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Relationship of Bactericidal Potency to Length of Fatty Acid Radical of Certain Quaternary Ammonium Derivatives.

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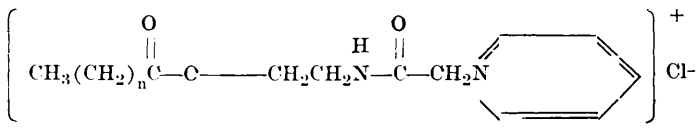
Within recent years, a number of quaternary ammonium derivatives have been introduced as antiseptic substances. These compounds are characterized in that they have a long chain lypophile group in the cation balanced with a hydrophile group as the anion. Some of these compounds have predominantly long chain lypophile alkyl groups attached directly to nitrogen.[1,2] Others have a long chain lypophile group interrupted by ether linkages or phenyl groups.[3,4]

Recently new types of quaternary ammonium cationic compounds have been introduced as bactericidal agents in which the lypophile group is interrupted by carboxyl and amide linkages.[5] They are characterized by the fact that in the cationic part of the molecule there is an acyl group, a colamine group and a formyl-methyl quaternary ammonium radical.

The introduction of the carboxyl and amide groups into the molecule has an effect upon the functional properties of the compound. A study was made of the effect on the bactericidal value of these compounds in relation to the chain length of the fatty acid radical which enters in the lypophile group, and their respective relations to detergency. The compounds were prepared according to the method outlined by Katzman.[6] The fatty acids were varied from C_8 to C_{18} and the bactericidal value of the homologous compounds was determined according to the official F.D.A.[7] Method. The results are tabulated in Table I in which it is shown that varying the fatty acid radical has a decided

TABLE I.
Effect of Changing the Fatty Acid Radical on Killing Power of



No. of carbons in $CH_3(CH_2)_nC(=O)O-$	*Average maximum dilution to kill organism in 10 minutes			
	20°C		37°C	
	S. aureus	*E. typhosa*	*S. aureus*	*E. typhosa*
8	1: 350	1: 550	1: 800	1: 1,300
10	1: 2,750	1: 2,000	1: 3,500	1: 4,000
12	1:14,000	1:10,000	1:25,000	1:30,000
14	1:30,000	1:30,000	1:55,000	1:55,000
16	1: 7,000	1: 3,500	1: 9,000	1:11,000
18	1: 4,000	1: 1,500	1: 7,000	1: 6,000

*According to F.D.A. official method.

1 Domagk, G., U. S. Patent No. 2,108,765, 1938.

2 Domagk, G., *Deut. Med. Wochenschr.*, 1935, **21**, 829.

3 Bruson, H. A., U. S. Patent No. 2,115,250, 1938.

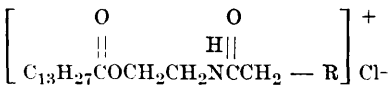
4 Rawlins, A. L., Sweet, L. A., and Joslyn, D. A., *J. Am. Pharm. Assn.*, 1943, **32**, 111.

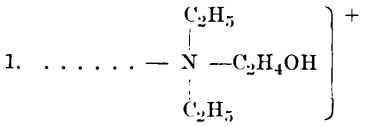
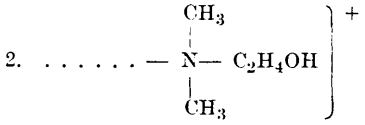
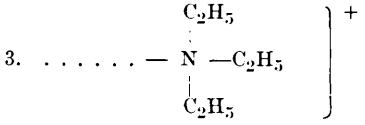
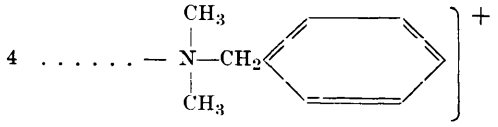
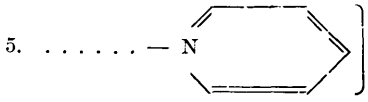
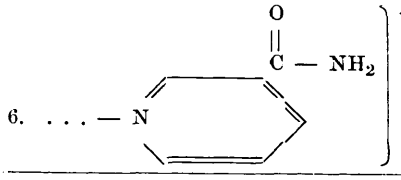
5 Epstein, A. K., and Harris, B. R., U. S. Patent No. 2,290,173, 1942.

6 Katzman, M., U. S. Patent No. 2,189,664, 1940.

7 Ruehle, G., and Brewer, C. M., 1931, U. S. Food and Drug Administration Method of Testing Antiseptics and Disinfectants, Circular No. 198, U. S. D. A.

TABLE II.
Effect of Changes in R on Killing Power of



R	Dilution required to kill organisms in 10 minutes at 20°C	
	Staph. aureus ×1000	*E. typhosa* ×1000
1. 	1:30	1:30
2. 	1:30	1:30
3. 	1:25	1:30
4 	1:25	1:30
5. 	1:30	1:30
6. 	1:10	1:15

effect on the bactericidal potency.

Kuhn and Dann[8] have shown, in studying the bactericidal effects of alkyl dimethyl sulphonium iodides on *B. coli* and *Staphylococcus aureus,* and varying the alkyl group from methyl to butyl, octyl, lauryl and cetyl, that the optimum bactericidal function for coli was obtained with the lauryl derivative, and for Staphylococci, the cetyl radical was most effective.

Kuhn, Jerchel and Westphal[9] reported on the series di-alkyl methyl benzyl ammonium chloride varying the alkyl radicals from C_4 to C_6, C_8, C_{12} and C_{16}, respectively. They found optimum potency at C_8 for Staphylococci, diphtheria, *B. coli,* Friedlander, but for paratyphoid, C_8 and C_{12} radicals were more effective. The same authors reported that in using C_8, C_{12} and C_{16} radicals in the alkyl groups of alkyl dimethyl benzyl ammonium chloride, the greatest bactericidal potency was obtained from the compound containing C_{16} in the alkyl chain. In the specific homologous series of the quaternary compounds which we studied, we found that in varying the fatty acids from C_8 to C_{18} re-

[8] Kuhn, R., and Dann, O., *Ber.*, 1940, **73**B, 1092.

[9] Kuhn, R., Jerchel, D., Westphal, O., Moeller, E. F., and v. Czernucki-Hrebeljanowitsch, M., *Ber.*, 1940, **73**, 1095.

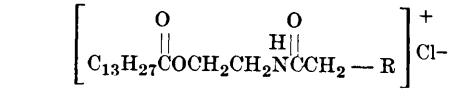
spectively, the optimum bactericidal function was obtained when the compound contained the fatty acid radical of C_{14} and that the compound which contained the C_8 radical had the lowest bactericidal value.

It is therefore evident that the bactericidal functions of these types of compounds depend upon the specific structure and the configuration of the molecule and also to a certain extent on the type of organism which is used in the test.

A compound designated as E 607 (Special) of the same series as shown in Table II was prepared from a mixture of C_{12} and C_{14} fatty acids and tested bacteriologically against *Staph. aureus* and *E. typhosa* at 20°C and 37°C respectively. The results show that the addition of C_{14} fatty acid ester increased the bactericidal potency of the C_{12} compound almost twofold.

One member of the series in Table I was selected for study of the effect of varying the groups attached to the pentavalent nitrogen on the bactericidal potency. For the acyl radical, C_{14} was selected as the fatty acid ester which gave the highest results. The compounds prepared are tabulated in Table II together with their bactericidal activity against *E. typhosa* and *Staph. aureus*.

It is noteworthy that there is little relative difference in the bactericidal potency of the quaternary ammonium derivatives in the series of



when the R radical is varied by substituting three lower molecular weight groups on the pentavalent nitrogen as shown in examples one, two, three, four and five respectively in Table II. If, however, R is nicotinic amide as shown in example No. 6 in Table II the bactericidal activity is reduced considerably. If compounds No. 5 and No. 6 in Table II are compared it appears that the substitution of an amide group in the beta position of the pyridine ring of compound No. 5 reduces the bactericidal activity of the compound. This is parallel to the vast reduction in the toxicity of pyridine when its beta hydrogen is substituted with $-C(=O)NH_2$ group to form nicotinic amide.

The phenol coefficient as determined by the standard F.D.A. method[7] may not be accepted in all laboratories as proof of the germicidal value of these compounds. The toxicity indices were therefore determined according to the technics of Welch and Brewer [10, 11] and Hirsch and Novak.[12]

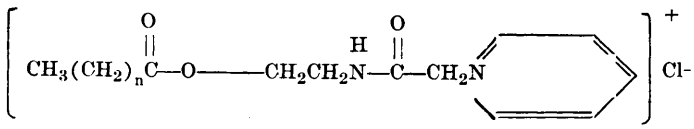
The lauric acid ester of colamino formyl methyl pyridinium chloride (which was designated as E 607) gave a toxicity index of less than .6 by both of these technics. A toxicity index which is less than unity is considered safe for tissue antisepsis.

Summary. (1) A number of homologs of quaternary ammonium derivatives were prepared of the series (see formula below) varying the carbon chain in the fatty acid radical from C_8 to C_{18} and the bactericidal potency of each compound was determined using *Staph. aureus* and *E. typhosa* as test organisms.

(2) In this series it was found that the C_{14} homolog possesses the maximum bactericidal activity and the sequence of diminishing bactericidal power is as follows:

$$C_{14}>C_{12}>C_{16}>C_{18}>C_{10}>C_8.$$

(3) A number of myristic acid esters of colamino formyl methyl quaternary ammonium chloride was prepared wherein the molecule as a whole remained constant in its structural configuration except as to the lower molecular weight substitution groups on the pentavalent nitrogen. The bactericidal ac-



[10] Welch, H., and Brewer, C. M., *J. Immunol.*, 1942, **43**, 25.

[11] Welch, H., Hunter, A. C., and Slocum, G. G., *J. Lab. and Clin. Med.*, 1942, **27**, 1432.

[12] Hirsch, M. M., and Novak, M. V., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 376.

tivity of this series of compounds was determined using the same test organisms and was found that there is no appreciable difference in the respective compounds with the exception of the nicotinic amide derivative.

(4) The substitution of beta hydrogen in the pyridine ring by $-\overset{\overset{O}{\|}}{C}NH_2$ reduces the bactericidal potency of the pyridinium derivative.

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Taxonomic Relationships in the Genus Proteus.

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Recently Cope and Kilander[1] described 83 strains isolated from gastroenteritis patients, contacts and foodhandlers with suggestive or definite history of enteric disorders as "atypical enteric organisms of the *Shigella* group." Stuart *et al.*[2] reported 19 comparable strains tentatively classed as "anaerogenic paracolon" cultures which were isolated in this laboratory or received from the laboratories of several states. To date the number of strains studied by us has increased to 48, including 5 strains kindly furnished by Dr. E. J. Cope. Forty-three of the strains were isolated from fecal specimens of gastroenteritis patients or foodhandlers, one from the blood of a patient and 4 from apparently normal contacts. To facilitate discussion the cultures studied in this laboratory will be called, type 33111.

Cultural Characteristics. Type 33111 cultures grew readily on eosin methylene blue agar and on Salmonella Shigella agar forming transparent and colorless colonies. All strains isolated in this laboratory were motile in semisolid, tryptone agar (0.25% agar) at 25°C. Several strains received from different laboratories as nonmotile on isolation were motile under the conditions specified. The motility of type 33111 cultures was usually poor and sometimes negative at 37°C. On agar slants an occasional strain showed slight spreading. On nutrient agar plates (1.0% agar) inoculated by touching the center of the plate with a needle, the growth of some strains covered the entire surface of the plate, others partially, others spread slightly while a few showed no tendency to spread after 48 hours at 25°C. The swarming characteristic of some strains was suggestive of the genus *Proteus* and flagella stains were made on representative strains of each species in this genus. *Pr. vulgaris, Pr. mirabilis, Pr. morganii* and type 33111 cultures showed peritrichous flagellation. *Pr. hydrophilus* and *Pr. ichthyosmius* possessed a single polar flagellum. Kulp and Borden[3] and Guthrie and Hitchner[4] found *Pr. hydrophilus* to be monotrichate.

Biochemical reactions. Type 33111 cultures fermented glucose and mannitol in 24 to 48 hours and sucrose in 14 to 21 days. Nineteen strains fermented salicin in 24 to 48 hours. Lactose and maltose were not attacked. Six strains produced a bubble to 10% gas in glucose and mannitol and one of these a bubble of gas in salicin but no gas was produced in sucrose by any culture. The remaining strains were anaerogenic in all fermentable carbohydrates.

Using a new urea medium Rustigian and Stuart[5] showed that the production of urease by *Pr. vulgaris* and *Pr. mirabilis* could be de-

1 Cope, J. E., and Kilander, K., *Am. J. Pub. Health*, 1942, **32**, 352.

2 Stuart, C. A., Wheeler, K. M., Rustigian, R., and Zimmerman, A., *J. Bact.*, 1943, **45**, 101.

3 Kulp, W. L., and Borden, D. G., *J. Bact.*, 1942, **44**, 673.

4 Guthrie, R., and Hitchner, E. R., *J. Bact.*, 1943, **45**, 52.

5 Rustigian, R., and Stuart, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 108.