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DIAMIDINES AS ANTIBACTERIAL COMPOUNDS

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Study of the diamidines was first directed to protozoal diseases, their usefulness in bacterial infections being discovered subsequently; some of our early results on propamidine were cited by Thrower and Valentine (1943), and its value in burns and surface infections has been confirmed. Fuller (1942) showed that Gram-positive were more susceptible than Gram-negative organisms to aliphatic diamidines, and we sought for aromatic diamidines with greater activity against coli, proteus, and pyocyanea as well as against staphylococcus. A preliminary account of our findings has been given in conjunction with clinical trials on two of the new compounds (Wien, Harrison, and Freeman, 1948). Now, while penicillin deals with many infections it has little effect against Gram-negative bacteria, and although streptomycin may be of more value there is still a need for other active compounds: we found certain diamidines which should be of value in local chemotherapy since our experimental studies have had clinical confirmation (Kohn and Cross, 1948).

Compounds.—They conform to the general formula Am.B.X.B.Am in which Am represents an amidine group in the 4 or 4′ position, B a benzene nucleus, and X a direct linkage, either an oxygen atom or an $-O(CH_2)_nO-$ group, where n is an integer from 1 to 10. Since all the derivatives described have substituents in the 2 or 2′ position in the benzene nucleus, they may be referred to for convenience in an abbreviated form, e.g., dibromopropamidine signifying 4:4′ - diamidino - 2:2′ - dibromo-diphenoxypropane, and a list of some of those examined will be seen in Table I. Their preparation and properties (Newbery and Berg, 1945) will be described elsewhere. The isethionates of the compounds are readily soluble, forming colourless, neutral, and stable solutions, but they are precipitated in normal saline; isotonic solutions may be prepared by the addition of 5 per cent (w/v) dextrose.

Materials and Methods

Antibacterial activity

Organisms.—These were derived mainly from the Lister Institute (for N.C.T.C. numbers see Table II), except for *Staph. aureus*, strain No. 19, which was obtained from a case of osteomyelitis, and strain No. 27, which was obtained from a case of staphylococcal septicaemia.

Media.—Hartley's broth was employed, with the addition of 2 per cent (w/v) glucose for streptococci; a thioglycollate medium was used for clostridia and human defibrinated blood was used in slide-cell experiments for staphylococci.

Methods.—(1) *Bacteriostatic activity* was measured by means of the two-fold serial dilutional method: a heavy inoculum, one drop of a 1:10 dilution of a 24-hour broth culture, was added to each tube, and the highest dilution completely inhibiting visible growth was recorded after incubation for 18 to 24 hours at 37° C.: these results are marked (*a*) in Table II. Bacteriostatic activity in blood was determined by Fleming's slide-cell method, the staphylococcal inoculum being adjusted to give about 40 colonies in the control cell: the highest effective dilution was read where 5 colonies or less were visible (Freeman, 1948).

(2) *Bactericidal activity* was determined by subinoculations on to trypsin-digest agar: growth on agar after 24 hours' incubation at 37° C. denoted lack of bactericidal activity. These results are marked (*b*) in Table II. The bactericidal properties were investigated also by observing the rate at which *Staph. aureus* and *B. coli* were killed from determinations of the minimal effective bactericidal concentrations at intervals up to 24 hours.

Antifungal activity

Method.—This consisted initially in incorporating serial dilutions of a compound in 2 per cent glucose-agar in small tubes infected with spores, which were then left at room temperature for 5 days. The highest dilution completely inhibiting growth was observed.

More reproducible results were obtained by employing a ditch-plate method.

The petri dishes contained 15 ml. of 2 per cent glucose-agar, and a ditch (3/8 in.) was cut right across the diameter of each agar plate. The ditches were then filled with 2 per cent glucose-agar containing solutions of the compounds to be tested; ten-fold dilutions were tested first and subsequently two-fold dilutions, after the approximate range of activity had been determined. The plates were then dried for one hour at 37° C. Inoculations were made with a platinum needle from ten-day-old glucose-agar slopes. Two species of fungi were employed for each plate, one streak being made of each fungus, crossing the ditch at a right-angle and continuing to the opposite edge of the plate. The plates were incubated at 37° C. for 5 days except for *Hormodendron langeronii*, which grew best at room temperature. *Fungistatic activity* was determined by noting the highest dilution which caused complete inhibition of growth of the fungus on the ditch containing the compound. A control was obtained by observing good growth of the streak on the agar which did not contain any compound. Some zonal inhibition was seen at the edges of the ditch owing to diffusion of the compound into the untreated agar, but these zones were disregarded in the assessment of fungistatic activity.

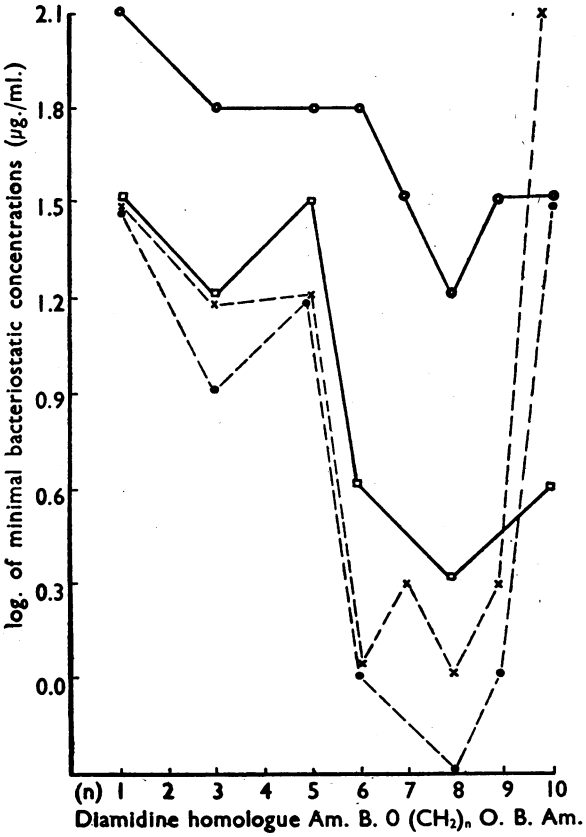

FIG. 1.—Bacteriostatic activities of homologous diphenoxyalkanes expressed as logarithms of minimal bacteriostatic concentrations. Note the increase in effect with increase in the length of the chain to a maximum at $n = 8$. o——o *B. coli* in broth. □——□ *B. flexneri* in broth. x- - -x *Staph. aureus* in blood. ●- - -● *Staph. aureus* in broth.

RESULTS

Bacteriostatic results on homologous diamidines.—Several points are worth noting from the bacteriostatic results shown in Fig. 1. Firstly, there was a graded increase in bacteriostatic activity against staphylococci with increase in the length of the chain to a maximum at $n=6$ to 9, followed by an abrupt decrease at $n=10$; against Gram-negative bacteria the maximum was maintained from $n=6$ to 10. Secondly, activity was retained in the presence of blood. Determination of the toxicities of these compounds on intravenous injection into mice showed an increase in toxicity in ascending the series from the methane (LD50, 30 mg./kg.) to the decane (LD50, 5 mg./kg.) derivative. It should be noted that propamidine, though less active than its higher homologues, was also less toxic to phagocytes (*vide infra*).

Bacteriostatic results on homologous diguanidines.—We found that homologous diguanidines displayed the same gradation in bacteriostatic activity as the diamidines against Gram-positive

TABLE I

BACTERIOSTATIC ACTIVITY OF HALOGENATED DIAMIDINES

Minimal concentrations in μg./ml. for complete inhibition of growth

Compound	*Staph. aureus*		*B. coli*	*Ps. pyocyanea*
	in broth	in blood	in broth	in blood
Phenamidine	128	64	256	512
Iodophenamidine	32	32	64	512
Stilbamidine	32	32	256	256
Iodostilbamidine	8	16	32	64
Bromopropamidine	4	2	32	256
Dibromopropamidine	1	4	4	32
Iodopentamidine	0.5	1.5	64	128
Diiodopentamidine	1	4	8	64
Iodohexamidine	0.5	1	16	8
Diiodohexamidine	1	8	4	16

organisms. Against *Staph. aureus* in blood, in slide-cell experiments, the minimal concentrations inhibiting growth were as follows:—4:4′-diguanidinodiphenoxyethane, 32 μg./ml.; propane homologue, 8 μg./ml.; butane homologue, 4 μg./ml.; pentane homologue, 2 μg./ml.; heptane homologue, 4 μg./ml.; and 256 μg./ml. for the nonane and decane homologues respectively.

Bacteriostatic and fungistatic results on halogenated diamidines.—Table I gives the results obtained with some of the halogenated diamidines examined: the diphenoxyalkanes were bacteriostatically more active than the stilbene compounds, and the diphenyl ethers were much less active. The halogenated diamidines were effective against certain pathogenic fungi, though not more so than the parent compounds; the degree of fungistatic action seemed to depend on the type of fungus, although some of the variation may have been due to the method employed.

Since the diphenoxyalkanes showed promising bacteriostatic activity, various substituted derivatives were examined; it was found that halogen in one or both benzene nuclei had a favourable effect, increasing bacteriostatic activity with little alteration in local toxicity to phagocytes. The mono-halogen derivatives were more active than the di-halogen derivatives against staphylococci, whereas the di-halogen derivatives were more active against Gram-negative bacteria.

Dibromopropamidine and iodohexamidine were found amongst the most effective of the compounds examined, and the results obtained with these and their parent compounds are shown in Table II. For comparison, penicillin (pure sodium salt) was effective in inhibiting the growth of *Staph. aureus* at a minimal concentration of 0.05 μg./ml., and streptomycin was bacteriostatically effective at 8 μg./ml. against *B. coli*, and at 4 μg./ml. against *Ps. pyocyanea*.

Bactericidal results on halogenated diamidines.—The bactericidal activity (results marked (*b*) in

TABLE II

ACTIVITY OF PROPAMIDINE, DIBROMOPROPAMIDINE, HEXAMIDINE, AND IODOHEXAMIDINE AGAINST VARIOUS BACTERIA AND FUNGI

(*a*) = bacteriostatic activity, (*b*) = bactericidal activity, (*f*) = fungistatic activity. Minimal concentrations in μg./ml. for complete inhibition of growth

Organism	N.C.T.C.* strain No.		Propamidine	Dibromo-propamidine	Hexamidine	Iodo-hexamidine
Strep. pyogenes	2432	(*a*)	4	1	0.5	0.5
Strep. viridans	3165	(*a*)	4	2	1	0.5
Staph. aureus	†19	(*a*)	8	1	1	0.5
		(*b*)	16	4	8	4
,, ,, (in 10% serum)	19	(*a*)	16	4	1	1
,, ,, (in blood)	19	(*a*)	16	4	1	1
,, ,, (in blood)	†27	(*a*)	16	16	4	2
Ps. pyocyanea	1999	(*a*)	256	32	16	8
		(*b*)	256	64	32	32
,, ,, (in 10% serum)	1999	(*a*)	256	128	32	32
Proteus vulgaris	3156	(*a*)	128	128	128	128
		(*b*)	256	256	256	256
B. coli	4144	(*a*)	64	4	64	16
		(*b*)	128	32	64	32
,, ,, (in 10% serum)	4144	(*a*)	128	8	64	64
B. flexneri	4835	(*a*)	32	8	4	16
B. enteritidis	4444	(*a*)	256	64	64	16
B. typhi-murium	2110	(*a*)	256	64	64	16
Cl. welchii	273	(*a*)	32	512	256	256
Cl. histolyticum	2915	(*a*)	256	256	256	256
Actinomyces kimberi	4583	(*f*)	100	10	20	20
,, *madurae*	3255	(*f*)	100	50	100	200
,, *hominis*	4525	(*f*)	1000	1000	10	10
Geotrichum dermatitidis	2787	(*f*)	25	200	20	20
Trichophyton tonsurans	2520	(*f*)	100	25	200	200
Hormodendron langeronii	2893	(*f*)	2	500	20	100

* N.C.T.C. (National Collection of Type Cultures). † Laboratory strain number.

Media.—Hartley's broth medium was used for bacteriostatic tests except where indicated; for streptococci 2 per cent (w/v) glucose was added, and for clostridia a thioglycollate medium was employed. Trypsin-digest agar was used for bactericidal tests and 2 per cent glucose agar for fungistatic tests.

TABLE III

ACQUIRED DRUG-RESISTANCE *in vitro*

Bacteriostatic activities: minimal concentrations in μg./ml. for complete inhibition of growth

Compound	Organism	Bacteriostatic activities		No. times increase in resistance	No. sub-cultures
		Initial	Maximal		
		μg./ml.			
Dibromopropamidine ..	*Strep. pyogenes*	1	128	128	24
Propamidine	*Staph. aureus*	8	128	16	10
5-aminoacridine	,, ,,	8	32	4	4
Penicillin	,, ,,	0.03 i.u./ml.	1400 i.u./ml.	46,666	40

Table II) of the halogenated diamidines was somewhat less (four to eight times) than their bacteriostatic activity. From determinations of the rate at which *Staph. aureus* and *B. coli* were killed by various concentrations of the compounds it was found that the maximum bactericidal effect was exerted fairly rapidly, within six to ten hours.

Effect of pH

We confirmed Elson's (1945) observation that the bacteriostatic action of diamidines was decreased in an acid medium and increased in an alkaline medium. At *p*H 6.3, 6.8, and 7.6 the minimal effective concentrations of dibromopropamidine against staphylococci in blood were 64 μg./ml., 4 μg./ml., and 1 μg./ml., respectively, and of propamidine 64 μg./ml., 16 μg./ml., and 4 μg./ml. respectively.

Since the diamidines inhibit the respiration of bacteria (*vide infra*), further evidence was obtained by measuring the oxygen uptake of *B. coli* in phosphate buffer at different *p*H values, in Warburg vessels, with various substrates. After initial incubation (37° C.) with the diamidine for 15 minutes the percentage inhibition of respiration was measured at 10-minute intervals for 2 hours. The manometer cups contained diamidine solution (0.00012 *M*), substrate solution (0.01 *M*), 0.5 ml. washed suspension of *B. coli* containing approximately 10^9 organisms per ml., phosphate buffer (0.033 *M*), and 0.2 ml. of 6 per cent (w/v) potassium hydroxide to absorb carbon dioxide; the volume was adjusted to 3 ml. with saline. The controls did not contain any diamidine. With sodium lactate as substrate, at *p*H 5.6, 6.8, and 7.8, the percentage inhibitions by propamidine after 2 hours were 10, 35, and 72 respectively; for dibromopropamidine they were 0, 51, and 82; for hexamidine 0, 26, and 77, and for iodohexamidine 29, 50, and 87. Similar results were obtained with alanine, glucose, and sodium acetate as substrates. It was evident that acidity decreased and alkalinity increased the inhibitory effect.

Acquired drug-resistance

Resistant-bacteria, whether naturally occurring or developed during treatment with chemical substances, are well recognized and may make selective treatment necessary. Accordingly, serial dilutions of the compounds in broth (2 per cent glucose broth for streptococci) were infected with the organisms (various strains of staphylococci and streptococci isolated from wounds were used), and after incubation at 37° C. for 24 to 48 hours the contents of the tube with the highest concentration of a compound showing growth was used for infecting a further series. The organisms were thus trained by repeated subcultivation to increasing concentrations of the compounds, and the results in Table III show the degrees of resistance attained. The resistance acquired by the various organisms was permanent and fairly readily induced. Cross-resistance experiments were also carried out, and these results are shown in Table IV. The following conclusions can be drawn: (1) staphylococci resistant to penicillin or to 5-aminoacridine were susceptible to diamidines; (2) staphylococci and streptococci resistant to one diamidine were resistant also to other diamidines; (3) staphylococci resistant to diamidines were not resistant to penicillin or 5-aminoacridine. Treatment with diamidines in the human subject might, but would not necessarily, produce similar resistant strains. We encountered some strains of pyocyanea from wounds which, although initially resistant to diamidines, readily lost this resistance when kept for only a week *in vitro* on a drug-free culture medium, showing that the resistance, in this instance, was not permanent. It should be noted that in our experiments the resistance produced by penicillin was much greater than that described by McIntosh and Selbie (1943).

TABLE IV

CROSS-RESISTANCE OF INDUCED DRUG-RESISTANT STRAINS OF *Staph. aureus* AND *Strep. pyogenes*

Figures indicate bacteriostatic activities: minimal concentrations in μg./ml. for complete inhibition of growth

Organism	*Strep. pyogenes*	*Staph. aureus*	*Staph. aureus*	*Staph. aureus*	*Strep. pyogenes*	*Staph. aureus*
Drug-resistance	Dibromo-propamidine resistant	Propamidine resistant	Penicillin resistant	5-amino-acridine resistant	Original unmodified strain	Original unmodified strain
Dibromopropamidine ..	128	8	1	1	1	1
Propamidine	256	128	4	16	4	8
Hexamidine	32	8	0.5	1	0.5	1
5-aminoacridine	8	8	8	32	4	8
Penicillin	0.1 i.u./ml.	0.01 i.u./ml.	1400 i.u./ml.	0.01 i.u./ml.	0.03 i.u./ml.	0.03 i.u./ml.

Toxicity and local tolerance (Table V)

(*a*) *Systemic toxicity for mice.*—The LD50 in mice was determined for both the intravenous and subcutaneous routes: symptoms were similar to those previously described for other diamidines (Wien, 1943). Both dibromopropamidine and iodohexamidine had a depressor effect in the chloralose cat; dibromopropamidine increased while iodohexamidine decreased the tone and movements of the isolated rabbit ileum.

(*b*) *Toxicity to human leucocytes.*—Killed staphylococci were added for 30 minutes to citrated human blood previously in contact with diamidine solution for 3 hours at 37° C. After lightly centrifuging the blood in capillary tubes, leucocyte films were prepared from the upper surface of the cellular deposit and stained by Gram's method. The average number of bacteria in 25 phagocytes was counted and the least toxic concentration was noted where the result showed one or less than one coccus per cell.

(*c*) *Toxicity to chick embryo.*—A window was cut in the shell of 10-day-old embryonated eggs, the shell membrane removed, and 0.3 ml. of a solution of a compound (two-fold serial dilutions, using at least 3 embryos for each dilution) dropped on to the collapsed chorio-allantoic membrane. The window was sealed with a waxed coverslip and the mimimum concentration causing death of the embryo within 3 to 4 days was determined.

(*d*) *Effect on guinea-pig skin.*—Intradermal injections (0.05 ml.) were made into the shaved skin of guinea-pigs and the least concentration causing erythema or necrosis was observed. The compounds were tested also for their effect on wound healing (described later), and it was found that there was no delay in healing with concentrations up to 0.4 per cent (in ointment base).

(*e*) *Effect on rabbit conjunctiva.*—Applied under the eyelids of rabbits, solutions of the diamidines caused no irritation of the conjunctiva in concentrations up to 1 : 1,000.

Antibacterial activity in vivo

By injection.—Although the diamidines display high bacteriostatic and bactericidal activity against

TABLE V

THE SYSTEMIC AND LOCAL TOXICITIES OF PROPAMIDINE, DIBROMOPROPAMIDINE, HEXAMIDINE, IODOHEXAMIDINE, AND 5-AMINOACRIDINE

Compound	Average lethal dose in mg./kg. for mice		Minimum toxic concentration (g./100 ml.) for:		
	(*a*) intravenous	(*a*) subcutaneous	(*b*) Human phagocytes	(*c*) Chick embryo	(*d*) Guinea-pig skin
Propamidine	42	55	0.6	0.4	0.1
Dibromopropamidine	10	300	0.1	0.8	0.05
Hexamidine	17	62	0.2	0.2	0.025
Iodohexamidine	6	150	0.1	0.4	0.05
5-aminoacridine	15	100	0.01	0.1	0.025

Gram-positive cocci, the ratio of antibacterial activity to systemic toxicity is such that little therapeutic activity can be demonstrated when they are administered parenterally. Hexamidine, given subcutaneously at the maximum tolerated dose, showed only slight therapeutic activity either in prolonging the survival time of mice infected with a virulent strain of staphylococci, or in reducing the incidence of kidney abscesses with a chronic strain. Against salmonella and clostridial infections in mice the results were similarly disappointing. Very slight virucidal activity was shown by hexamidine, but not by the others, against influenza virus infections in mice, but the effect was barely significant.

Locally.—Compounds, in solution and in ointment base, were examined initially for local tolerance on burn-wounds in guinea-pigs by Ungar's (1944) method. It was found that they did not delay the rate of healing after treatment for 4 days at concentrations twice those toxic to phagocytes. Infected wounds were produced on both flanks of the shaved skin of rabbits by scarifying the skin tissue and then infecting with *Staph. aureus* or *Ps. pyocyanea.* The wound was kept moist and enclosed in a plastic cover (Robson, 1946); solutions of the compounds were applied through a hole in the cover which could be closed with a screw. Against staphylococcal infections the diamidines were effective in concentrations of 1:1,000, but no effect could be demonstrated at this concentration against pyocyaneal infections. Better results, however, were obtained in the human subject (Kohn and Cross, 1948).

Mode of action

Bernheim (1944) found that propamidine inhibited the oxidative metabolism of bacteria, and we found that the halogenated diamidines had a similar inhibitory effect. We confirmed, also, that a 2-hour period of incubation was necessary in order to obtain maximal effects, probably in order to allow diamidines to penetrate the bacterial cell. In studying the inhibition by diamidines of oxidative systems various substrates were used, in order to discover the effect on the most important dehydrogenases present in the bacterial cell. The substrates used were: glucose, sodium lactate, sodium pyruvate, sodium glutamate, sodium acetate, sodium malate, sodium succinate, and alanine.

The oxygen uptake of *B. coli* was measured in Warburg vessels after a 2-hour period of incubation at *p*H 7.8 in order to obtain optimum effects; the manometer cups contained 0.00012 *M* diamidine solution, 0.01 *M* substrate, 0.5. ml. of a washed bacterial suspension of *B. coli* containing approximately 10^9 organisms per ml., 0.2 ml. of 6 per cent potassium hydroxide, and 0.033 *M* phosphate buffer.

It was found that the diamidines caused marked inhibition and to a similar degree with all the substrates examined. For instance, with glucose and sodium lactate as substrates the percentage inhibitions caused by propamidine after 2 hours at 37° C. were 69 and 72 respectively; for dibromopropamidine the percentage inhibitions were 85 and 87, for hexamidine 83 and 80, and for iodohexamidine 98 and 90. There did not seem to be any relationship between the degree of inhibition and the bacteriostatic activities of these diamidines, although it should be noted that only one organism (*B. coli*) was employed in the manometric experiments.

Similar experiments were carried out with tissue preparations. The substrates were sodium lactate, glucose, sodium succinate, and choline chloride. The lactic and glucose dehydrogenases were obtained from rat brain tissue, and the succinic and choline dehydrogenases were obtained from rat liver. The manometer flasks were set up containing 1 ml. of 0.022 *M* phosphate buffer *p*H 7.8, 0.2 ml. of 0.01 *M* substrate, 0.00012 *M* (and 0.0012 *M*) diamidine solution, Locke's solution, 1 ml. of enzyme preparation, and 0.2 ml. of 6 per cent potassium hydroxide; the volume was adjusted to 3 ml. The flasks were incubated at 37° C. for two hours. No inhibition of oxygen uptake was observed with any of the substrates except choline, with which 72 per cent inhibition was obtained.

In bacteriostatic experiments, with staphylococci in blood, *p*-aminobenzoic acid had no inhibitory effect on the bacteriostatic activity of propamidine, hexamidine, or their halogenated derivatives. But Bichowsky (1944) found that nucleic acid reduced the antibacterial action of propamidine, and Elson (1945) suggested that phospholipides competed with diamidines for the anionic position on the cell. We found in manometric experiments with sodium lactate as substrate that 0.1 per cent of nucleic acid reduced the inhibitory effect of propamidine on the oxygen uptake of *B. coli* by 14 per cent after 1 hour at 37° C. and by 43 per cent after 2 hours. It is suggested, therefore, that the diamidines may act by deranging some phase in the metabolism of bacteria involving nucleic acid.

Discussion

The diamidines are interesting compounds since they have such a wide range of action: they are effective not only against protozoa but also against

bacteria and fungi. The use of the diamidines as local bactericidal compounds requires the fulfilment of certain conditions—i.e., the compounds should show high bacteriostatic and bactericidal activity, they should be effective in body tissues, and they should be well tolerated locally. Tests should parallel as far as possible the conditions of actual use; results obtained, therefore, in serum or blood have more significance than those in broth, and in surface infections local toxicity to tissues is of more importance than systemic toxicity. Although the diamidines are regarded as potentially toxic compounds for parenteral use they are well tolerated when applied locally; the small amounts absorbed from the surface are unlikely to produce any systemic effects.

In the investigation of many substituted diamidines it was found that the introduction of halogen in the 2-position into one or both benzene nuclei had a favourable effect. The introduction of two halogen atoms into the same benzene nucleus in the 2:6 positions was less beneficial—the minimal effective concentration for 2-iodopropamidine against staphylococci in blood was 4 μg./ml., whereas the minimal effective concentration for the 2:6 diiodo derivative was 16 μg./ml. The halogenated stilbene compounds possessed less bacteriostatic activity than the diphenoxyalkane compounds; the halogenated diphenyl ethers were even less active (Table I). We observed that the homologous diguanidines displayed the same gradation in bacteriostatic activity as the diamidines; similar results have been obtained by Fuller (1942) for aliphatic amidines and guanidines.

In an attempt to elucidate their mode of action we found that the diamidines exerted a general inhibitory effect, to a similar degree, on a fairly wide range of oxidizable substrates. The number of substrates involved may be an indication that an effect was exerted directly on a respiratory mediator common to a number of systems, and not on a specific dehydrogenase enzyme system. No parallelism was found between the effects obtained with tissue and bacterial enzymes; there may, however, be some relationship between the effects of the diamidines in causing both an inhibition of choline oxidase and fatty degeneration of the liver. The experiments with induced drug-resistant strains of bacteria threw little light on the problem except to emphasize that dissimilar substances like penicillin and the diamidines probably have different types of action, since an organism made resistant to one diamidine was also resistant to other diamidines but was sensitive to penicillin. Gale (1947) has demonstrated a relationship between the assimilation of glutamic acid by staphylococci and their sensitivity to penicillin, but we have not as yet investigated whether diamidines can similarly block glutamic acid assimilation.

Summary

1. A study of the antibacterial properties of the diamidines showed that bacteriostatic activity in the diphenoxyalkanes rose to a maximum from the propane to the hexane and nonane derivatives. This increased bacteriostatic activity was accompanied by an increase in intravenous toxicity, but by only a relatively small increase in local toxicity to phagocytes. Gram-positive bacteria were more susceptible than Gram-negative bacteria, and the bacteriostatic activity was maintained in the presence of blood.

2. The introduction of halogen into one or both benzene nuclei in the diphenoxyalkanes further increased the bacteriostatic effect against *Staph. aureus* as well as against *B. coli, Proteus vulgaris,* and *Ps. pyocyanea,* with little alteration in local toxicity.

3. Two new derivatives, dibromopropamidine and iodohexamidine, were studied more closely, in comparison with their parent compounds, for their possible use in surface infections. They showed both bacteriostatic and bactericidal effects which appear to be due to inhibition of the oxidative metabolism of bacteria. Small differences in *p*H markedly influenced both the inhibitory and the bacteriostatic effects, which were increased in an alkaline medium and decreased in an acid medium.

4. Drug-resistant strains of bacteria could readily be induced by repeated subcultivation *in vitro*; a diamidine-resistant strain, although resistant to other diamidines, was sensitive to penicillin and a penicillin-resistant strain was sensitive to the diamidines.

We are indebted to Miss Pattinson, Mrs. Bradish, and Mrs. Oakley for valuable assistance; to Dr. Gordon and Miss Sowden for the biochemical part of this investigation; to Dr. J. N. Ashley and his colleagues for the preparation of the compounds; to Dr. W. R. Thrower for initiating the clinical trials, and Dr. A. J. Ewins for his interest throughout the investigation.

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Since the completion of this paper we have noted an article by Bichowsky-Slomnitzki (*J. Bact.*, 1948, **55**, 33), who found that nucleic acid and certain polyamines (spermine and spermidine) antagonized the inhibitory effect of diamidines (stilbamidine and pentamidine) on the growth of *B. coli*. It was assumed that the diamidines caused metabolic disturbances of the cell nucleotides by fixation of nuclear substances, whereas a different mechanism of action existed in the antagonism between diamidines and polyamines, which was probably associated with their competition for the same cellular substance.