

THE EFFECT OF AROMATIC DIAMIDINES ON BACTERIAL GROWTH

I. THE MECHANISM OF ACTION

LEAH BICHOWSKY-SLOMNITZKI

Department of Hygiene and Bacteriology, The Hebrew University, Jerusalem

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It was observed by Fuller (1942) that certain long-chained aliphatic diamidines exert an antibacterial action on gram-positive as well as on gram-negative microorganisms *in vitro*. Thrower and Valentine (1943) showed that propamidine (an aromatic diamidine, 4-4-diamidino-diphenoxy-propane), in addition to its high trypanocidal effect, also inhibits the multiplication of gram-positive cocci *in vitro* as well as *in vivo* in wounds and burns. These findings have been confirmed by many authors. The purpose of the experiments reported below was to test the influence of certain aromatic diamidines on bacteria *in vitro* and to study the mechanism of their action.

EXPERIMENTAL RESULTS

Five aromatic compounds were tested: 4-4-diamidino-diphenyl-ether, 4-4-diamidino-diphenyl-benzyl-ether, 4-4-diamidino-diphenoxy-ether, 4-4-diamidino-stilbene (stilbamidine), and 4-4-diamidino-diphenoxy-pentane (pentamidine).

The microorganisms employed for the *in vitro* tests were *Escherichia coli* and *Staphylococcus aureus*. The medium used for the experiments with *E. coli* contained ammonium sodium phosphate, 0.5 per cent; sodium chloride, 0.2 per cent; $MgSO_4 \cdot 7H_2O$, 0.03 per cent; and glucose, 0.2 per cent; whereas that employed for *S. aureus* was prepared according to Knight (1937). Both media were adjusted to pH 7.3 to 7.4. The experiments were conducted in standard test tubes and each contained 5.0 ml of medium to which phenol red had been added. The diamidines were added under sterile conditions. A quantity of each drug was weighed in a sterile Erlenmeyer flask; ether was then added to a depth of about 1 cm, and the flasks were kept in the dark until all the ether had evaporated. Sterile water was added to produce the required dilutions. Fresh solutions of drugs were prepared for each experiment.

The influence of diamidine compounds on bacterial growth. The bacterial suspensions were prepared as follows: bacteria were removed from a 20- to 24-hour agar slant with 10 ml of saline, and this stock suspension was diluted 1:10,000. One-tenth ml of this final dilution was added to each test tube. The results of the *in vitro* experiments were read after 24- and 48-hour incubation at 37 C. The minimal drug concentration at which no growth appeared after 48 hours was noted as the minimal inhibiting concentration. The results of these experiments are summarized in table 1.

Table 1 shows that the compounds most active against *E. coli* and *S. aureus* were stilbamidine and pentamidine. But when the test with *E. coli* was re-

TABLE 1
Inhibition of bacterial growth by aromatic diamidines

ORGANISM TESTED	COMPOUNDS	MINIMAL EFFECTIVE MOLAR CONCENTRATION
<i>E. coli</i>	4-4-diamidino-diphenyl-ether	2×10^{-4}
	4-4-diamidino-diphenyl-benzyl-ether	4×10^{-4}
	4-4-diamidino-diphenoxy-ether	2×10^{-5}
	4-4-diamidino-stilbene (stilbamidine)	4×10^{-5}
	4-4-diamidino-diphenoxy-pentane (pentamidine)	4×10^{-5}
<i>S. aureus</i>	4-4-diamidino-diphenyl-ether	2×10^{-4}
	4-4-diamidino-diphenyl-benzyl-ether	2×10^{-4}
	4-4-diamidino-diphenoxy-ether	1×10^{-4}
	4-4-diamidino-stilbene (stilbamidine)	4×10^{-5}
	4-4-diamidino-diphenoxy-pentane (pentamidine)	1×10^{-5}

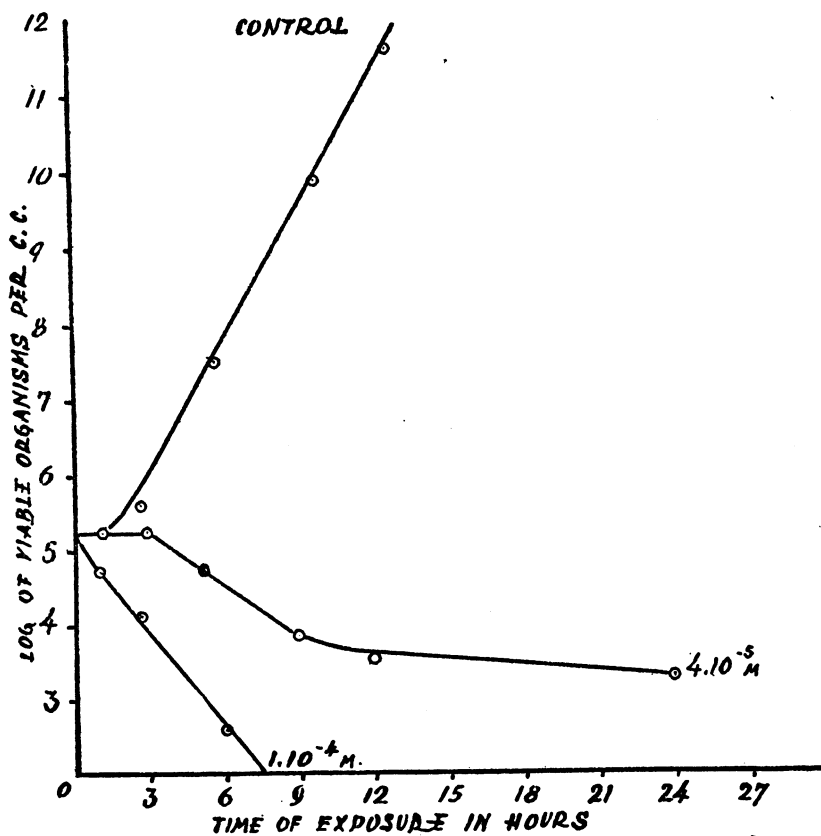


FIG. 1. THE MAINTENANCE OF *E. COLI* IN THE PRESENCE OF INHIBITING CONCENTRATIONS OF STILBAMIDINE

peated on Knight's medium (1937), the activity of these compounds was considerably depressed and their minimal effective molar concentration was 1×10^{-4} .

Survival of microorganisms in the presence of diamidine compounds. In the following experiments only the most active compounds (stilbamidine and pentamidine) were employed. The purpose of these experiments was to determine whether the action of the compounds is bactericidal or bacteriostatic. Media and culturing procedure were identical with those in the experiment described

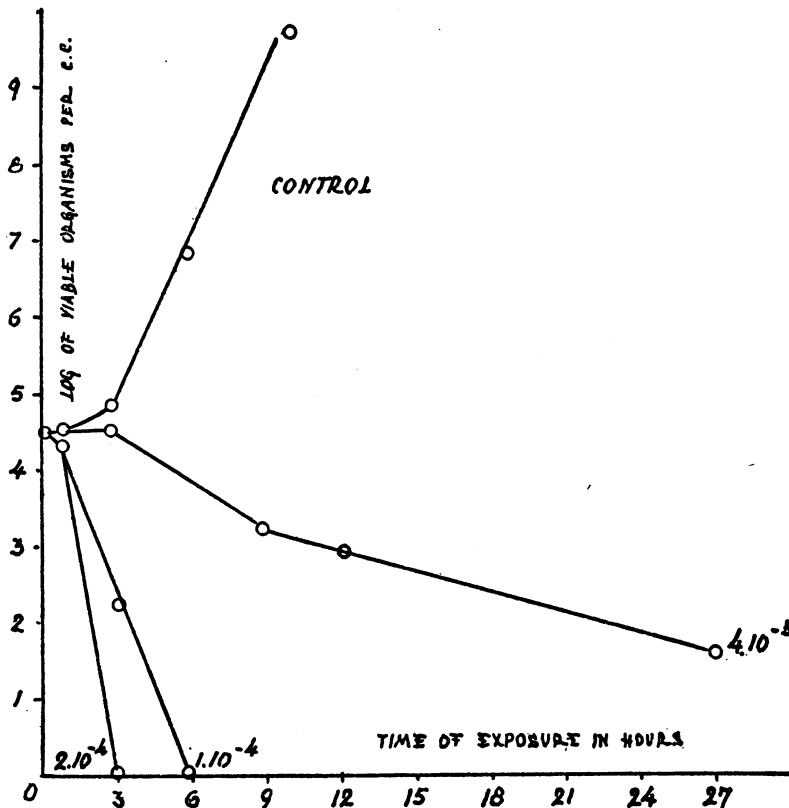


FIG. 2. THE MAINTENANCE OF *E. COLI* IN THE PRESENCE OF INHIBITING CONCENTRATIONS OF PENTAMIDINE

above. After varying incubation periods at 37 C in the presence of varying quantities of drug, aliquot portions of the incubated mixtures were removed and the bacterial count was estimated by plating.

Figures 1 through 4 show the results of these experiments. There is full agreement between the actions of both compounds on *E. coli* suspended in its synthetic medium. Although the 1×10^{-4} molar concentration exerts a typical bactericidal effect, expressed by a logarithmic death curve, the 4×10^{-5} concentration, which we had found to be the minimal growth-inhibiting concentration, exerted quite a different effect. After an initial period lasting for about

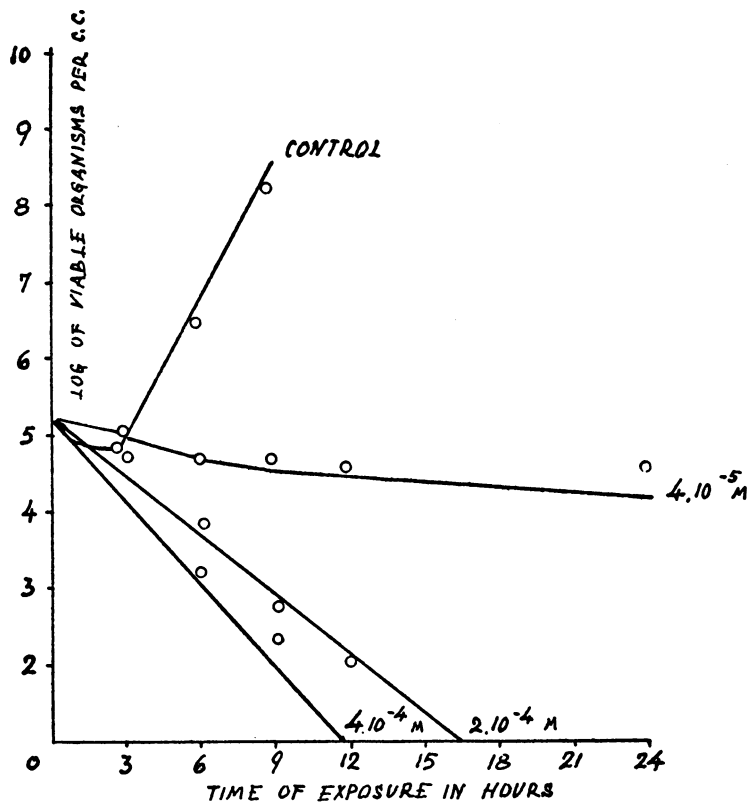


FIG. 3. THE MAINTENANCE OF *S. AUREUS* IN THE PRESENCE OF INHIBITING CONCENTRATIONS OF STILBAMIDINE

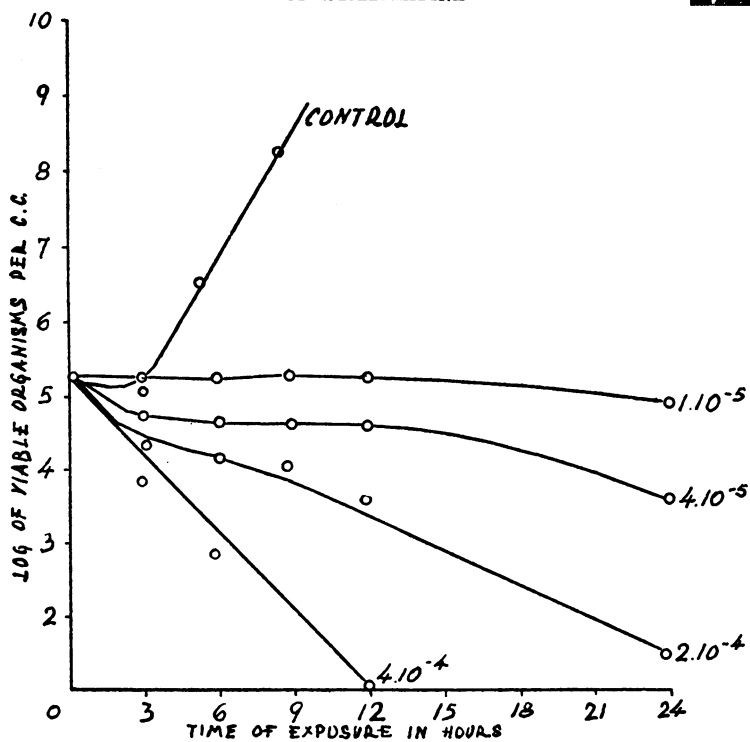


FIG. 4. THE MAINTENANCE OF *S. AUREUS* IN THE PRESENCE OF INHIBITING CONCENTRATIONS OF PENTAMIDINE

3 hours, during which no change was noted in the bacterial count, there followed a period of about 6 hours during which the death rate was logarithmic, and this was followed by a period of low mortality, which lasted for at least 48 hours since at the end of this time living bacteria were still found. When these experiments were repeated on Knight's medium (1937), similar results were obtained, but higher drug concentrations were required to exert a bacteriostatic or a bactericidal effect.

S. aureus behaved quite differently in the presence of pentamidine and stilbamidine. The zone of drug concentrations inhibiting bacterial growth while permitting survival of the microorganisms after 48 hours was much wider than in the case of *E. coli*, as demonstrated in figures 3 and 4.

SUMMARY

Five aromatic diamidine compounds were tested for their antibacterial effect on *Escherichia coli* and *Staphylococcus aureus*.

Stilbamidine and pentamidine were found to be the most active of the five compounds.

Under identical conditions, *E. coli* is more resistant to the growth-inhibiting action of these compounds than is *S. aureus*. However, the zone of inhibition of growth unaccompanied by bactericidal action is narrower in the case of *E. coli* than in that of *S. aureus*.

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