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Relationship of Chemical Structure and Antimicrobial Activity of Alkyl Amides and Amines

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Contrary to the limited effects of alkyl amides and their corresponding *N*-derivatives, alkyl amines affected both gram-positive and gram-negative organisms. As with other alkyl derivatives the most sensitive gram-negative bacteria were usually more resistant than the most resistant gram-positive bacteria.

Compounds with a chain-length of 11 to 15 are most active. Although some of the general properties relating the activity of fatty acids to their antimicrobial action are similar to those of amine compounds, the amines are unique in that monounsaturation *does not* increase compound activity.

The possible modes of action of these compounds are discussed.

Fatty acids are known to possess antimicrobial activity (4, 5, 19, 28). In an attempt to study structure-activity relationships for fatty acids, a number of fatty acid derivatives were screened (18). From this study and others (17, 30), the wide-spectrum antimicrobial action for nitrogen compounds became of special interest. Therefore, a more extensive study of amine and amide compounds was undertaken.

The present report concerns itself with two variables: (i) change of antimicrobial activity of amines and amides with changes in chain length and unsaturation, and (ii) the effect of these same compounds on different species of a given genus and on different strains of the same species.

MATERIALS AND METHODS

Compounds. Amines and amides of high purity (95 to 99.5%) were obtained, except where noted, from Lachat Chemicals, Inc., Chicago Heights, Ill. Lauric acid, lauryl *N*,*N*-dimethyl amide, pentylamine, and *n*-nonylamine were obtained from Sigma Chemical Co., St. Louis, Mo. Eastman Chemicals of Rochester, N.Y., supplied the docecylamine·HCl.

A 0.2-g amount of amine or amide compound was dissolved in 0.5 ml of absolute methanol and quantitatively transferred to 200 ml of Trypticase soy broth (TSB; BBL). If the resulting suspension was granular or turbid, the suspension was carefully heated (approximately 70 C) to increase drug solubilization. Standard solutions (or suspensions) containing 1,000 μg/ml were serially diluted with additional broth to achieve desired concentrations. The serial dilutions were then dispensed into screw-cap tubes (16 by 125 mm), and the tubes were autoclaved at 15 psi for 15 min. After sterilization, all tubes were cooled, incubated overnight at 35 C, and examined for demonstration of sterility. Control experiments indicated no change of compound activity after sterilization and comparison with an unheated control.

Organisms. The organisms used in our survey were clinical isolates which have been maintained in our laboratory (18). The organisms used in the intraspecies survey were also clinical isolates (Providence Hospital, Southfield, Mich., 1970–1972); these had been stored in skim milk broth at −80 C.

Inoculum. A test inoculum consisted of 0.05 ml of an 18- to 24-hr TSB culture (approximately 10^9 organism/ml). The inoculum was aseptically delivered into all dilutions of the compound, well mixed, and incubated at 36 C in a 5% CO_2 atmosphere. A tube of inoculated broth without drug served as a positive control; also, an uninoculated set of dilutions was incubated. After 18 hr of incubation, the minimal inhibitory concentration (MIC) of each compound against each organism was determined. In our study, the MIC is defined as the lowest concentration of compound at which no macroscopic evidence of growth was observed when turbidity of the inoculated broth dilutions was compared with that of the control tubes.

In those cases in which the test compound itself caused turbidity so that the MIC could not accurately be determined, a sample (0.015 ml) of the well-agitated broth in question was inoculated onto a Trypticase soy agar plate containing 5% defibrinated sheep blood, inoculated at 35 C, and examined after 18 hr for bacteriostatic and bactericidal end points. There was usually only a one-tube difference between the bactericidal and bacteriostatic concentrations.

It was found that turbidity owing to the compounds

did not "confuse the readings." Most of the compounds were inhibitory at low concentrations with which solubility was almost complete.

The *p*H of the broth was monitored throughout the study by the use of an Accutint set (Anachemia, Montreal, Que., Canada) and was found to be within the range of 7.3 ± .2. Also, at the concentration used, methanol was found to be noninhibitory, as demonstrated by controlled test experiments.

RESULTS

The MIC (micromoles per milliliter) of the compounds studied is given in Tables 1 and 3. As a link to a previous report (18), lauric acid, lauryl dimethyl amide, and dodecylamine hydrochloride were used for comparativeness and as chemical controls.

Amines. Although both gram-positive and gram-negative organisms were affected by amine compounds, the gram-positive group was more susceptible to the antibacterial action of amines than were gram-negative organisms (Table 1). A comparison of the straight-chain amines from C_8 to C_{20} is given in Fig. 1. As can be seen in Fig. 1, the most resistant organisms were most susceptible to chain length of 10 to 12 carbons. On the other hand, the more susceptible organisms were affected by chain lengths 2 to 3 carbon atoms longer.

Against gram-negative bacteria, hendecylamine (Table 1) showed the most activity (average MIC, 1.14 μmoles/ml). Against gram-positive bacteria, the most active were tridecylamine (0.13 μmole/ml) and tetradecylamine (0.16 μmole/ml). On the basis of activity against all of the microorganisms tested, hendecylamine showed the most inhibitory activity (average MIC, 0.60 μmole/ml).

Comparatively (Table 2), the most susceptible gram-negative bacteria were usually more resistant than the most resistant gram-positive bacteria. The notably resistant gram-negative bacteria were *Proteus mirabilis* and *Pseudomonas*. The most resistant gram-positive organisms were *Bacillus subtilis* and *Streptococcus faecalis*. Susceptible gram-negative bacteria were *P. rettgeri* and *Escherichia coli*; susceptible gram-positive bacteria were *S. pyogenes* (group A and nongroup A) and *Diplococcus pneumoniae*.

Amide derivatives. The amide derivatives of previously active fatty acids were tested (Table 3). In general, the amide derivatives were less active than the amines, and only the more susceptible of the gram-positive organisms were affected. The more resistant gram-positive bacteria and all of the gram-negative bacteria were not inactivated by amides of fatty acids. The lower chain amide (C_{12}) was more active than the longer chain lengths. However, since not all members of the amide series were available for testing, a comparison similar to that made for the amine series was not possible.

From the limited series studied, methylation of the amide nitrogen produces a more active compound. The *N'*,*N'*-dimethyl amide was more active than the *N*-methyl compound. The addition of a hydroxyl group to oleylamide did not enhance activity and may even lessen it.

Isovalerylamide was more active than its corresponding normal, secondary, or tertiary derivative.

It should be noted that the relative antimicrobial order of activity of amide compounds towards a specific genus was the same as that of amine derivatives, but amides were less active.

Microorganisms versus susceptibility. The susceptibility of a given species of organism is a function of several variables. It was of interest, therefore, to determine the relative susceptibility of different strains of a given species. Three strains of *Pseudomonas aeruginosa* were tested. For the genus *Proteus*, we examined the effect of drugs on one strain of *P. vulgaris*, five strains of *P. mirabilis*, and three strains of *P. rettgeri*. Six strains of *E. coli* were tested for susceptibility. Within the *Klebsiella-Enterobacter* group, eight strains were examined. Eight different clinical isolates from patients with staphylococcal infection were tested.

A number of different streptococci were also examined: group D, group A, and other beta-hemolytic (non-A) streptococci. A total of six strains of beta-hemolytic streptococci were tested for susceptibility.

Since a consistent pattern in compound effect on these strains was found, only a summary statement of the results need be made: except for some minor deviations, which are not statistically significant, a compound which is active against a particular species retains the same or a similar order of activity against a variety of different strains of that same species.

DISCUSSION

Previous studies with anion compounds (fatty acids) indicated that their antimicrobial activity was limited chiefly to gram-positive microorganisms (4, 19, 28). Whereas amide derivatives have a similar spectrum of activity as fatty acids, the *N*-methylated derivatives and the amine compounds have a wider spectrum of antimicrobial activity. For any chemical family so far tested in our series, the gram-positive organisms are more susceptible than gram-negative ones. It is conceivable that the considerable amount of lipids in gram-negative organisms protects them from inactivation by lipophilic compounds (32).

TABLE 1. *Minimal inhibitory concentrations (micromoles per milliliter) for amine series (initial screening)*[a]

Organism	*n*-Heptylamine	*n*-Octylamine	*n*-Nonylamine	*n*-Decylamine	*n*-Hendecylamine	*n*-Dodecylamine	Dodecylamine-HCl	1,12-Dodecane diamine	*n*-Tridecylamine	*n*-Tetradecylamine	Myristoleylamine	*n*-Pentadecylamine	Hexadecylamine	Palmitoleylamine	Octadecylamine	Oleylamine	Eicosanylamine	Lauric acid	Lauryl dimethyl amide
Gram-negative bacteria																			
Proteus vulgaris	NI	7.7	3.5	1.59	1.46	1.35	1.12	5.0	NI	NI	NI	4.4	NI	NI	NI	NI	NI	NI	NI
P. mirabilis	11.4	7.7	1.7	1.59	1.46	NI	NI	5.0	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
P. rettgeri	NI	3.8	3.5	0.79	0.58	0.54	0.22	5.0	0.25	NI	NI	4.4	0.4	NI	NI	NI	NI	NI	NI
Escherichia coli	NI	3.8	1.7	0.79	0.58	0.54	0.22	5.0	0.5	2.34	NI	2.2	NI	NI	NI	NI	NI	NI	NI
Klebsiella-Enterobacter sp.	NI	7.7	1.7	1.59	0.58	1.59	0.56	5.0	1.25	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Pseudomonas	11.4	3.8	3.5	3.18	2.9	NI	NI	5.0	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Serratia marcescens	NI	7.7	1.7	1.59	0.73	5.4	0.22	NI	5.0	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Salmonella typhimurium	NI	3.8	1.7	0.79	0.58	0.54	0.22	5.0	0.5	NI	NI	NI	1.03	NI	NI	NI	NI	NI	NI
Gram-positive bacteria																			
Streptococcus faecalis	11.4	3.8	1.7	0.79	0.58	0.67	0.11	5.0	0.25	0.058	0.12	0.014	0.1	0.2	0.93	0.09	NI	4.9	0.2
S. pyogenes (group A)	1.4	0.96	0.17	0.159	0.07	0.27	0.05	0.6	0.06	0.015	0.004	0.0017	0.006	0.013	0.046	0.047	NI	0.24	0.05
S. pyogenes (nongroup A)	5.7	0.96	0.69	0.159	0.146	0.27	0.028	1.25	0.06	0.015	ND	0.0035	ND	ND	ND	ND	ND	0.24	0.05
Streptococcus (Viridans group)	5.7	3.8	0.69	0.6	0.29	0.135	0.22	2.5	0.125	0.058	0.03	0.0035	0.025	0.026	0.23	0.047	0.42	0.624	0.1
Diplococcus pneumoniae	2.8	0.96	0.17	0.3	0.07	0.135	0.22	0.5	0.016	0.015	ND	0.0035	ND	ND	ND	ND	ND	0.06	0.1
Micrococcus sp.	2.8	1.9	0.69	0.79	0.146	0.54	0.56	0.5	0.06	0.058	ND	0.007	ND	ND	ND	ND	ND	2.49	0.2
Sarcina sp.	5.7	1.9	1.7	0.6	0.58	0.67	0.45	2.5	0.125	0.058	0.059	0.007	0.05	0.2	0.93	0.094	3.26	1.24	0.2
Staphylococcus aureus	2.8	1.9	0.87	0.6	0.29	0.27	0.11	2.5	0.125	0.058	0.12	0.007	0.012	0.05	0.046	0.18	NI	1.24	0.2
S. epidermidis	5.7	1.9	1.7	0.79	0.58	0.54	0.11	2.5	0.06	0.058	0.059	0.007	0.025	0.05	0.046	0.18	NI	2.49	0.2
Corynebacterium sp.	2.8	0.38	0.35	0.79	0.018	0.27	0.11	0.5	0.03	0.058	ND	0.0035	ND	ND	ND	ND	ND	0.624	0.67
Nocardia asteroides	5.7	3.8	1.7	0.79	0.29	0.54	0.45	1.25	0.25	0.47	2.36	2.2	1.03	2.09	NI	1.87	NI	0.624	0.1
Bacillus subtilis	NI	3.8	1.7	0.79	0.58	0.54	0.22	5.0	0.5	1.17	ND	4.4	ND	ND	ND	ND	ND	NI	NI
Candida purapsilosis	ND	1.9	ND	0.07	0.073	0.06	0.22	ND	0.03	0.029	ND	ND	ND	ND	ND	ND	ND	2.49	0.1

[a] NI = not inhibitory under test conditions; ND = not done.

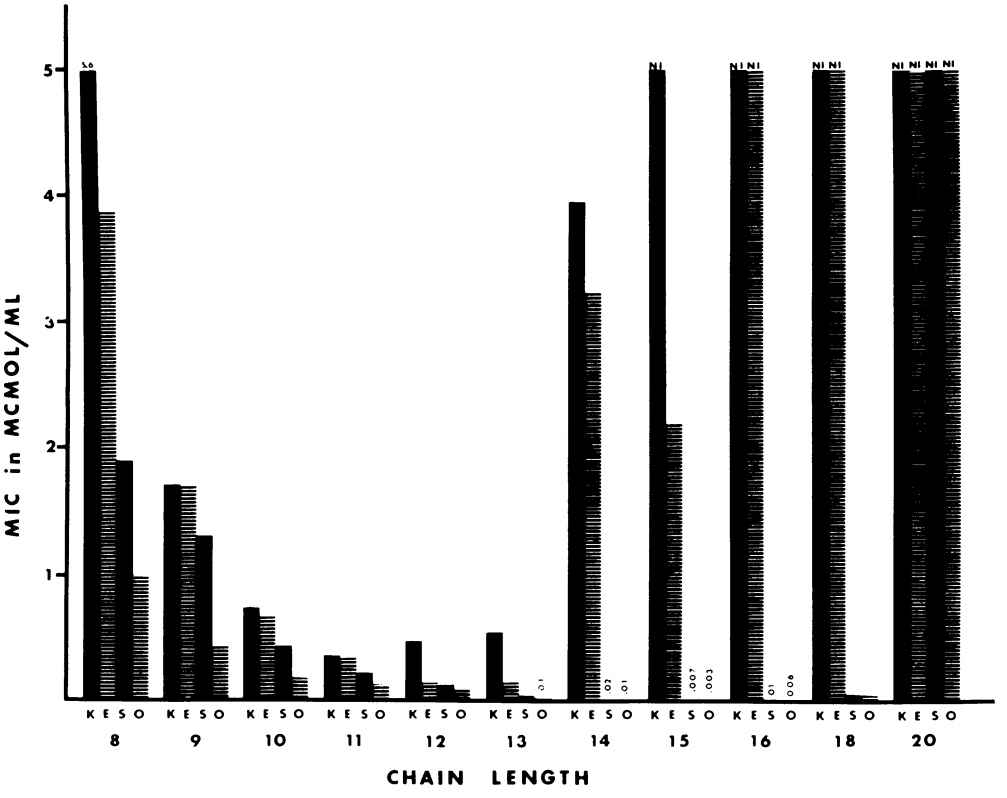

FIG. 1. *Effect of chain lengthening on amine activity tested against Klebsiella-Enterobacter sp. (K), E. coli (E), S. aureus (S), and S. pyogenes group A (O).*

TABLE 2. *MIC averages and ranges for amine compounds*[a]

Compound	Gram-negative bacteria		Gram-positive bacteria		Total species tested	
	Avg	Range	Avg	Range	Avg	Range
Heptylamine	11.4	11.3–NI	5.3	1.4–NI	7.75	1.4–NI
Octylamine	5.8	3.8–7.7	2.19	0.38–3.8	3.57	0.38–7.7
Nonylamine	2.37	1.7–3.5	1.01	0.17–1.7	1.55	0.17–3.5
Decylamine	1.49	0.79–3.18	0.68	0.159–0.79	0.99	0.159–3.18
Hendecylamine	1.14	0.58–1.46	0.28	0.018–0.58	0.60	0.018–1.46
Dodecylamine	2.56	0.54–NI	0.38	0.135–0.67	1.2	0.135–NI
Tridecylamine	2.80	0.5–NI	0.13	0.03–0.5	1.16	0.03–NI
Tetradecylamine	4.40	2.3–NI	0.16	0.015–1.17	1.77	0.015–NI
Pentadecylamine	4.10	2.2–NI	0.55	0.0017–4.4	1.9	0.0017–NI
Dodecylamine HCl	1.44	0.22–NI	0.22	0.028–0.56	0.69	0.028–NI

[a] NI = not inhibitory under test conditions. Listings are in micromoles per milliliter.

Although it is not known with any degree of certainty just what the law(s) governing antimicrobial action(s) is, there have been a number of mechanisms postulated. Gilby and Few (10, 11) have established a relationship between bactericidal action of detergents and their lysis of protoplast of *M. lysodeikticus*. Within the dodecyl series, the lytic action was

$$NH_3 + > - N - Me_3 + > SO_4 = > SO_3$$

Some differences were observed with the anionic surface agents sodium dodecyl sulfate and dodecylamine hydrochloride on isolated membranes (12). Anionic detergents completely

TABLE 3. *Minimal inhibitory concentrations (micromoles per milliliter) for amide series (initial screening)*[a]

Organism	Dodecanamide	Tetradecanamide	Ricinoleamide	Oleylamide	*N,N*-dimethyl oleylamide	Dimethyl, methyl isovalerylamide
Streptococcus faecalis	NI	NI	NI	NI	0.4	NI
S. pyogenes (group A)	0.5	NI	1.77	0.08	0.04	1.08
S. pyogenes (nongroup A)	0.5	NI	1.77	0.35	0.04	1.08
S. viridans	NI	NI	NI	NI	0.4	NI
Diplococcus pneumoniae	0.62	4.4	0.44	0.88	0.02	2.17
Micrococcus sp.	NI	NI	3.5	NI	0.08	4.2
Sarcina sp.	NI	NI	NI	NI	0.32	NI
Staphylococcus aureus	NI	NI	NI	NI	0.16	NI
S. epidermidis	NI	NI	NI	NI	0.16	NI
Corynebacterium sp.	2.5	NI	NI	NI	0.04	1.08
Nocardia asteroides	2.5	NI	NI	NI	3.2	1.08
Candida purapsilosis	NI	NI	NI	NI	ND	4.3

[a] NI = not inhibitory under test conditions: ND = not done. *Bacillus subtilis* was not inhibited by any of these compounds. The following compounds had no effect on any of the organisms tested; *n*-methyl, *n*-valerylamide: *n*-dimethyl, *t*-valerylamide: *n*-dimethyl, *sec*-valerylamide: and *n*-hexadecanamide.

disperse a membrane, whereas cationic compounds do not. Gilby and Few (12) concluded that cationic detergents react with the phosphatidic and lipid components of the cytoplasmic membrane, thus disrupting its permeability properties; it was proposed that the action of anionic detergents involves the protein moiety (possibly as lipoprotein) of the membrane, which was thus completely dissolved.

Novak et al. (30) reported that many *NN* disubstituted decanamides exhibit a broad spectrum of activity against bacteria, yeasts, and molds. In an earlier report (29), derivatives of the C_{18} fatty acids were tested and also found to be active. In these studies, the highest overall antimicrobial activity was exhibited by the substituted amides of C_8 to C_{12}. As in our own study, unsaturation of the amide did not appear to contribute any detectable enhancement to antimicrobial activity.

In the C_{18} *N*-amide series (31), the insertion of an epoxy group appears to contribute more antimicrobial activity than does unsaturation or halogenation. The addition of a second epoxy group does not improve activity beyond that achieved in the monoepoxide. The insertion of a second amide group in the 9 or 10 position of the fatty acid molecule appears to increase compound activity.

As in our findings, amides are generally less active than the corresponding fatty acid (9). However, Goettsch and Wiese (13) prepared several amide derivatives and found them to be excellent fungistats. However, direct comparisons with fatty acid activities were not made.

Cronk et al. (6) synthesized and tested a number of nitrogen derivatives of sorbic acid. Two compounds, *N*-isobutylamine sorbate and morpholine sorbate, exhibited a greater antifungal activity than did the sorbic acid.

Morgan et al. (26) also prepared a number of amide and amine derivatives of sorbic acid. The amine salt *N*-methylfurfurylamine sorbate and amide, however, showed less activity than undecylenic acid in disc-plate testing. It should be pointed out that, in using this method, the diffusion of a compound is important to its measured activity. Consequently, the order of potency found may be a reflection of this variable rather than antibacterial property of the compound per se.

Poly-amine compounds have been tested as potent antibacterial agents (1). According to these authors, an aliphatic chain of seven carbons separating the two amino groups seemed optimal for inhibition of *E. coli*. However, longer-chain compounds were not tested. From this research, it is evident that the two primary amino groups were essential for the antibacterial action; the substitution of an amino group by OH radical decreases activity. In our hands, longer- rather than short-chain mono-amine compounds were more effective against gram-negative bacteria. It should be noted in our mono-amine series that the addition of a second amine group (position 12) to *n*-dodecylamine lowered activity of the parent mono-amine compound. A similar conclusion was reached for dicarboxyclic acids.

It is of interest that cadaverine($NH_2[CH_2]_5NH_2$) was most effective in protecting protoplast-infecting agent against thermal inactivation (8). This has been attributed to the *N* to *N*

distance (0.73 nm) in the cadaverine molecule, which is similar to the distance between the phosphate oxygens in the deoxyribonucleic acid molecule.

Little is known about the mechanism of antimicrobial action of polyamines. Previous studies have shown certain similarities between the action of spermine on bacteria and that of another cationic antibiotic, streptomycin. Both agents are bactericidal and have early effects on protein synthesis and ribonucleic acid (RNA) synthesis, as well as on potassium flux (25). These compounds are presumably bound to the ribosomes on identical sites (21), and both cause extensive misreading of the genetic code (7). However, the relationship between misreading, inhibition of protein synthesis, and cell death has not been fully elucidated for streptomycin or spermine. Also, the stimulation of RNA synthesis by polyamines needs to be explained (1). At this stage, it is difficult to distinguish between the alternative explanations. The reason for the stimulation of RNA synthesis in the presence of polyamines must await the results of further experiments.

Other biological effects of polyamines relate to their effects in neutralizing, stabilizing, or labeling lipid and membrane structures (34–36). Aliphatic polyamines such as spermidine, $H_2N(CH_2)_4NH(CH_2)_3NH_2$, and spermine, $H_2N(CH_2)NH(CH_2)_4NH(CH_2)_3NH_2$, are without effect on *E. coli* active transport systems unless added in concentrations approaching 10^{-2} M. It seems reasonable to attribute the efficacy of the *N*-acyltriamines at 1,000-fold lower concentrations to the lipophilic fatty acids covalently bound to the triamines. This rationale is similar to the argument offered to explain the potency of steroidal diamines as inhibitors of active transport systems (34). In fact, the fatty acid-conjugated triamines are inhibitory at concentrations an order of magnitude lower than the steroidal diamines (33).

The membrane effects of ammonia (20) and amines (14) could explain their enhancement of the rate of electron flow in chloroplasts concomitant with an inhibition of phosphorylation. The uncoupling of photophosphorylation by amines is caused by the free base of the amine rather than by the amine cation (16). Amines and NH_4Cl inhibit the light-induced uptake of H^+ in chloroplasts (27). Amines containing polar groups are much less effective inhibitors of photophosphorylation than nonsubstituted amines. Thus, the penetration of the uncharged form of the amines and their uncoupling activity may be related to their lipoidal solubility. Certain *N,N'*-bis(dichloracetyl) diamines inhibit spermatogenesis (3), block alcohol metabolism (15), and possess potent amoebicidal activity (2). It has been shown that some of these compounds also inhibit drug metabolism in vitro and in vivo (24). It was recently reported that several members of this group of compounds are inhibitors of pyridine nucleotide-linked electron transport and also uncouple phosphorylation from oxidation in beef heart mitochondria (22). Data obtained at that time and since suggest that these drugs act differently from the more classical uncouplers such as 2,4-dinitrophenol (DNP) and carbonyl cyanide *m*-chlorophenyl hydrazone (CCP). There appears to be a very strict requirement for uncoupling phosphorylation from oxidation. Lipid solubility is, of course, important in this system also, but other parameters emerge which play a greater role in determining activity. Substitution of an alkyl group to give a tertiary amide causes a nearly complete loss of activity, even though the compounds may possess solubilities, based on partition data, nearly identical to very active secondary amides. A very strict requirement in the nature of the acyl group also exists. Of the derivatives tested, only this derivative stimulates Mg^{2+} adenosine triphosphatase and induces a rapid *p*H change reminiscent of the change induced by DNP or CCP in weakly buffered media (23).

The explanation of drug toxicity as related to their action on mitochondrial respiration cannot fully be accepted without further proof. What remains of interest with these amine compounds, however, is their wide spectrum of antimicrobial activity regardless of mechanisms involved. Additional data relating structure and biological activity need to be accumulated before any hypothesis can be formulated. To date, the unsubstituted short-chain alkyl amines, chain length 11 to 15, represent active compounds. Although some of the general properties relating the activity of fatty acids to their antimicrobial properties are similar for amine compounds, the amines are unique in that mono-unsaturation *does not* increase compound activity. This single difference may be fruitful for further investigation in our attempt to link structure and antimicrobial activity.

LITERATURE CITED

1. Bachrach, U., and A. Weinstein. 1970. Effect of aliphatic polyamines on growth and macromolecular synthesis in bacteria. J. Gen. Microbiol. 60:159–165.
2. Berberian, D. A., R. G. Slighter, and A. R. Surrey. 1961. *In vitro* and *in vivo* amebicidal activity of N,N'-bis-(dichloroacetyl) diamines. Antibiot. Chemother. 11:245–255.
3. Beyler, L. A., G. O. Potts, F. Coulston, and A. R. Surrey. 1961. The selective testicular effects of certain bis-(dichloroacetyl) diamines. Endocrinology 69:819–833.
4. Boylis, M. J. 1936. Effect of the chemical constitution of soaps upon their germicidal properties. J. Bacteriol. 31:485–504.

5. Clark, J. F. 1899. On the toxic effect of deleterious agent on the germination and development of certain filamentous fungi. Botan. Gaz. **28**:289–327.
6. Cronk, D. H., L. C. Zopf, and J. W. Jones. 1959. Synthesis and antifungal studies on sorbic acid derivatives. J. Amer. Pharm. Ass. Sci. Ed. **48**:455–457.
7. Davies, J., L. Gorini, and B. D. Davis. 1965. Misreading of RNA codewords induced by amino glycoside antibiotics. Mol. Pharmacol. **1**:93–00.
8. Fraser, D., and H. R. Machler. 1958. Effect of diamines on the protoplast infecting agent derived from T_2 bacteriophage. Amer. Chem. Soc. **80**:6456.
9. Gershon, H., R. L. Rodin, R. Parmegiani, and P. K. Godfrey. 1970. IV. Synthesis and antifungal properties of 2-fluoro fatty acid amides. J. Med. Chem. **13**:1237–1239.
10. Gilby, A. R., and A. V. Few. 1957. Reactivity of ionic detergents with micrococcus lysodeikticus. Nature (London) **179**:422–423.
11. Gilby, A. R., and A. V. Few. 1957. Surface chemical studies on the protoplast membrane of Micrococcus lysodeikticus. Proc. Int. Congr. Surface Activity, 2nd, vol. 4, p. 262–269.
12. Gilby, A. R., and A. V. Few. 1960. Lysis of protoplasts of Micrococcus lysodeikticus by ionic detergents. J. Gen. Microbiol. **23**:19–26.
13. Goettsch, R. W., and G. A. Wiese. 1958. The synthesis of some substituted thianaphthene-2-carboxamides and their antifungal properties. J. Amer. Pharm. Ass. Sci. Ed. **47**:319.
14. Good, N. E. 1960. Activation of the hill reaction by amines. Biochim. Biophys. Acta **40**:502–517.
15. Heller, C. G., B. Y. Falgeolle, and L. J. Matson. 1963. Histopathology of the human testes as affected by bis-(dichloroacetyl) diamines. J. Exp. Mol. Pathol. Suppl. **2**:107–114.
16. Hind, G., and C. P. Whittingham. 1965. Reduction of ferricyanide chloroplasts in the presence of nitrogenous bases. Biochim. Biophys. Acta **75**:194–202.
17. Hueck, H. J., D. M. M. Adema, and J. R. Wiegmann. 1966. Bacteriostatic, fungistatic, and algistatic activity of fatty nitrogen compounds. Appl. Microbiol. **14**:308–319.
18. Kabara, J. J., D. M. Swieczkowski, A. J. Conley, and J. P. Truant. 1972. Fatty acids and derivatives as antimicrobial agents. Antimicrob. Ag. Chemother. **2**:23–28.
19. Kodicek, E. 1949. The effect of unsaturated fatty acids on gram-positive bacteria. Soc. Exp. Biol. Symp. **3**:217–232.
20. Krogmann, D. W., A. T. Jagendorf, and M. Avron. 1959. Uncouplers of spinach chloroplast photosynthetic phosphorylation. Plant Physiol. **34**:272–277.
21. Mager, J., M. Benedict, and M. Artman. 1962. A common site of action for polyamines and streptomycin. Biochim. Biophys. Acta **62**:202–204.
22. Merola, A. J., and G. P. Brierley. 1970. Inhibition of mitochondrial oxidation and uncoupling of phosphorylation by antispermatogenic bis-dichloroacetamides. Biochem. Pharmacol. **19**:1429–1442.
23. Merola, A. J., K. M. Hwang, M. Jerkowitz, and G. P. Brierley. Structural requirements in the uncoupling of oxidative phosphorylation by N, N′-bis-(dichloroacetyl) diamines. Biochem. Pharmacol. **20**:1393–1403.
24. Merola, A. J., and J. D. Turnbull. 1967. The inhibition of drug metabolism by antispermatogenic N,N′-bis-(dichloroacetyl) diamines. Biochem. Pharmacol. **16**:211–215.
25. Mills, J., and D. T. Dubin. 1966. Some effects of spermine on *E. coli*. Mol. Pharmacol. **2**:311–318.
26. Morgan, L. W., D. H. Cronk, and R. P. Knott. 1969. Synthesis and *in vitro* fungistatic evaluation of some N-substituted amides and amine salts of sorbic acid. J. Pharm. Sci. **58**:942–945.
27. Neumann, J., and A. T. Jagendorf. 1964. Light-induced pH changes related phosphorylation by chloroplasts. Arch. Biochem. Biophys. **107**:109–119.
28. Nieman, C. 1954. Influence of trace amounts of fatty acids on the growth of microorganisms. Bacteriol. Rev. **18**:147–163.
29. Novak, A. F., M. J. Fischer, S. P. Fore, and H. P. Dupuy. 1964. Antimycotic activity of some fatty acid derivatives. J. Amer. Oil Chem. Soc. **41**:503–505.
30. Novak, A. F., and J. M. Solar. 1969. Antimicrobial activity and physical characteristics of some N,N-disubstituted decanamides. J. Amer. Oil Chem. Soc. **46**:249–251.
31. Novak, A. F., J. M. Solar, R. R. Mod, F. C. Magne, and E. L. Skau. 1969. Antimicrobial activity of some *N*-substituted amides of long-chain fatty acids. Appl. Microbiol. **18**:1050–1056.
32. Russell, A. D. 1969. The mechanism of action of some antibacterial agents. Progr. Med. Chem. **6**:135–199.
33. Silva, S., and M. L. Kralovic. 1969. Fatty acid conjugated polyamines that alter cell permeability and active transport properties of *E. coli*. Mol. Pharmacol. **5**:300–302.
34. Silver, S., and E. Levin. 1968. Actions of steroidal diamines on active transport and permeability properties of *E. coli*. J. Bacteriol. **96**:338–345.
35. Tabor, C. W. 1962. Stabilization of protoplasts and spheroplasts by spermine and other polyamines. J. Bacteriol. **83**: 1101–1111.
36. Tabor, H., and C. W. Tabor. 1964. Spermidine, spermine and related amines. Pharmacol. Rev. **16**:245–000.