fig. 8 in accordance with an expression derived from Eq. 21:

$$1/k = [H^+]/(k^* K_a) + 1/k^* \qquad \text{(Eq. 22)}$$

The pKa and k^* values of clindamycin, derived from the slopes and intercepts of the plots for drug-affected *Staph. aureus* (curves B and C in Fig. 9) and *E. coli* (curve A in Fig. 9) are given in Table III. The kinetically derived pKa values are in reasonably good agreement with the 7.0 value (6) obtained by potentiometric titration. It is concluded that Eq. 21 holds and that the unprotonated form of the clindamycin molecule contributes to activity because of its ready penetrability through cell membranes of Gram-positive and Gram-negative organisms.

Table III—Values of pKa and Intrinsic Inhibitory Rate Constants (k^*) for Clindamycin Action on *Staph. aureus* and *E. coli*

Organism	$10^5 k_c^{*a}$	$10^5 k_a^{*b}$	Kinetically[c] Determined pKa	Potentiometrically[d] Determined pKa
Staph. aureus	—	3333.34	7.2	7.0
Staph. aureus	1666.67	—	7.4	7.0
E. coli	—	6.67	6.9	7.0

[a] As determined from the intercept of the plot of $1/k_c$ *versus* $[H^+]$ in accordance with Eq. 22, where k_c is obtained from the data of Fig. 7 and in accordance with Eq. 2. [b] As determined from the intercept of the plot of $1/k_a$ *versus* $[H^+]$ in accordance with Eq. 22, where k_a is obtained from the data of Fig. 7 for *Staph. aureus* or from the data of Ref. 6 for *E. coli* and in accordance with Eq. 3. [c] Calculated from the quotient of the slope and intercept of the plots in Footnote *a* or *b*, whichever is applicable. [d] Reference 6.

REFERENCES

(1) J. D. Mason, A. Dietz, and C. DeBoer, *Antimicrob. Ag. Chemother.*, **1962**, 554.

(2) W. E. Herrell, "Lincomycin," Modern Scientific Publications, Chicago, Ill., 1969, p. 36.

(3) B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *Antimicrob. Ag. Chemother.*, **1966**, 727.

(4) B. Weisblum and J. Davies, *Bacteriol. Rev.*, **32**, 493(1968).

(5) M. L. Celma, R. E. Monro, and D. Vazquez, *FEBS Lett.*, **13**, 247(1971).

(6) S. M. Heman-Ackah and E. R. Garrett, *J. Med. Chem.*, **15**, 152(1972).

(7) S. M. Heman-Ackah, *J. Pharm. Sci.*, **63**, 1077(1974).

(8) E. R. Garrett, *Progr. Drug. Res.*, **15**, 27(1971).

(9) Parkinson, Ph.D. thesis, London, England, 1954; cited by A. M. Cook, *J. Pharm. Pharmacol.*, **6**, 629(1954).

(10) E. R. Garrett and A. Richards, *J. Pharm. Sci.*, **63**, 884(1974).

(11) A. Richards and E. R. Garrett, *ibid.*, **63**, 894(1974).

(12) O. Rhan, *J. Gen. Physiol.*, **13**, 179(1929).

(13) C. Hinshelwood, *Nature*, **167**, 666(1951).

(14) O. Rhan, *Biodynamica*, **4**, 81(1943).

(15) W. B. Hugo, in "Disinfection," M. A. Benarde, Ed., Dekker, New York, N.Y., 1970, p. 31.

(16) T. D. Brock, "Biology of Microorganisms," Prentice-Hall, Englewood Cliffs, N.J., 1970, p. 237.

(17) H. Chick, "A System of Bacteriology in Relation to Medicine," I. H. M. Stationary Office, London, England, 1930.

(18) E. R. Withell, *J. Hyg.*, **42**, 124(1942).

(19) H. Berry and I. Michaels, *Quart. J. Pharm.*, **20**, 348(1947).

(20) G. S. Wilson and A. A. Miles, "Principles of Bacteriology and Immunity," vol. I, Williams & Wilkins, Baltimore, Md., 1964, p. 152.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 23, 1974, from the *Department of Pharmacy, College of Pharmacy and Pharmacal Sciences, Howard University, Washington, DC 20059*

Accepted for publication February 5, 1975.

The author is grateful to Dr. Ira C. Robinson, Dean of the College, for his interest and assistance in this research project. He is also indebted to Miss Patricia Ann Wright for technical assistance.