Bactericidal Activity of Certain Fatty Acids[1]

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THE bactericidal activity of the salts of fatty acids has been the subject of a critical review by Klarmann (1), who summarized and interpreted the controversial early literature. The sodium salts of certain unsaturated fatty acids and abietic acid were considered bactericidal to *Staphylococcus aureus* and *Escherichia coli* by Bayliss (2). In 1941 Klarmann and Shternov (3) studied the germicidal activity of potassium salts of saturated fatty acids from 1- to 18-carbon atoms and concluded that, in general, soaps should not be classified as disinfectant, antiseptic, or germicidal although potassium salts of the 8-, 9-, and 10-carbon fatty acids showed evidence of germicidal activity in solutions of fairly high concentration and the activity varied with different organisms. No attempt was made to correlate bactericidal activity with pH. Tetsumoto (3a) had previously indicated that saturated solutions of undecylic and lauric acids possessed sterilizing action against *Bacterium typhosa* and *Vibrio cholerae* but that the effect of pH was not large. Later Stuart and Pohle (4) investigated germicidal activity of soaps prepared from a number of fatty acids and rosin acids and found that activity varied somewhat with the pH of the alkaline soap solutions. They also found that rosin acid soaps had better germicidal activity than fatty acid soaps.

In the references cited, no tests were apparently conducted on germicidal activity of fatty acids adjusted to lower pH values even though several workers had shown previously that such antiseptics as benzoic and salicylic acids and phenols (5) are much more bactericidal in acidic media than in the neutral or alkaline range. For example, Degering and his coworkers (6-10), who later studied this phenomenon quite thoroughly, attribute it to a "hydrogen-ion effect" while other workers (11) regard the undissociated benzoic or salicylic acid molecule as the toxic germicidal principle. The fungistatic properties of the fatty acids and phenols (12, 13, 14) are likewise enhanced with increasing acidity; fatty acids from 8- to 12-carbon atoms were most effective for the inhibition of mold growth. Likewise the fungistatic principle (15-17) of human hair has been shown to consist of fatty acids in the C_7 to C_{13} range. The sodium salts of the fatty acids with an odd number of carbon atoms, *i.e.*, enanthic, pelargonic, undecylic, and tridecylic were more fungistatic against *Microsporon audouini* than those with an even number of carbon atoms.

An excellent study on the inhibition of bacterial metabolism by various cationic and anionic detergents was carried out by Baker, Harrison, and Miller (18), who pointed out that the inhibitory action is markedly influenced by hydrogen-ion concentration. They showed that cationic detergents exhibited maximum activity in the alkaline pH range and the anionic in the acid range.

In connection with a study on the bactericidal activity of polyethenoxy tallate ozonides (19) it was noted that pelargonic acid, a degradation product, possessed bactericidal activity against *Staphylococcus aureus* and that this activity was materially enhanced by acidity. In view of the above-described controversial literature on the bactericidal activity of soaps and also the effect of pH on other acidic bactericides, it seemed desirable to study the effect of the acidic medium on the bactericidal activity of a number of fatty acids in this range, particularly since this phase seems to have been neglected heretofore. The results of this study and a possible explanation of bacterial inhibition are presented herein.

Experimental Details

Materials. The fatty acids were pure samples obtained commercially and possessed the following melting points: pelargonic, 10-12°; capric, 29-30°; undecylic, 27.5-28.5°; lauric, 42-43°. A 1-500 solution of each of the acids was prepared by dissolving 1.000 g. of the fatty acid in a sufficient quantity of 1.000 *N* sodium hydroxide solution to neutralize the acid exactly, and the sodium salt was diluted to 500 ml. with sterile distilled water.

The organisms *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus* A.T.C.C. No. 6538, FDA strain 209, 1938) and *Escherichia coli* (A.T.C.C. No. 11229, W. C. Morse AMSGS strain 198) were obtained from the American Type Culture Collection. The remaining organisms (Table II) were obtained through the kindness of Sr. M. Vincent of the College of St. Francis, Joliet, Illinois.

Procedure. Various dilutions of each of the alkaline soap stock solutions (1:500) were prepared, and the volume of acetic acid (100%, 20%, or 2%) necessary to bring each solution to a pH of 3, 4, 5, or 6 without altering the total volume materially was predetermined. A series of titration curves was thus obtained for each dilution of each acid. The bactericidal tests were performed essentially according to the FDA method (20) except that in the actual procedure 5 ml. of the particular dilution of each fatty acid (sodium salt) was placed in the test tube and the predetermined amount of acetic acid was added accurately with a micropipet in order to adjust the solution to the desired pH. One-tenth ml. of a 24-hr. culture of bacteria was transferred to the acidic fatty acid solution, and one loopful of this solution was transferred to culture tubes in 5-, 10-, and 15-minute intervals, and the pH of the remaining fatty acid solution was again ascertained. Addition of the bacteria culture raised the pH from 0.05 to approximately 0.15 of a pH unit, but this was not taken into account in preparing Figures 1 and 2. The culture tubes were then incubated for 48 hours at 37°C., and bactericidal activity was determined by the appearance or absence of turbidity. It was noted that some of the culture tubes containing the transfers, allowed to incubate for 14 days, gave no apparent change from results obtained after 48 hours of incubation. In order to eliminate the possibility of bacteriostatic action, control experiments were set up in which the acidified fatty acid solutions were neutralized to a pH of 8.5 at one-minute intervals after inoculation fol-

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TABLE I

Bactericidal Activity of Fatty Acids Against *Staphylococcus Aureus* in Acidic Solution

Fatty Acid	pH 3		pH 4		pH 5		pH 6	
	A	B	A	B	A	B	A	B
Pelargonic	1-12,000	1-13,000	1-11,000	1-12,000	1- 7,000	1- 8,000	1- 500	1- 1,000
Capric	1-65,000	1-66,000	1-48,000	1-50,000	1-17,000	1-18,000	1-13,000	1-14,000
Undecylic	1,88,000	1-90,000	1-59,000	1-60,000	1-26,000	1-27,000	1-20,000	1-21,000
Lauric	1-48,000	1-50,000	1-10,000	1-11,000	1- 2,000	1- 4,000	<1- 500	<1- 500

A. Highest dilution for negative growth after 10 minutes' exposure.
B. Lowest dilution for positive growth after 10 minutes' exposure.

lowed by immediate transfer to the culture tubes. It was found that after a 3- to 4-minute contact of *Staphylococcus aureus* with the fatty acids at pH 3 the culture exhibited no growth even after a 14-day incubation whereas a shorter period of contact resulted in growth in the culture tubes within 24 hours. Since it was also considered possible that bacteria agglutinated with fatty acid might adhere to the test tube walls, a swab technique was used which indicated the absence of this phenomenon.

The bactericidal activity of phenol was determined daily in order to check the activity of the bacteria. The highest dilution killing in 10 minutes but not in five was usually found to be in the order of 1-120.

In determining bactericidal activity of the fatty acids against *Staphylococcus aureus*, the highest dilution which gave negative growth and the lowest dilution which gave positive growth after a 10-minute interval was determined for each acid at the various pH's, using acetic acid. Table I records these observations, which represent the average of a large number of determinations, and the results are also depicted graphically in Figures 1 and 2.

A pH of 3 was chosen as the lower limit in the above experiments to avoid any killing action by the acetic acid alone. In a number of control experiments it was noted that acetic acid was not bactericidal to *Staphylococcus aureus* at a pH of 2 or 3, but a pH of 1.5 appeared to inhibit the growth of this organism.

Tests with undecylic acid against several other organisms were conducted at a pH of 3, using acetic acid, and are recorded in Table II. However no attempt was made to obtain the highest dilution for negative growth and the lowest dilution for positive growth as in the preceding table. The dilutions given in Table II only give indication of the order of magnitude for negative or positive growth for the bacteria presented.

TABLE II

Bactericidal Activity of Undecylic Acid Against Various Microorganisms at pH 3

Organism	Gram Stain	Dilution giving Negative Growth after 10 Minutes	Dilution giving Positive Growth after 10 Minutes
Staphylococcus aureus	Gm.(+)[a]	1- 88,000	1- 90,000
Escherichia coli	Gm.(−)	1- 85,000	1- 95,000
Salmonella paratyphi	Gm.(−)	1- 95,000	1-100,000
Shigella flexner	Gm.(−)	1- 85,000	1- 95,000
Serratia marcescens	Gm.(−)	1- 85,000	1- 95,000
Sarcina lutea	Gm.(+)	1-100,000	1-105,000

[a] Gm.(+), Gram Positive; Gm.(−), Gram Negative Organisms.

It is interesting that *Bacillus staerothermophilus* seems to be killed in dilutions in excess of 1-130,000. However this may be due to acetic acid itself or to a complementary action of acetic and undecylic acids.

Although most of the tests at pH 3 were conducted throughout this study with acetic acid, it seemed of interest to effect the acidity with other acids to determine whether this was actually a pH effect or some specific synergistic action of acetic acid with other fatty acids. The tests with several acids recorded in Table III, using *Staphylococcus aureus*, indicate that bactericidal activity is independent of the nature of the acid.

The bactericidal activity of undecylic acid to *Staphylococcus aureus* was also ascertained by the plate-count method (21). The results in terms of % surviving bacteria are listed in Table IV.

The agar plate method devised by the U. S. Public Health Service for water-insoluble antiseptics (22) was carried out with the fatty acids. The results with petroleum jelly (Vaseline), in which the width of the

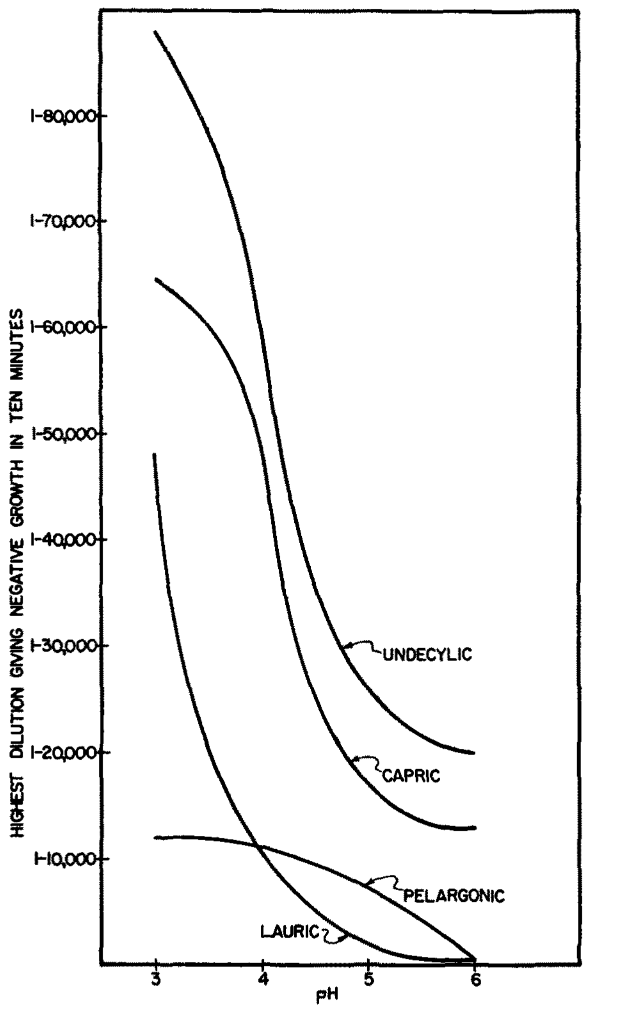


FIG. 1. Variation of bactericidal activity (*Staphylococcus aureus*) with pH of various fatty acids.

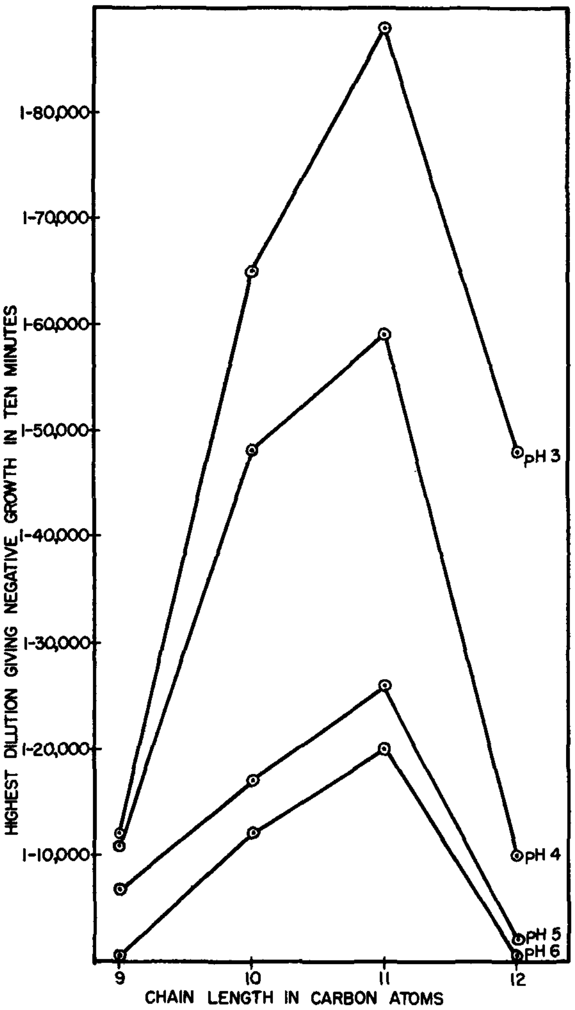


FIG. 2. Variation of bactericidal activity *(Staphylococcus aureus)* with fatty acid chain length.

clear zone indicates the inhibitory effect of the fatty acid to *Staphylococcus aureus,* are shown in Table V.

Other ointments prepared with a hydrogenated vegetable fat and with lanolin gave similar results. For example, a hydrogenated vegetable oil "Crisco" containing 3% acetic acid and 5% lauric acid gave a zone of inhibition of 3 mm. while pelargonic acid under similar conditions gave a value of 4 mm.

Discussion of Results

From the results depicted in Figure 1 it becomes evident that the bactericidal activity of the fatty acids from 9- to 12-carbon atoms increases substantially with increasing hydrogen-ion concentration. Furthermore Figure 2 indicates the chain length of the fatty acid necessary for optimum bactericidal activity, namely undecylic. In accord with the theory about the germicidal action of fatty acids to be presented in the following discussion, it would seem that the fatty acids lower than undecylic are of increasing water-solubility and hence form a film which would be more easily penetrable or the fatty acids are more easily solubilized in the culture media while those of greater chain length than undecylic are water-insoluble to such an extent that they do not participate as fully in the germicidal mechanism. The effect of acidity on germicidal activity of other acidic substances is attributed to "hydrogen-ion effect" (9) or the toxicity of the undissociated acidic (11) principle. The explanation presented below embodies both of these theories. It is to be particularly noted that the activity of the 9- to 12-carbon fatty acids at pH 3 or 4 is of the same order as that of the quaternary ammonium salts. Furthermore it should be noted that the latter bactericides are more effective in the alkaline range (23). In view of these facts it would seem that fatty acids in acid solution behave in a manner similar to long-chain quaternary amines in alkaline solution. An explanation for bactericidal action of both fatty acids and the quaternary amines is presented with the idea of further stimulating work on the mechanism of bactericidal action.

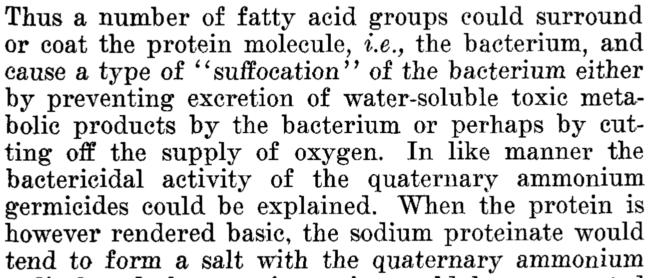
TABLE III
Effect of Various Acids Upon Bactericidal Activity of Undecylic Acid on *Staphylococcus Aureus* at pH 3

Acid	Dilution of Undecylic Acid	Time of Exposure in Minutes		
		5	10	15
Acetic	1-85,000	−	−	−
	1-95,000	+	+	+
Citric	1-85,000	−	−	−
	1-95,000	+	+	+
Oxalic	1-85,000	−	−	−
	1-95,000	+	+	+
Phosphoric	1-85,000	−	−	−
	1-95,000	+	+	+
Hydrochloric	1-85,000	−	−	−
	1-95,000	+	+	+

TABLE IV
Effect of Undecylic Acid on *Staphylococcus Aureus* at pH 3

Dilution of Undecylic Acid	% Organisms Surviving		
	5 min.	10 min.	15 min.
1- 50,000	0	0	0
1- 85,000	24	0	0
1-180,000	74	50	22
Control	100	100	100

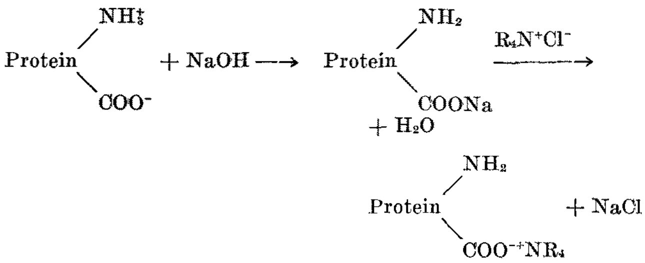
Consider the bacterium as a large protein molecule with amino and carboxylic acid groups arranged in Zwitterion formation. Acidification of the protein with any acid HX should have a "hydrogen-ion effect," *i.e.,* increase the tendency for the Protein-NH_3^+ radical to form a salt with the "undissociated" long-chain fatty acid R-COOH.



Thus a number of fatty acid groups could surround or coat the protein molecule, *i.e.,* the bacterium, and cause a type of "suffocation" of the bacterium either by preventing excretion of water-soluble toxic metabolic products by the bacterium or perhaps by cutting off the supply of oxygen. In like manner the bactericidal activity of the quaternary ammonium germicides could be explained. When the protein is however rendered basic, the sodium proteinate would tend to form a salt with the quaternary ammonium radical and the protein again would become coated with long chain fatty groups.

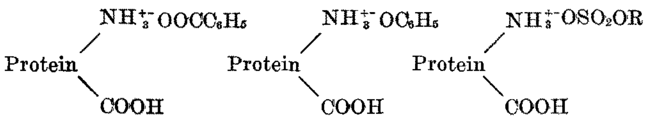
TABLE V
Bactericidal Effect of Undecylic Acid in a Vaseline Ointment Composition

Composition of Ointment	Width of Inhibited Zone in mm.	Interpretation
Vaseline	0	None
Vaseline + 5% Phenol	1	Poor
Vaseline + 5% Undecylic Acid	1½	Poor
Vaseline + 1% Acetic Acid	0.75	Poor
Vaseline + 2% Acetic Acid	1	Poor
Vaseline + 3% Acetic Acid	1½	Poor
Vaseline + 1% Acetic + 5% Undecylic Acid	2	Fair
Vaseline + 2% Acetic + 5% Undecylic Acid	3	Good
Vaseline + 3% Acetic + 5% Undecylic Acid	7	Excellent



The statement that "bacterial cells enter into some combination with the quaternary ammonium compounds which precipitates them from their suspension" was actually made by Klarmann and Wright (24). In connection with bactericidal activity of cationic and anionic detergents (18) it was also noted that "it is possible that hydrogen-ion concentration directly affects the bacteria, altering either membrane or protoplasm in such a manner as to render the microorganisms more susceptible to the action of the detergent." These observations lend additional support to the above theory. However it should be pointed out that a fatty acid coating as indicated above on any protein molecule essential to the bacteria, such as an enzyme, *e.g.*, catalase, could conceivably deactivate the enzyme and thus endanger the life of the bacteria.

It should also be indicated that the bactericidal activity of benzoic acid, salicylic acid, anionic detergents, and perhaps even of the phenols, all of which inhibit bacterial metabolism in the acid range, might be explained by this type of hypothesis except that the bacterium would become coated with the respective benzoyl, phenolic, or alkyl sulfate groups, *e.g.*:



In order to show whether possible chemical combination of fatty acid with bacteria could occur, the following experiment was performed. A 24-hr. culture of *Staphylococcus aureus* was prepared in the customary manner. To 25 ml. of this suspension was added 50 ml. of the 1-500 sodium undecylate solution (100 mg.). The mixture was adjusted to a pH of 3 with acetic acid and incubated over-night at 37°C. The bacterial cells were then separated by centrifugation and pipetting, and the clear supernatant liquor was assayed for undecylic acid using *Staphylococcus aureus* at pH 3. The total weight of undecylic acid in the centrifugate amounted to 2.9 mg. by the microbiological assay method. As a further check, the centrifuged cells themselves were washed five times with 25-ml. portions of light-boiling petroleum ether by trituration and centrifugation in order to remove any unreacted undecylic acid, and the combined extracts were concentrated in a stream of air to a residual syrup, which was dissolved in 20 ml. of 0.1 *N* sodium hydroxide solution and assayed for undecylic acid. The petroleum ether extract contained 95.5 mg. of the 100 mg. of undecylic acid originally present in the bacterial suspension. The bacterial cells themselves were then washed once with 20 ml. of 0.1 *N* sodium hydroxide solution by centrifugation, and the alkaline solution was filtered through celite, acidified to a pH of 3 with acetic acid, and also assayed for undecylic acid. The results showed that 1.13 mg. of the original undecylic acid had been adsorbed by the bacterial cells, most probably by salt formation with the protein; a total weight of 99.53 mg. of undecylic acid from the original 100 mg. was accounted for by the assay method. The above experiment was also repeated by adding 4 mg. of undecylic acid to 25 ml. of bacterial suspension. After similar processing 0.85 mg. of the acid was obtained in the supernatant liquor, 2.05 mg. in the petroleum ether; and the bacteria had absorbed a total weight of 1.14 mg. However it should be pointed out that the exact mechanism by which the bacteria are actually killed by the fatty acids is yet to be determined. It is interesting to note that the bactericidal activity of the fatty acids is inhibited by surface-active agents, such as nonionic detergent oils and even by certain polyphosphates, depending of course upon concentration. This fact would indicate that the surface-active agents tend partially to solubilize the fatty acid coating on the bacterium.

The binding of the bacterium by the fatty acids is also in keeping with the work of Davis and Dubos (25), who showed that serum albumin could be coated with oleic acid. Further work by Davis (26) indicated that tubercle bacilli could remove oleic acid from his medium.

Rancidity of Fats

In a recent study Roth and Halvorson (27) indicated that unsaturated fatty acids, *e.g.*, oleic acid, as well as rancid fats, upon treatment with oxygen gas and warming, produced a substance which inhibited the growth of various bacteria. In view of the results obtained in the present study, a sample of oleic acid was oxidized with ozone gas, and the product was observed to be highly bactericidal to *Staphylococcus aureus*, especially in acidic media. The oleic acid ozonide was then steam-distilled after preliminary treatment with hydrogen peroxide and acetic acid, and it was noted that the bactericidal principle was largely volatile with steam. The volatile product was identified as pelargonic acid, which was previously known to be one of the oxidation products of oleic acid. It is entirely conceivable that unsaturated fatty acids upon air oxidation, *i.e.*, upon rancidification, liberate a sufficient quantity of pelargonic acid to render them bactericidal, especially in the presence of other acidic products liberated in the same manner. Assuming the pH of rancid fat to be in the neighborhood of 5, only 1 part of pelargonic acid per 7,000 parts of fat would need be formed for bactericidal activity.

Microbiological Method of Assay for Certain Fatty Acids

It should be emphasized that when the nature of the fatty acid is known and the pH is controlled, the bactericidal activity of the fatty acid, *e.g.*, with *Staphylococcus aureus,* may be utilized as illustrated in Figures 1 and 2 in order to assay for micro quantities of the individual acids especially in the C_9 to C_{12} range. For example, since undecylic acid possesses a killing dilution of 1:88,000 at a pH of 3, 8.8 ml. of solution will contain 0.1 mg. of the acid. Thus under these conditions, if 10 ml. of a solution would require a 1-88 dilution to reach the highest killing dilution, then a total of 10 mg. of undecylic acid would be present in the original solution.

Other Bacteriological Applications

Previous workers (28) have demonstrated the usefulness of a lauryl sulfate-tryptose broth for detection of coliform organisms. It is quite possible that certain dilutions of fatty acids at various pH's might also give selective media for differentiation and isolation of certain microorganisms. However the concentration of fatty acid should be sufficiently high to account for possible combination with proteins present in the media.

Other practical applications resulting from the bactericidal activity of fatty acids can best be applied by workers in their respective fields.

Summary

Fatty acids in the C_9 to C_{12} range are bactericidal to a number of different organisms, and this activity is markedly enhanced with increasing acidity. Optimum bactericidal activity to *Staphylococcus aureus* was exhibited by undecylic acid. Since the activity is of the same order as that of the quaternary ammonium germicides in alkaline solution, an explanation for both types of germicidal activity is presented in which the bacteria or some protein essential to the bacteria are considered to be "suffocated" by a "coating" of fatty groups in chemical combination with the protein.

The application of these findings to bactericidal activity encountered in rancid fats is discussed briefly in connection with the oxidative degradation of oleic acid derivatives to pelargonic acid. In addition, the possible usefulness of the bactericidal activity as a microbiological assay method for certain fatty acids is pointed out.

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A Laboratory Tool to Study the Plodding Characteristics of Soap Formulations

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RECENT introductions to the consumer market of bars based partly or wholly on synthetic detergents indicate the interest of soapers and others in solving the age-old problem of lime soap formation which has plagued the users of classical soaps wherever waters containing relatively small amounts of hardness occur. Those who have attempted formulations of an all-synthetic or soap-synthetic bar have been struck by the changes in physical appearance of the plodded bar that take place when small amounts of some compounds are added, for example, sodium chloride. The introduction of some organics produces what appears to be a desired improvement in the milled flake, but subsequent attempts at plodding the improved flakes are fruitless because the mass fails to coalesce to homogeneity as evidenced by feathering, cracking, and friability of the plodded bar.

In view of the consideration that we had at our disposal no reliable screening technique for plodder bar character, it was mandatory for us to rely on pilot plant equipment for every evaluation. Since the minimum requirements of this machine are about 10 lbs. of product, the method proved to be time-