THE GERMICIDAL AND HEMOLYTIC ACTION OF α-BROM SOAPS.

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This paper is the first of a contemplated series of papers describing the germicidal action of various soap derivatives. The ordinary soaps are salts of fatty acids, the fatty acid radical consisting of a straight chain of carbon atoms to the first of which two oxygen atoms are attached, while the remainder are united to each other and to hydrogen. It is possible to replace one or more of these hydrogen atoms of the soap molecule by some other atom, such as a halogen, or a group of atoms, such as the hydroxy, amino, or other groups. If the substitution takes place at the carbon atom adjacent to the carboxyl group, the resulting substance is designated as an alpha substituted compound. As the parent unsubstituted fatty acids form an homologous series, the members of which differ from each other in the number of carbon atoms they contain, so the derivatives also form homologous series. By making a systematic study of several such series, it is hoped to throw some light on the relationship of chemical structure to germicidal action. The soaps are unusually favorable for such a study because they, more than any other germicidal substances we know, exhibit the interesting phenomenon of selective germicidal action (Walker, 1924).

In the present investigation, the alpha-brom derivatives of the ordinary saturated soaps have been prepared and tested. These are the simplest of the soap derivatives; they are also intermediate products in the synthesis of other series.

Preparation of the Alpha-Brom Fatty Acids.

Fatty acids are best brominated by the method of Hell (1881) by

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the addition of bromine and red phosphorus. The bromine first combines with the phosphorus forming PBr_5; this reacts with the fatty acids forming the acid bromides. An atom of bromine then replaces an atom of hydrogen, but only at the alpha position. The final product is an α-brom-acid bromide:

$$3\ RCH_2COOH + P + 11\ Br = 3\ RCHBrCOBr + HPO_3 + 5\ HBr$$

On pouring this reaction product into water, the acid bromide is converted to the fatty acid. Exact details for the preparation and purification of the various α-brom fatty acids are given by the following authors: α-bromobehenic acid, by Fileti (1907); α-bromoarachidic acid, by Baczewski (1896); α-bromostearic acid, by Hell and Sadomsky (1891); α-bromopalmitic acid, by Hell and Jordanoff (1891); α-bromomyristic acid, by Le Sueur (1905); α-bromolauric acid, by Auwers and Bernhardi (1891); α-bromocapric acid, by Bagard (1907). The preparation of α-bromocaprylic acid is not described in the literature; it is, however, easily prepared in the usual way and purified by distilling at 155°C. at a pressure of 10 mm. of mercury. α-Bromocaproic acid was purchased from the Eastman Kodak Co. A complete series of all the even numbered α-brom fatty acids from the behenic with 22 carbon atoms to caproic, with 6 carbon atoms, was thus obtained. Very careful purification of these acids is important, as partially purified products in several cases gave higher titers than the pure substances.

To form the corresponding soaps, the theoretical amount of KOH solution was added to weighed quantities of the fatty acids. The soaps were made up fresh for each experiment. Excess of alkali and high temperatures must be avoided, as hot alkali converts the brom to the hydroxy acids. However, the soaps of bromoarachidic and bromobehenic acids require a short boiling to bring them into solution.

The α-brom fatty acids and their soaps differ markedly from their parent unsubstituted fatty acids. The brom acids have a lower melting point and are stronger acids than the parent substances; their soaps are much more soluble in water and are far less hydrolyzed. For these reasons the α-brom soaps are much more satisfactory to work with than the unsubstituted ones. When the brominated soaps are added to acid buffer solutions, the brom fatty acids are liberated as fine and stable colloidal emulsions.

Technic of Germicidal Tests.

The potassium soaps were prepared, and serial dilutions made with sterile distilled water. Buffer solutions containing N/10 potassium phosphate and N/20 glycine were used to maintain the pH; the exact composition of these solutions is given in a previous paper (Eggerth, 1926). The sterile buffer fluids were inoculated with 24 hour broth cultures of the test organisms, and 0.5 cc. quantities were pipetted into series of small test-tubes; to these, 0.5 cc. quantities of the soap dilutions were added and the contents of the tubes well mixed. The tests were incubated at 37°C. and subcultured on glucose blood agar plates at the end of 30 minutes, 2 hours, and 18 hours.

The following test organisms were used:

1. *Streptococcus hæmolyticus,*—"Gay" strain.
2. *B. diphtheriæ,*—"Park-Williams No. 8" strain.
3. *B. typhosus,*—"Pfeiffer" strain.
4. *Staphylococcus aureus,*—old laboratory culture.
5. *Diplococcus pneumoniæ,* Type I,—recently isolated strain.
6. *Vibrio choleræ,*—American Type Culture Collection No. 528.
7. *Micrococcus ovalis,*—recently isolated strain.
8. *B. melitensis,*—old laboratory culture.
9. *B. lepisepticus,*—a virulent strain from Dr. Gay's laboratory.

The first four organisms are the same strains as used in previous investigations with soaps (Eggerth, 1926, 1927).

The inoculations in the germicidal tests were such that each cubic centimeter of final test fluid contained 0.02 cc. of a 24 hour broth culture, with the exception of *Staphylococcus aureus* and *B. typhosus*, where the inoculum was 0.01 cc.

Hemolytic tests were done by preparing a 2 per cent suspension of washed sheep red cells in 0.85 per cent NaCl solution containing N/20 potassium phosphate at pH 7.5. The soaps were diluted with salt solution, and 2 cc. of red cell suspension added to 2 cc. of soap dilution. The tubes were incubated at 37°C. and read.

DISCUSSION.

The effect of introducing a halogen atom into the molecule of an organic germicide is usually to increase germicidal action. This occurs when the soaps are brominated (Figs. 1 to 4), the only exception being the bromocaproate, which is, in most cases, non-germicidal in a concentration of N/10. Also, when *B. typhosus* is the test organism, bromination does not increase toxicity (compare Table I with Fig. 4 of previous article, Eggerth, 1926). In most cases, however, germicidal action is considerably increased by bromination. Thus, potassium stearate has little or no action upon *Streptococcus* and *B. diphtheriæ* at pH. 7.5, whereas the corresponding brom derivative is highly active (Figs. 1 and 2). Potassium palmitate has no action upon *Staphylococcus aureus* at pH 7.5 in 2 hours, whereas the bromopalmitate is rapidly toxic in high dilutions (Fig. 3 and Table II). It is interesting to note that the bromostearate at pH 7.5 is five to ten times as germicidal as potassium oleate, a very soluble soap having the same number of carbon atoms (Eggerth, 1926).

Figs. 1 to 4 and Table I bring out a fact that is of great interest. For any given organism, germicidal action increases regularly as the number of carbon atoms of the soap increases, up to a certain point, after which it diminishes more or less rapidly. Thus, with *Diplococcus pneumoniæ*, the maximum is reached with α-bromostearate (Table I). With *Streptococcus hæmolyticus*, the bromopalmitate and bromostearate are about equally germicidal (Fig. 1), while with *B. diphtheriæ* (Fig. 2), *Staphylococcus aureus* (Fig. 3), and *Micrococcus ovalis* (Table I), the maximal titer occurs at the bromopalmitate. The bromomyristate is most germicidal for *B. lepisepticus*, and the bromolaurate for *V. choleræ* and *B. typhosus* (Table I). All of the Gram-negative organisms of the series tested have their highest titers with soaps of 12 or 14 carbon atoms, and the Gram-positive organisms with soaps of 16 or 18 carbon atoms. Higher titers are obtained with the Gram-positive than with the Gram-negative organisms.

The following hypothesis is suggested as an explanation of the above facts: The toxicity of the brom soaps for all species of bacteria increases rapidly with the length of the fatty acid chain. The ability to penetrate into the bacteria, however, diminishes with equal rapidity. Bacteria vary considerably in the permeability of their limiting membranes, the Gram-negative organisms being less permeable than the Gram-positive ones. The point of maximal germicidal titers, then, will be determined by the permeability of the organisms to the soaps.

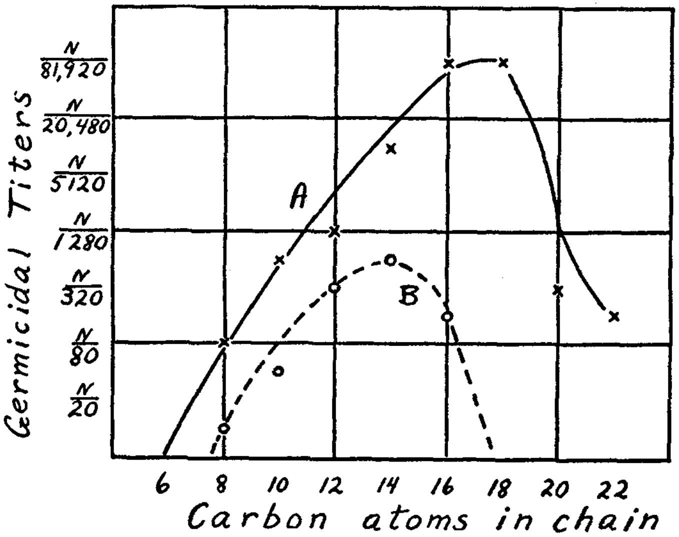

FIG. 1. The germicidal titers of α-brom soaps (*A*) and of unsubstituted saturated soaps (*B*) for *Streptococcus hæmolyticus*, at pH 7.5. The soaps are designated by the number of carbon atoms in their molecule. Time of test, 2 hours; temperature, 37°C.

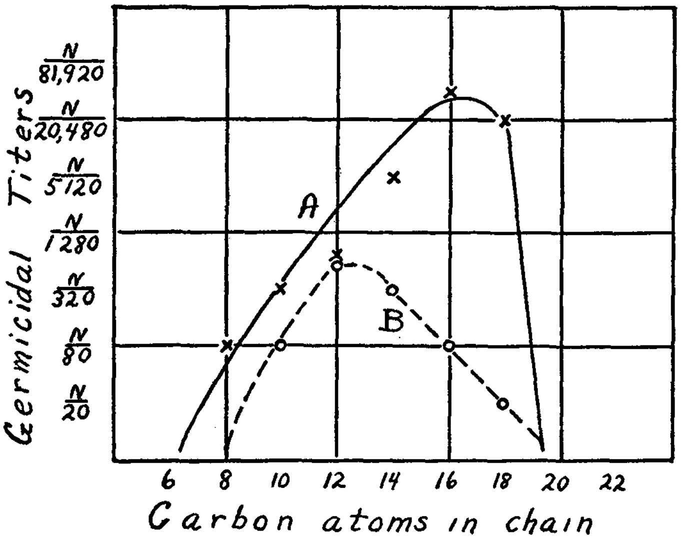

FIG. 2. The germicidal titers of α-brom soaps (*A*) and of unsubstituted saturated soaps (*B*) for *B. diphtheriæ*, at pH 7.5. The soaps are designated by the number of carbon atoms in their molecule. Time of test, 2 hours; temperature, 37°C.

TABLE I.

The Germicidal and Hemolytic Titers of α-Brom-Soaps.

	α-bromo-caproate	α-bromo-caprylate	α-bromo-caprate	α-bromo-laurate	α-bromo-myristate	α-bromo-palmitate	α-bromo-stearate	α-bromo-arachidate	α-bromo-behenate
No. of carbon atoms..	6	8	10	12	14	16	18	20	22
Diplococcus pneumoniæ									
pH									
6.5	0	N/640	N/5120	N/20,480	N/163,840	N/327,680	N/327,680	N/1280	N/1280
7.5		N/160	N/1280	N/5120	N/40,960	N/81,920	N/163,840	N/640	N/640
8.5		N/80	N/640	N/2560	N/40,960	N/40,960	N/81,920	N/640	N/640
Streptococcus hæmolyticus									
6.0	0	N/320	N/2560	N/5120	N/40,960	N/163,840	N/163,840	N/640	N/160
7.5		N/80	N/640	N/1280	N/10,240	N/81,920	N/81,920	N/320	N/160
8.5		N/40	N/320	N/640	N/5120	N/40,960	N/40,960	N/160	N/80
Staphylococcus aureus									
6.0	0	N/320	N/1280	N/2560	N/20,480	N/81,920	N/320	0	0
7.5		N/80	N/320	N/640	N/5120	N/40,960	N/320	0	0
8.5		N/40	N/320	N/640	N/2560	N/20,480	N/80	0	0
B. diphtheriæ									
6.0	0	N/320	N/1280	N/2560	N/20,480	N/81,920	N/40,960	0	0
7.5		N/80	N/320	N/640	N/5120	N/40,960	N/20,480	0	0
8.5		N/40	N/160	N/320	N/2560	N/20,480	N/10,240	0	0
Micrococcus ovalis									
6.0	0	N/320	N/640	N/1280	N/10,240	N/20,480	N/10,240	0	0
7.5		N/80	N/160	N/320	N/2560	N/10,240	N/5120	0	0
8.5		N/40	N/80	N/160	N/1280	N/5120	N/2560	0	0
Vibrio choleræ									
6.0	N/20	N/320	N/1280	N/10,240	N/10,240	N/5120	N/320	0	0
7.5		N/80	N/320	N/2560	N/1280	N/640	N/80	0	0
8.5		N/40	N/160	N/1280	N/640	N/320	N/80	0	0

Where permeability is low, so that only the lower soaps can act, the maximal titers will be low; where permeability is high and the longer chained soaps can act, the maximal titers will be high.

TABLE I—*Concluded.*

	α-bromo-caproate	α-bromo-caprylate	α-bromo-caprate	α-bromo-laurate	α-bromo-myristate	α-bromo-palmitate	α-bromo-stearate	α-bromo-arachidate	α-bromo-behenate
No. of carbon atoms..	6	8	10	12	14	16	18	20	22
					B. lepisepticus				
6.0	0	N/160	N/2560	N/5120	N/10,240	N/1280	N/160	0	0
7.5		N/80	N/320	N/2560	N/5120	N/640	N/80	0	0
8.5		N/40	N/160	N/1280	N/2560	N/320	N/80	0	0
					B. melitensis				
pH									
6.0	N/10	N/320	N/640	N/5120	N/10,240	N/10,240	0	0	0
7.5		N/80	N/320	N/2560	N/2560	N/2560	0	0	0
8.5		N/40	N/160	N/1280	N/2560	N/1280	0	0	0
					B. typhosus				
6.0	0	N/40	N/80	N/80	0	0	0	0	0
7.5		N/20	N/40	N/80	N/20	0	0	0	0
8.5		N/20	N/40	N/80	N/20	0	0	0	0
					Hemolytic titer for sheep red cells				
7.5	0	N/160	N/320	N/2560	N/5120	N/20,960	N/20,960	N/640	N/640

The lowest dilutions tested were N/10 for the bromocaproate, N/40 for the bromoarachidate and bromobehenate; all others, N/20. Time of germicidal tests, 2 hours; time of hemolytic tests, 30 minutes. Temperature, 37°C.

It was observed that when the titer of a soap for an organism fell on the ascending limb of the curve (as in Figs. 1 to 4), such a titer was very constant in different experiments, never in any case varying by more than one tube in the series. But when the titers fell on the descending limb of the curve, the results in different experiments were extremely variable; those given in the figures and tables represent the mean of several observations. Thus, the bromoarachidate gave, with *Streptococcus*, at pH 7.5 for 2 hours, a titer of N/2560 in one experiment, N/640 in a second, N/160 in two experiments, N/80 in a fifth. The bromostearate gave with *Staphylococcus* the following titers in successive experiments: N/40, N/2560, N/320, N/640, N/80, N/640. The

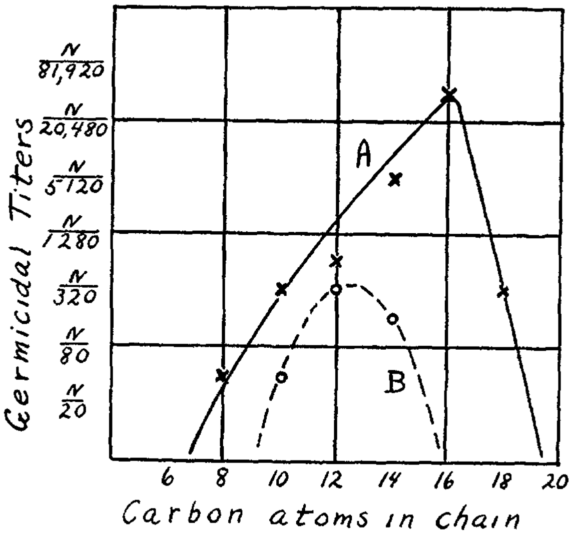

FIG. 3. The germicidal titers of α-brom soaps (*A*) and of unsubstituted saturated soaps (*B*) for *Staphylococcus aureus*, at pH 7.5. The soaps are designated by the number of carbon atoms in their molecule. Time of test, 2 hours; temperature, 37°C.

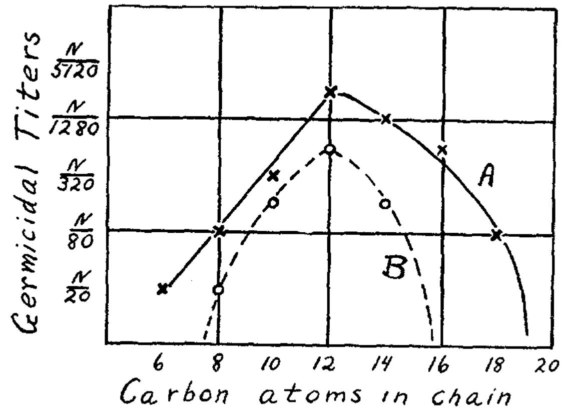

FIG. 4. The germicidal titers of α-brom soaps (*A*) and of unsubstituted saturated soaps (*B*) for *Vibrio choleræ*, at pH 7.5. The soaps are designated by the number of carbon atoms in their molecule. Time of test, 2 hours; temperature, 37°C.

same thing occurred in hemolytic tests, the titers of the bromoarachidate and bromobehenate varying between N/80 and N/1260, while with the other soaps the hemolytic titer was very constant in different experiments.

In general, the brom soaps are very rapid germicides. In most cases, the 30 minute titer was found to be one-half the 2 hour titer. Shorter periods than 30 minutes were tried with two organisms, *Staphylococcus* and *Streptococcus;* the results are given in Table II. The extremely rapid action of high dilutions of the bromopalmitate against these two pyogenic organisms is noteworthy; a concentration of N/2560 being about 1 part by weight to 7500 of water. Two other recently isolated strains of *Staphylococcuss aureus* gave identical results with this soap.

TABLE II.

Germicidal Titers of Potassium α-Bromopalmitate at pH 7.5 for Different Time Intervals.

Test organism......................	*Staphylococcus aureus*	*Streptococcus hæmolyticus*
Time		
15 sec.	N/1280	N/2560
30 sec.	N/2560	N/2560
1 min.	N/5120	N/5120
5 min.	N/5120	N/5120
10 min.	N/10,240	N/10,240
30 min.	N/20,480	N/40,960
2 hrs.	N/40,960	N/81,920
18 hrs.	N/40,960	N/163,840

Temperature, 37°C.

As can be seen in Table I, the effect of the pH on the germicidal action of the brominated soaps is similar to that of the parent unsubstituted soaps; if anything, the results are more clear-cut. It was found in a previous paper (Eggerth, 1926) that the lower members of the saturated series of soaps were most germicidal in acid reactions, while the longer chained soaps were most germicidal at an alkaline pH. It was suggested that this difference was due to the high insolubility of the higher fatty acids liberated at the acid pH. The brom fatty acids, however, are more soluble, hence, with certain organisms such as *Diplococcus pneumoniæ* and *Streptococcus hæmolyticus*, the entire series of brominated soaps including the behenic with 22 carbon atoms is more active in acid than in neutral or alkaline reactions. The data obtained in this investigation, therefore, confirm and strengthen the conclusion previously arrived at: that whenever they are sufficiently soluble to be tested, the fatty acids are more germicidal than the corresponding soaps.

The hemolytic titers of the α-brom soaps are given in Table I; the results are in every way similar to those obtained in the germicidal tests.

SUMMARY AND CONCLUSIONS.

1. The soaps of the α-brom fatty acids are usually more germicidal than the unsubstituted soaps. Only when *B. typhosus* was the test organism was there no increase in germicidal action.

2. For any test organism, germicidal action of the brominated soaps increases rapidly with increasing molecular weight up to a certain point, then diminishes. This is likewise true of the hemolytic titer. The point of maximal germicidal action varies with the different species of tests organisms. In the series studied, brominated soaps of 12 and 14 carbon atoms were most germicidal for the Gram-negative organisms, while soaps of 16 and 18 carbon atoms were most germicidal for Gram-positive organisms.

3. The brominated soaps are, in general, more active in acid that in neutral or alkaline reactions. The reasons for this have been discussed in a previous paper, in which a similar phenomenon with unsubstituted soaps was observed.

4. For certain organisms, the brominated soaps are among the most rapid and potent germicides known.

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