Kinetics and Mechanisms of Drug Action on Microorganisms XXII: Effects of Aminosidine with and without Organism Pretreatment with Bacteriostatic Agents

EDWARD R. GARRETT ˣ and JOHN K. LEWIS

Abstract □ *Escherichia coli* generation in the logarithmic growth phase was inhibited in peptone broth USP at pH 7.0 without kill below 3.0 μg/ml of aminosidine. Above this value, the logarithms of the number of viables of the drug-treated culture ultimately decreased linearly with time and the slopes of these plots were independent of concentration. A concentration-dependent lag in the time of attainment of the cidal action was observed, and the extent of this lag was related to the ease of emergence of resistant organisms. The minimal concentration for cidal action increased with increasing concentrations of nutrients and with decreasing pH. Pretreatment of the cultures with novobiocin and tetracycline lessened the minimum bactericidal concentration of aminosidine whereas chloramphenicol pretreatment increased it. Tetracycline pretreatment inhibited the emergence of aminosidine-resistant organisms.

Keyphrases □ Aminosidine—kinetics and mechanisms of action, microorganisms, with and without pretreatment □ Antibiotics—aminosidine, kinetics and mechanisms of action, microorganisms, with and without pretreatment □ *Escherichia coli*—kinetics and mechanisms of aminosidine action, with and without pretreatment

Aminosidine is an oligosaccharide antibiotic isolated from *Streptomyces chrestomyceticus* (1). Structural studies indicated that the major component of aminosidine is identical with that of paromomycin and that aminosidine belongs to the neomycin group of aminoglycoside antibiotics (2). The nature of aminosidine or paromomycin activity indicates that it is closely related to streptomycin in its mode of action (3) in causing misreading of messenger-RNA. The bactericidal action may be due primarily to an irreversible binding to the ribosomes (3, 4).

The purpose of this work was to study the effects of aminosidine on a balanced growth culture of *Escherichia coli* as part of ongoing research into the kinetics and mechanisms of antibiotic action on microbial generation (5–7).

EXPERIMENTAL

Bacterial Cultures—The test organism, *Escherichia coli* (ATCC 12407) (5, 6), and the antibiotic medium 3[1] rehydrated to peptone broth USP (6) were as described previously. Studies were also conducted at twice and half the normal nutrient composition. The pH of the autoclaved medium, normally 7.0, was adjusted between 6.1 and 7.8 with sterile filtered 1 *N* HCl and 1 *N* NaOH for studies on pH effects

An aliquot (10 ml) of culture medium was inoculated from a fresh agar slant, and the culture generated for 15 hr at 37.5°. An aliquot (0.5 ml) was then diluted into 49.5 ml of fresh culture medium and generated to 2×10^7 *E. coli*/ml. An aliquot (0.5 ml) of this culture was treated similarly, and an aliquot of this final culture was diluted 100-fold into bulk broth. The suspension was dispensed by a sterile 49.5-ml pouring head into separate, sterile, loosely capped conical culture flasks maintained at 37.5 ± 0.01° in a constant-temperature water bath equipped with a shaker. After 25 min, when the organisms were in the logarithmic growth phase, 0.50 ml of an appropriate stock solution of antibiotic was added. In addition, the antibacterial effects of aminosidine[2] were studied at various inoculum concentrations of organisms.

The generation of viable and total *E. coli* as affected by aminosidine sulfate[2] concentrations was monitored by previously described methods (5–7); typical examples are shown in Figs. 1 and 2. Similar studies were conducted with paromomycin sulfate[3].

Bacterial Resistance to Aminosidine Action—Cultures of resistant organisms were prepared by overnight growth in the presence of 4.0, 5.0, and 6.0 μg/ml of aminosidine. Aliquots (0.5 ml) were diluted 100-fold with drug-free medium and subcultured twice at 1×10^7 organisms/ml. The presumed resistant organisms, inoculated at 1×10^5 organisms/ml in the logarithmic growth phase, were permitted to grow in fresh medium. At 60 min, drug concentrations ranging from 3.5 to 6.0 μg/ml were effected in different flasks and the generation rates were monitored. The various flasks were filtered at 1×10^9 organisms/ml, and the filtrates were stored at 4° for 17 hr. They then were inoculated with logarithmically generating naive organisms at 37.5°, and the generation rates were monitored.

Aminosidine Combinations with Bacteriostatic Agents—The effects on generation rates of aminosidine combinations with growth-inhibiting concentrations of the bacteriostatic chloramphenicol[4], tetracycline[5], or novobiocin[6] at pH 7.00 were studied by adding aminosidine subsequent to the establishment of drug-affected steady-state generation rates with one of the former.

Cell Size Distribution—The size–frequency distributions and total counts of the generating organisms in the presence of aminosidine were recorded with time using a pulse height analyzer[7]. This instrument had been calibrated with 1.305-μm diameter polytoluene latex beads.

Aminosidine pKa Values—Nitrogen-purged aminosidine sulfate solutions (10 ml containing 3 mg), to which a measured quantity of 1 *N* HCl had been added, were potentiometrically titrated[8] with 0.10 *N* NaOH. Volumes without drug and treated similarly were also titrated.

RESULTS AND DISCUSSION

Effect of Aminosidine on Microbial Generation—The numbers of *E. coli* as a function of time as affected by various concentrations of aminosidine are plotted semilogarithmically in Figs. 1 and 2. At bactericidal concentrations, the action of the drug demonstrated an initial lag period with a subsequent increasing diminution of generation rate. The numbers of viables tended to increase to a maximum and then decrease to approach an apparent first-order rate of dying, $k_{app} = -5.36 \times 10^{-4}$/sec at 37.5°, which was independent of drug concentration above the minimum necessary to exercise killing action. However, there was a relationship between the time required to achieve this first-order process of dying and drug concentration in the medium (Figs. 1 and 3), which

[1] Difco Laboratories, Detroit, Mich.

[2] Lot 515, Farmitalia Research Laboratories, Milan, Italy.
[3] Lot EPP96-I, Parke-Davis and Co., Detroit, MI 48232
[4] Lot H22254, Parke-Davis and Co., Detroit, MI 48232
[5] Lot U5965 (HCl) 4335R, The Upjohn Co., Kalamazoo, Mich.
[6] Albamycin U-6591, Lot 1FT33, The Upjohn Co., Kalamazoo, Mich.
[7] Coulter Channelyzer, Coulter Electronics Inc., Hialeah, FL 33010
[8] Radiometer TTTlc titrator with SBR2C titrigraph and SBU1a syringe burette, The London Co., Westlake, Ohio.

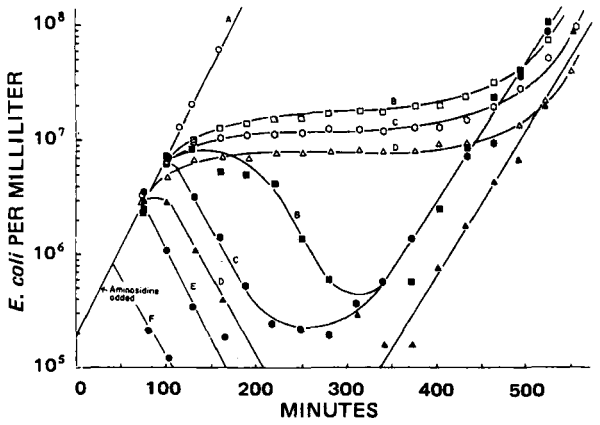

Figure 1—*Drug-affected death rates of* E. coli *in the presence of aminosidine sulfate at pH 7.00 in peptone broth USP. Open symbols are total counts, and solid symbols are viable counts. Key: A, 0.0 μg/ml; B, 4.0 μg/ml; C, 4.5 μg/ml; D, 5.0 μg/ml; E, 5.5 μg/ml; and F, 15 μg/ml.*

can be assigned to concentration-dependent processes of rates of drug permeation into the cell and reaction with receptor sites.

The fact that, after a definitive period of organism kill, there is a subsequent generation of viable organisms (Fig. 1) indicates: (*a*) development of drug-resistant organisms, (*b*) drug degradation in the culture with time, or (*c*) production of antagonists by the culture. The inoculation of fresh medium with these filtered organisms demonstrated generation rates ($k_o = 5.80 \times 10^{-4}$/sec) equivalent to those of naive organisms in the absence of drug, even at drug concentrations more than twice the minimum inhibitory concentrations for these organisms. When the filtrates of these cultures were inoculated with naive organisms, the action of aminosidine was the same as when equivalent amounts of fresh drug were added to fresh cultures.

It was concluded that negligible aminosidine degradation or antagonist production occurred *in situ* and that the later generation of organisms in the presence of bactericidal concentrations of drug was due to the production of resistants. The fact that extrapolation of the semilogarithmic plots for the generation of these resistant organisms (Fig. 1) gave different intercepts for the same inoculum size implied that resistant organisms developed during the generation observed in the presence of drug prior to the establishment of the first-order rate of kill. The longer the time at the lower bactericidal concentrations to obtain this maximum kill rate, the greater was the rate of production of resistant organisms.

Studies on aminosidine-affected microbial generation conducted at drug concentrations below the minimum (3.0 μg/ml) necessary to effect killing of *E. coli* at pH 7.0 demonstrated inhibition of generation rates with no significant kill. The evidence was the coincidence of viable and total counts of organism with time in these dose ranges.

There was a significant difference in the minimum concentration necessary to effect killing at pH 7.0 with various nutrient concentrations of medium. At twice the normal concentration of the nutrient medium, the value was 14 μg/ml but diminished to less than 3.0 μg/ml at half the normal nutrient concentration. This finding is strong evidence for antibiotic binding to the constituents of the medium.

Size distribution plots indicated that at the low drug concentrations studied, the mean size of the organisms decreased from 1.5 to 0.75 μm^3 as the population of the culture approached 1×10^8 *E. coli*/ml. This finding is consistent with the size distribution data observed for drug-free cultures.

At the higher aminosidine sulfate concentrations (*e.g.*, 15 μg/ml), which correspond to the plasma concentrations reached in humans under the recommended therapeutic regimen (8), the mean size remained the same after drug addition but the dispersion increased.

The organism appears to have an ability (Fig. 2) to survive with lessened generation rate until a critical concentration of aminosidine is reached in its biophase, at which time and concentration the process of dying becomes irreversible.

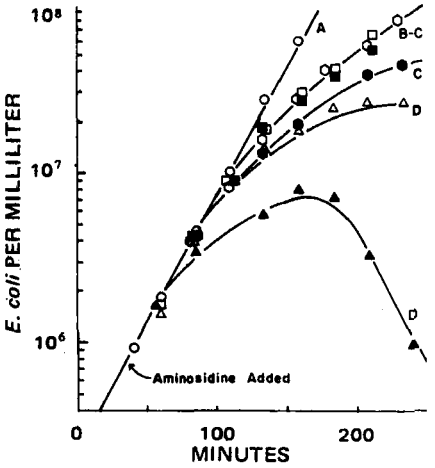

Figure 2—*Drug-affected generation of* E. coli *in the presence of aminosidine at pH 7.00 in peptone broth USP. Open symbols are total counts, and solid symbols are viable counts. Key: A, 0.0 μg/ml; B, 2.75 μg/ml; C, 3.0 μg/ml; and D, 3.25 μg/ml.*

There was no significant difference between the aminosidine- and paromomycin-affected generation curves for *E. coli* for all drug concentrations at pH 7.0 in the normal medium.

pH Effects of Aminosidine Action—Decreasing the pH of the culture medium from 7.8 to 6.1, which had no effect (9) on the generation rate of *E. coli*, increased the time necessary to reach the aminosidine-affected first-order rate of dying (Fig. 3) and increased the minimum concentrations of aminosidine necessary for observation of death from 3.0 to 12 μg/ml. The rate of death at bactericidal concentrations of aminosidine was independent of pH. These facts imply that the degree of protonation of the polyamine aminosidine decreases its rate of permeation into the cell, which may be dependent on the concentrations of species that are least ionized.

Analysis of the potentiometric titration curve (Fig. 4) of aminosidine by the difference method of Parke and Davis (10) permitted estimation of the pKa′ values for each of the five amino groups: 5.47, 6.24, 7.17, 8.15, and 9.07. These pKa′ values were estimated from the pH values at the midpoints of the five equal segments of the difference in titer necessary to achieve the same pH value for 10 ml of the aminosidine solution (0.300 mg/ml) and an equal volume of blank solution. The stoichiometric titer demonstrated that the active aminosidine was 706 μg/mg of the aminosidine sulfate used, which was within 5% of the estimated purity by the manufacturer's microbial assay.

Effects of Aminosidine Combinations with Bacteriostatic

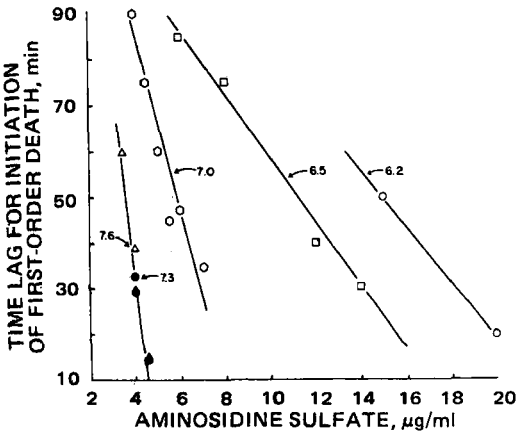

Figure 3—*Dependence of time lag to achieve first-order decay of* E. coli *on aminosidine concentrations. The lag was estimated from the intersection of the extrapolated linear segment of the decreasing log (numbers of organisms) against time with the semilogarithmic plot of the generation of organisms in the absence of drug. Curves are labeled with pH values of the medium.*

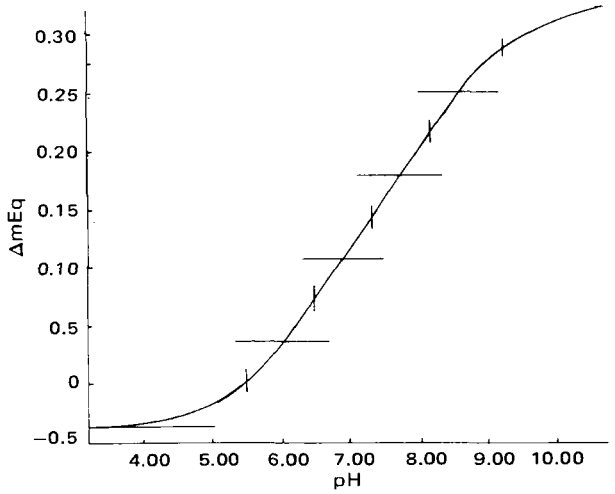

Figure 4—*Plots of the difference in milliequivalents of titer, ΔmEq, necessary to achieve a given pH for 10 ml of 0.300 mg/ml of aminosidine and an equal volume of blank solution against pH. The designated values are apparent pKa′ values at midpoints of five equal segments of the stoichiometric titer.*

Agents—The curves labeled A in Figs. 5–7 represent the generation rate of *E. coli* in the absence of any drug. The curves labeled B represent the action of the bacteriostatic agent alone. The curves labeled C, D, *etc.*, represent the action of aminosidine in combination with that same amount of bacteriostatic agent. The amount of aminosidine increased in the alphabetical order of the labeled curves. The minimum concentration for the ultimate killing rate of aminosidine alone on *E. coli* was 3.5 μg/ml, but it diminished to 1.0 μg/ml in the presence of 90 μg/ml of novobiocin (Fig. 5) and to 2.5 μg/ml in the presence of 0.3 μg/ml of tetracycline (Fig. 6); these combinations may be considered synergistic.

Only in the tetracycline case did combinations with 2.5, 3.0, and 3.5 μg/ml of tetracycline not show the development of resistant organisms on the following day. However, concentrations as high as 4.5 μg/ml of aminosidine in the presence of 1.98 μg/ml of chloramphenicol had less significant effects on *E. coli* generation (Fig. 7), and even this combination demonstrated the ultimate emergence of resistant organisms.

Rationalizations of Aminosidine Action—A toxicity model, similar to that proposed by Jusko (11), can rationalize the ultimate first-order dependency of the aminosidine-affected death rate, $-dN/dt$ (Fig. 1).

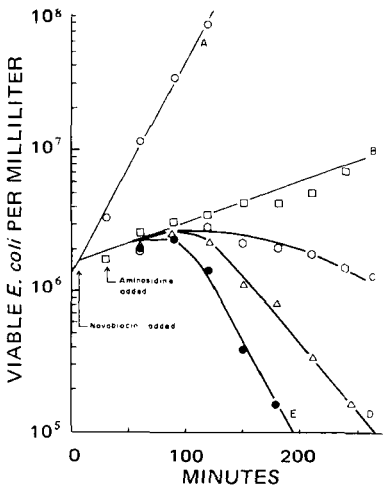

Figure 5—*Generation curves for combinations of aminosidine sulfate and 90 μg/ml of novobiocin. Curve A is for the control culture, and curve B is for novobiocin alone. Curves C, D, and E are for novobiocin combinations with 0.5, 1.0, and 2.0 μg/ml of aminosidine sulfate, respectively. All showed the development of drug-resistant* E. coli *on the following day.*

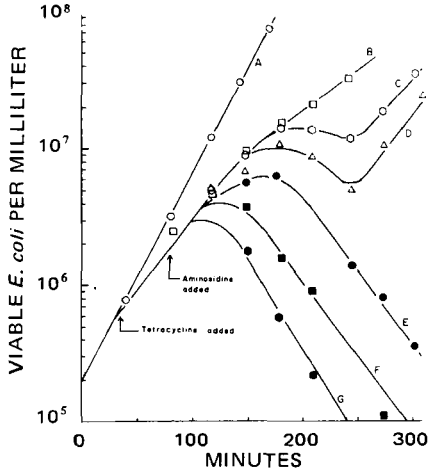

Figure 6—*Generation curves for combinations of aminosidine sulfate and 0.3 μg/ml of tetracycline hydrochloride. Curve A is for the control culture, and curve B is for tetracycline hydrochloride alone. Curves C, D, E, F, and G are for tetracycline hydrochloride combinations with 1.0, 2.0, 2.5, 3.0, and 3.5 μg/ml of aminosidine sulfate, respectively. The cultures of curves E, F, and G showed no subsequent development of resistant organisms.*

If the number of organisms dying per unit of time is proportional both to the net degree of complete inhibition, ΔI, of the biological or metabolic action necessary for organism survival and to the number of organisms, N, then:

$$-dN/dt = k_D(\Delta I)N = k_D(I - I_m)N \qquad \text{(Eq. 1)}$$

when $I > I_m$. The net degree of complete inhibition, ΔI, is a finite value when the degree of inhibition, I, exceeds a minimum value, I_m, below which the repair or homeostatic capacity of an individual organism permits the biological processes to continue at a level that maintains survival. If I is less than I_m, $-dN/dt = 0$ in the absence of generation.

These postulates include the premises that the repair capacity in every individual organism is the same and that a larger net degree of inhibition results in death in a shorter time than a smaller net degree of inhibition. The inhibitory process eventually results in the critical depletion of some biologically necessary product or effect, and thus death results ultimately in all organisms where $I > I_m$.

It follows that for $I > I_m$:

$$\int_{N_{t_1}}^{N} dN/N = \int_{t_1}^{t} k_D(I - I_m)dt \qquad \text{(Eq. 2)}$$

where N_{t_1} is the number of organisms alive at time t_1, and N is the number of organisms alive at time t. Thus:

$$\ln N/N_{t_1} = -\int_{t_1}^{t} k_D(I - I_m)dt \qquad \text{(Eq. 3)}$$

and the fraction, f_A, of organisms at time t_1 alive at any time t is:

$$f_A = N/N_{t_1} = e^{-\int_{t_1}^{t} k_D(I-I_m)dt} \qquad \text{(Eq. 4)}$$

where all ultimately die if $I > I_m$ and $f_A = 0$ at infinite time. Also $f_A = 1$ for all values of $I \leq I_m$ for all times.

If the net degree of inhibition is maintained constant after $t = t_1$, as by a constant concentration of aminosidine in the biophase of the organism, Eq. 4 reduces to:

$$\log N = -[k_D(I - I_m)/2.303](t - t_1) + \log N_{t_1} \qquad \text{(Eq. 5)}$$

which is consistent with the linear plot of log N against time after a time t_1 observed previously for other bactericidals (7) and for all bactericidal concentrations of aminosidine in Fig. 1 when the data are corrected for the emergence of resistant organisms. Thus, for aminosidine at 37.5°:

$$k_{app} = k_D(I - I_m) = 5.36 \times 10^{-4}/\text{sec} \qquad \text{(Eq. 6)}$$

The degree of inhibition of the necessary biological process should be related to a concentration, C', of the toxic agent in the

biophase. In an occupied receptor site model with a finite number of receptor sites, the degree of inhibition can be expressed in terms of the fraction of receptor sites occupied, which is a function of this concentration (12), so that:

$$I = aC'/(1 + aC') \qquad \text{(Eq. 7)}$$

and if the concentration in the biophase is proportional to the concentration in the nutrient medium, $C = C'/\alpha$:

$$I = a\alpha C/(1 + a\alpha C) = a'C/(1 + a'C) \qquad \text{(Eq. 8)}$$

Thus, at very high concentrations, $I = 1$; at low concentrations, $I = a'C$.

Substitution of forms such as Eq. 8 into Eq. 6 results in:

$$k_{app} = k_D \frac{a'C}{1 + a'C} - k_D \frac{a'C_m}{1 + a'C_m} \qquad \text{(Eq. 9)}$$

where C_m is the concentration in the nutrient medium that gives the steady-state concentration C_m' in the biophase that results in the inhibition, I_m, that does not cause death.

At high concentration, the maximum apparent mortality rate constant is:

$$\lim_{c \to \infty} k_{app} = (k_{app})_\infty = k_D - k_D \frac{a'C_m}{1 + a'C_m} \qquad \text{(Eq. 10)}$$

Thus, on consideration of Eqs. 9 and 10:

$$1/[(k_{app})_\infty - k_{app}] = 1/k_D + (a'/k_D)C \qquad \text{(Eq. 11)}$$

and a plot of the reciprocal of the difference between the maximum mortality rate constant, $(k_{app})_\infty$, and the constant, k_{app}, against the concentration where the latter was observed gives a slope of a'/k_D and an intercept, $1/k_D$, from which the values of k_D and a' can be calculated. These calculated values plus the known $(k_{app})_\infty$ permit the calculation of C_m from a rearrangement of Eq. 10:

$$C_m = [k_D - (k_{app})_\infty]/a'(k_{app})_\infty \qquad \text{(Eq. 12)}$$

Thus, from Eq. 12, when $C_m = 0$, $k_D = (k_{app})_\infty$, and from Eq. 9 or 11:

$$1/k_{app} = [1/a'(k_{app})_\infty](1/C) + 1/(k_{app})_\infty \qquad \text{(Eq. 13)}$$

and a plot of the reciprocal of the constant, k_{app}, against the reciprocal of the concentration gives a slope of $1/a'(k_{app})_\infty$ and an intercept of $1/(k_{app})_\infty$, from which the values of a' and $(k_{app})_\infty$ can be calculated when the latter equals k_D. The apparent linear dependency of kill rate on bactericidal concentrations of penicillin, kanamycin, and rifampin (7) at their studied concentrations implies that $a'C$ and $a'C_m \ll 1$, so Eq. 9 can be modified in these cases to:

$$k_{app} = k_D a'(C - C_m) \qquad \text{(Eq. 14)}$$

Aminosidine (Figs. 1 and 2) and also sulfisoxazole (13) are bacteriostatic below a minimum value, but the organisms demonstrate an invariant ultimate first-order dying rate above a certain minimum bactericidal concentration. These observations imply that the saturation of receptor sites, resulting in complete inhibition of the generation rate, results in a statistical probability of dying with organisms inhibited by these agents. Ribosomal binding of aminosidine that results in the misreading of the messenger-RNA that interferes with proper protein synthesis (4) must be initially reversible or else viable and total counts would not be coincident in the subbactericidal concentration range. An additional irreversible process must occur with aminosidine when generation is completely inhibited so as to account for the bactericidal action.

The fact that the time of achievement of the terminal first-order rate of dying decreases with increasing aminosidine concentrations (Fig. 3) implies a concentration-dependent process of aminosidine transfer from the medium into the biophase to effect antibacterial action. This process is confirmed further by the number of viables achieving a smaller maximum with increasing drug concentrations. At aminosidine sulfate concentrations of 15 μg/ml, the mean organism size did not increase after drug addition, implying a more abrupt halt to metabolic activity. This phenomenon is graphically shown in Fig. 8, where the ratio, N_p/N_o, of total organisms (N_o) produced prior to the emergence of resistants with bactericidal concentrations (see plateau values on plots of total organisms against time in Fig. 1) to the inoculum size at drug addition (N)

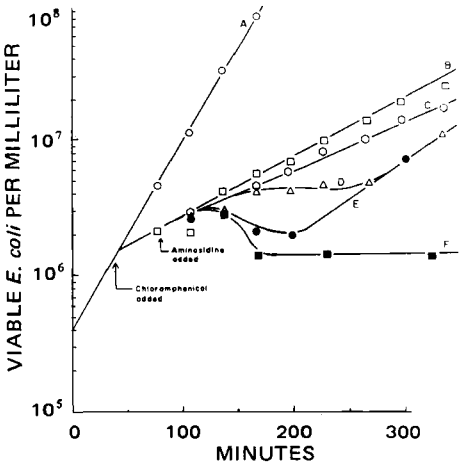

Figure 7—*Generation curves for combinations of aminosidine sulfate and 1.9 μg/ml of chloramphenicol. Curve A is for the control culture, and curve B is for chloramphenicol alone. Curves C, D, E, and F are for chloramphenicol concentrations with 3.0, 3.5, 4.0, and 4.5 μg/ml of aminosidine, respectively. All cultures showed the development of drug-resistant* E. coli *on the following day.*

decreases with aminosidine concentration. This ratio decreases with increasing inoculum size, which may indicate a possible drug binding or drug deactivation dependent on numbers of organisms or of structural mutants present at the time of drug addition. The hypothesis of drug inactivation appears to be negated by the fact that antibiotic-containing medium filtered from drug-affected organisms appeared to maintain its potency against naive organisms. An inoculum size effect of similar small magnitude was shown previously for many organisms (11).

The fact that other bacteriostatic agents, such as tetracycline and novobiocin, decrease the minimum bactericidal concentration of aminosidine implies that generation in their absence produces more receptor sites than necessary to be affected by aminosidine or that the generation process permits repair of the insult engendered by aminosidine.

Aminosidine (4) and tetracycline (3) both bind to the 30S ribosome. The action of novobiocin is not antagonized by tetracycline but is antagonized by chloramphenicol and other 50S binding inhibitors of protein synthesis (7). Thus, by analogous reasoning, it is not unexpected that chloramphenicol lessens the bactericidal activity of aminosidine, and it would be of interest to ascertain if other 50S binding antibiotics, such as erythromycin or lincomycin, act similarly.

The fact that aminosidine acts on organisms affected by such bacteriostatic agents denies a general premise (15) that bactericidals will not be active on organisms whose generation rate is inhib-

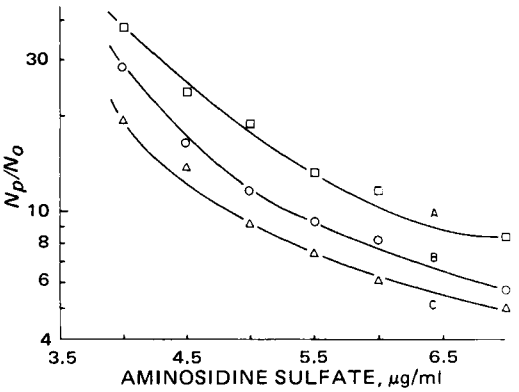

Figure 8—*Effect of inoculum size on aminosidine action. The ratio,* N_p/N_o, *of the plateau value,* N_p, *in total counts to the population,* N_o, *of* E. coli *at the time of drug addition is used as a measure of drug action. The various inoculum sizes were, in* E. coli *per milliliter: A,* 2.8×10^5; *B,* 6.4×10^5; *and C,* 1.29×10^6.

ited by such agents. In fact, the decreased emergence of resistants and lessened minimum concentrations of a drug to affect bactericidal action may indicate the advantages of such combinations.

The emergence of resistant organisms at earlier times with lower bactericidal concentrations of aminosidine (Fig. 1) implies that resistance is acquired in the presence of subbactericidal concentrations of the drug. It is most probably a property exhibited by generating organisms with aminosidine-lessened rates of generation and is consistent with the argument given (4) that altered ribosomes may be produced only by growth in the presence of drug. Aminosidine concentrations of 15 and 25 μg/ml, typical of the plasma levels achieved by the recommended therapeutic regimens (8), caused a rapid initiation of microbial death and did not permit that growth of resistants within 24 hr observed for concentrations below 7 μg/ml. This phenomenon was also observed (7) when penicillin or kanamycin was added to organisms previously treated with bacteriostatic agents, although the final killing rate was also not modified by such prior treatment.

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GLC Determination of Carbamazepine Suitable for Pharmacokinetic Studies

ANDRÉ GÉRARDIN [x], FRANÇOISE ABADIE, and JOËLLE LAFFONT

Abstract □ A GLC determination of carbamazepine in plasma and urine is described. It is performed by injecting the cyano derivative, which, in contrast to carbamazepine, has good GLC properties. The method is suitable for pharmacokinetic studies, since it is specific and accurate down to 0.27 μg/ml.

Keyphrases □ GLC—analysis, carbamazepine derivative, plasma and urine □ Anticonvulsants—carbamazepine derivative, GLC analysis, plasma and urine □ Carbamazepine—derivatization, GLC analysis, plasma and urine

Many GLC methods have been described for the determination of anticonvulsants in plasma or serum. Most of them are concerned with the monitoring of the effects of antiepileptic therapy. Some of these methods are suitable for the determination of carbamazepine[1] (I), which is injected either directly or after derivatization (1–12).

Reports on the GLC behavior of I indicate that its thermal stability is weak (6, 8, 9, 13, 14). As a result, some workers have used derivatives such as silylated I (6), dibenz[*b,f*]azepine, which is the thermal decomposition product of I (9), or its methylated derivative, obtained by flash reaction with a quaternary ammonium hydroxide (10). However, flash-reaction techniques sometimes require very precise conditions and may lead to poorly reproducible results. Moreover, these derivatives apparently cannot be obtained in a good state of purity (9). Recently, dimethylformamide dimethylacetal was reported to react with I, giving a derivative suitable for GLC (12).

These techniques generally meet clinical requirements, since the plasma concentrations in patients under continuous therapy (usually 2–10 μg/ml) do not require a high sensitivity. A method for the determination of I in plasma by mass fragmentography has been reported (14). The structure of the selected internal standard is closely related to I to compensate for its loss by thermal decomposition by an equivalent loss of the internal standard.

This paper describes a GLC method for the accurate and sensitive determination of I in plasma and urine, suitable for pharmacokinetic studies and bio-

[1] Tegretol, Geigy.