was said to be debilitated. Four rats were used for each dose of drug, and the data were analyzed for NTD_{50} in rotarod performance by Horn's (6) method.

RESULTS

The selectivity of mouse-killing behavior can be calculated by dividing the rotarod NTD_{50} dose by the muricide MKD_{50} dose. A ratio close to or below 1.00 indicates a nonspecific blockade of mouse killing, the muricidal block being indistinguishable from debilitation or depression of the animal. A ratio significantly greater than 1.00 indicates selectivity of a drug, mouse-killing blockade at a nonneurotoxic dose.

The effects of the four anorectic compounds examined for muricide blockade and rotarod performance are given in Table I. With dextroamphetamine, the smallest dose (0.46 mg./kg.) of all the anorectics tested was needed to produce mouse-killing blockade in 50% of the rats and also the smallest dose was needed for rotarod debilitation. However, the separation between those two doses was great enough to produce a ratio of NTD_{50}/MKD_{50} of 10.09. Only the ratio for diethylpropion was larger (17.78) than that of amphetamine. Aminorex (7.25) and fenfluramine (4.65) also demonstrated ratios well above 1.00.

DISCUSSION

Of the four anorexigenic agents tested, only the dextroamphetamine data can be compared with results of other investigators. Even though different methods of calculation of the MKD_{50} and the NTD_{50} were used, Sofia (4) and Horovitz *et al.* (1) demonstrated a ratio significantly separating the antimuricide and neurotoxic doses for dextroamphetamine. Our data confirm this separation.

All of the anorexigenic drugs examined in this study illustrated selectivity determined by the NTD_{50}/MKD_{50} ratio in mouse killing in the rat. Muricide behavior was blocked at doses that did not render the rat physically debilitated. The relative selectivity of the anorectics studied is as follows: diethylpropion > dextroamphetamine > aminorex > fenfluramine.

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Synthesis and *In Vitro* Antiplaque Activity of Methylene Homologs of Chlorhexidine

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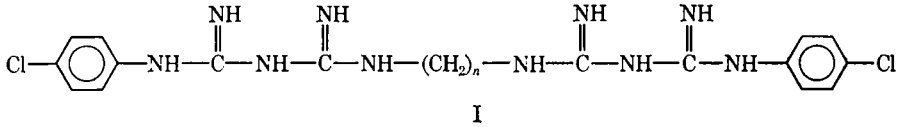
Abstract □ Five straight-chain homologs of chlorhexidine, containing from two to 12 methylene groups, were synthesized and evaluated along with chlorhexidine for *in vitro* antiplaque activity. Maximum antiplaque activity was exhibited by the compounds containing six (chlorhexidine) and 12 methylene groups.

Keyphrases □ Chlorhexidine methylene homologs—synthesized and tested *in vitro* for antiplaque activity □ Antiplaque activity—synthesis and *in vitro* testing of chlorhexidine methylene homologs □ Plaque inhibitors—synthesis and *in vitro* testing of chlorhexidine methylene homologs

Dental plaque is a soft, tenacious, bacterial deposit which forms on the surface of teeth. A close correlation exists between dental plaque and the principal diseases of the mouth: caries and periodontal disease (1). The high incidence of these diseases among the general population (2) is ample evidence that the current approaches to plaque control based on the use of mechanical aids are not effective. In this report, some efforts to develop long-acting chemical inhibitors of plaque formation are described. Recent *in vitro* and clinical studies (2–5) have shown that chlorhexidine [1,6-bis-(N^5-*p*-chlorophenyl-N^1-biguanido)hexane, I, $n = 6$], an antibacterial bisbiguanide, is an effective inhibitor of dental plaque. Three factors are apparently responsible for the antiplaque activity of chlorhexidine: (*a*) the antibacterial activity of chlorhexidine (6), (*b*) the ability of chlorhexidine to bind to the tooth surface (7), and (*c*) the largely bacterial nature of plaque (8).



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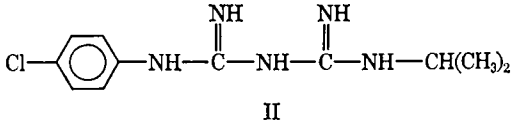


II

Table I—Bis(N^3-cyano-N^1-guanidino)alkanes

Methylene Groups, *n*	Reaction Time, hr.	Yield (Crude), %	Melting Point	Lit. (11) Melting Point	Recrystallization Solvent[a]
2	12	32	245–247°	248–250°	A
4	18	15	191–194°	197–199°	A
8	16	35	117–147°	—	A–B
10	16	10	164–182°	184–186°	A–C
12	18	46	136–169°	—	A–C

[a] A = water, B = ethanol, and C = 2-ethoxyethanol.

Table II—Bis(N^5-*p*-chlorophenyl-N^1-biguanido)alkanes

Methylene Groups, *n*	Reaction Time, hr.	Yield, %	Melting Point	Lit. (11) Melting Point	Recrystallization Solvent[a]	Formula	Analysis, %	Calc.	Found
2	6	46	255–259°	245–246°	A	$C_{18}H_{22}Cl_2N_{10}\cdot 2HCl\cdot H_2O$	C	40.02	40.04
							H	4.85	4.87
							N	25.92	25.85
4	3	40	251.5–255°	253–254°	A	$C_{20}H_{26}Cl_2N_{10}\cdot 2HCl$	C	43.65	43.50
							H	5.13	5.18
							N	25.45	25.25
8	8	9	212–227°	—	A	$C_{24}H_{34}Cl_2N_{10}\cdot 2HCl$	C	47.53	47.67
							H	5.98	6.30
							N	23.09	23.04
10	6	16	239–245°	246–248°	A–B	$C_{26}H_{38}Cl_2N_{10}\cdot 2HCl$	C	49.22	49.03
							H	6.35	6.56
							N	22.08	21.73
12	6	18	221–227.5°	—	A–B	$C_{28}H_{42}Cl_2N_{10}\cdot 2HCl$	C	50.76	50.87
							H	6.69	6.95
							N	21.14	20.91

[a] A = water and B = ethanol.

Structure–activity studies with biguanides have shown that molecules containing only one biguanide residue, *e.g.*, proguanil (II), do not inhibit plaque formation (4) and that the nature of the substituents attached to the biguanide groups in bisbiguanides has a marked effect on antiplaque (9, 10) and antibacterial activity (6). In addition, the distance between the biguanide residues in bisbiguanides affects antibacterial activity (6). These findings support the hypothesis that two properly substituted biguanide residues separated by a specific distance must be present in this type of molecule for optimal antiplaque activity.

To determine the optimal separation of the two biguanide groups, a series of chlorhexidine methylene homologs (I, n = 2, 4, 8, 10, 12) was synthesized and evaluated for *in vitro* plaque inhibition relative to chlorhexidine.

EXPERIMENTAL[1]

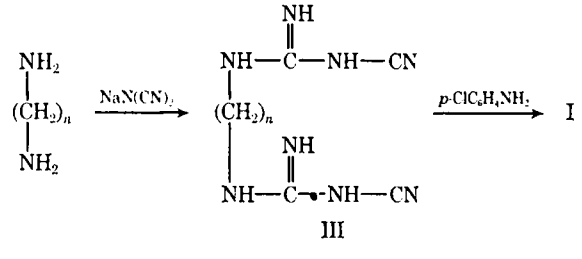
The homologs were synthesized using the method developed by Rose and Swain (11) as outlined in Scheme I. Treatment of the appropriate diaminoalkane with sodium dicyanamide gave the corresponding intermediate bis(N^3-cyano-N^1-guanidino)alkanes (III) (Table I) which, on treatment with *p*-chloroaniline, gave the desired bisbiguanides (I) (Table II).

Bis(N^3-cyano-N^1-guanidino)alkanes (III)—An intimate mixture of the appropriate diaminoalkane dihydrochloride (1.0 mole) and sodium dicyanamide (2.0 moles) was stirred in refluxing 1-butanol (15 ml./0.01 mole) for 12–18 hr. The cooled reaction mixture was filtered and, where the product was soluble (III, n = 2, 4), the filtrate was concentrated *in vacuo* and recrystallized to give the crude biscyanoguanidine which was used without further purification. Where the product was insoluble, the solid on the filter was washed with water to remove sodium chloride and recrystallized (Table I).

Bis(N^5-*p*-chlorophenyl-N^1-biguanido)alkanes (I)—A mixture of the appropriate bis(N^3-cyano-N^1-guanidino)alkane (1.0 mole) and *p*-chloroaniline hydrochloride (2.0 moles) was stirred in refluxing 2-ethoxyethanol (20 ml./0.01 mole) for 3–8 hr. Concentration *in vacuo* followed by recrystallization gave the pure bis(N^5-*p*-chlorophenyl-N^1-biguanido)alkane dihydrochlorides (Table II).

Antibacterial Activity—Antiplaque activity as displayed by chlorhexidine requires that a compound be an antibacterial agent. Therefore, the homologs were first evaluated for their *in vitro* antibacterial activity against *Streptococcus mutans* No. 6715, a pure strain of plaque-forming bacteria[2]. Chlorhexidine[3] and a solvent control were tested concurrently. One milliliter of a solution of the test compound was added to a tube containing 7.85 ml. of trypticase broth, 1 ml. of 50% sterile sucrose solution, and 0.15 ml. of a 24-hr. culture of *S. mutans* No. 6715, and this mixture was incubated under anaerobic conditions[4] for 48 hr.

Antiplaque Activity—The *in vitro* antiplaque activity of these same compounds was evaluated using the method of Turesky *et al.*



Scheme I

[1] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are corrected. Microanalyses were performed by Scandinavian Microanalytical Laboratory, Herlev, Denmark, and by Midwest Microlab, Ltd., Indianapolis, Ind.

[2] Isolated at, and made available to us by, the National Institute of Dental Research.

[3] Supplied by Ayerst Laboratories, Inc., New York, N. Y.

[4] BBL-Gaspak, BBL, Division of BioQuest, Cockeysville, Md.

Table III—*In Vitro* Antiplaque Activity[a]

Methylene Groups, *n*	—Percent Inhibition[b] at 10^{-5} *M*— 24 hr.	48 hr.
2	20	0
4	60	0
6	100	40
8	80	20
10	20	0
12	100	20

[a] All compounds were evaluated as their hydrochloride salts in dimethyl sulfoxide solution. Solvent controls exhibited 0% inhibition. Each compound was tested on five teeth. [b] Percentage of teeth that did not show plaque formation after stated incubation period.

(12). Sterilized extracted human teeth were immersed in solutions of the test compounds for two 1-min. periods, each of which was followed by a 1-min. exposure to air. These treated teeth were washed with 250 ml. of distilled water for 5 min. and then incubated with *S. mutans* No. 6715 under anaerobic conditions. The teeth were suspended in test tubes on orthodontic wire[5] (0.71-mm. diameter) threaded through a hole in the root so that the entire tooth was completely immersed. Subjective estimates were made of adherent microbial growth on the test tube walls, wires, and the teeth and of nonadherent growth in the broth using a scale from 0 (no growth) to 4 (maximum growth). Because in an overwhelming number of tests, growth ratings were either 0 or 4 both in the broth and on the hard surfaces, the findings were expressed as either + (growth) or − (no growth). The total microbial accumulation was considered as *in vitro* plaque.

RESULTS AND CONCLUSIONS

The five homologs and chlorhexidine all inhibited bacterial growth at a concentration of 10^{-5} *M* but not at 10^{-6} *M*. As shown in Table III, antiplaque activity was greatest when the biguanide groups were separated by either six or 12 methylene groups. Since all six compounds were comparable in antibacterial activity at the concentrations tested, the observed differences in antiplaque activity may reflect better binding to the tooth surface by the more active compounds.

[5] Rocky Mount.

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