THE GERMICIDAL ACTION OF HYDROXY SOAPS

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This paper is the fourth of a series of papers dealing with the germicidal action of soaps and soap derivatives. In the present investigation, the α-hydroxy soaps, and the soap of ricinoleic acid, were prepared and tested.

Preparation of Hydroxy Fatty Acids

The α-hydroxy fatty acids are prepared by heating the α-brom fatty acids with an excess of dilute aqueous sodium hydroxide. This is best done by heating in the Arnold steam sterilizer for several hours. The bromine is split off by the alkali, forming sodium bromide and the hydroxy soap. A small amount of the alpha unsaturated soap is likewise formed. The hydroxy acid is liberated with dilute sulphuric acid, filtered, washed with water, and dried. One has a choice of several satisfactory methods of purification: the most convenient is to dissolve the product in a small quantity of ether and then add a large excess of petroleum ether, which precipitates the hydroxy acids. This process is repeated until a constant melting point is obtained. Further details for the preparation and purification of the various hydroxy acids are given by the following authors: α-hydroxybehenic acid, by Fileti (1907); α-hydroxystearic, -palmitic, and -myristic acids, by Le Sueur (1905); α-hydroxylauric acid, by Guerin (1903); α-hydroxycapric acid, by Bagard (1907); and α-hydroxycaprilic acid, by Ley (1877). The hydroxy acid with 20 carbon atoms was not prepared. (In the preceding investigation (Eggerth, 1929), the α-bromoarachidic acid was prepared and tested, on the assumption that it was a normal acid having 20 carbon atoms. The writer at that time was not acquainted with the work of Ehrenstein and Stuewer (1922) who have shown that arachidic acid is actually iso-behenic, with 22 carbon atoms. It is interesting to note that the α-bromoarachidic soap actually gave germicidal titers that were in every case practically the same as those of the α-bromobehenate.)

The α-hydroxy fatty acids have higher melting points and are stronger acids than the parent unsubstituted acids. Their soaps are somewhat less hydrolysed by water. Nevertheless, those soaps having 14 or more carbon atoms are strongly alkaline to phenolphthalein. The sodium soaps are very little soluble in water; the potassium soaps are much more so.

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Ricinoleic acid is a normal fatty acid having 18 carbon atoms, with a double bond between the 9th and 10th carbon atoms, and a hydroxyl group attached to the 12th carbon atom. It is a hydroxylated oleic acid. It seemed desirable to study it here, inasmuch as it is the only member of its series that is known and available. It was prepared from castor oil by saponification; the liberated crude acid was thoroughly extracted with petroleum ether, converted to the barium salt, recrystallized several times from alcohol, and liberated with sulphuric acid.

Parallel tests were made with soaps of saturated unsubstituted fatty acids and oleic acid. The former were purchased from the Eastman Kodak Co.; the latter was Kahlbaum's best grade of oleic acid.

Technic of Germicidal Tests

The potassium soaps were made by adding the theoretical amount of KOH solution to weighed quantities of the fatty acids. Serial dilutions were made in sterile distilled water just prior to making the germicidal tests.

Buffer solutions set at pH 6.5, 7.5, and 8.5 were prepared. These contained N/10 potassium phosphate and N/20 glycine; details of their composition are given in a previous paper (Eggerth, 1926). When the test organism was *Diplococcus pneumoniæ*, the buffer solution was made to contain N/20 phosphate. The sterilized buffer fluids were inoculated with 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. Thus the final concentration of potassium phosphate was N/20, except in the case of *Diplococcus pneumoniæ*, where the final concentration was N/40. (*Diplococcus pneumoniæ* frequently failed to survive for 18 hours in the N/20 potassium phosphate controls, whereas it always survived in the N/40 phosphate.) The tests were incubated at 37°C. and one loopful subcultured on glucose blood agar plates at the end of 30 minutes, 2 hours, and 18 hours.

The following test organisms were used: *Diplococcus pneumoniæ; Streptococcus hæmolyticus; B. diphtheriæ; Staphylococcus aureus; Micrococcus ovalis; B. typhosus; Vibrio choleræ; B. lepisepticus; B. melitensis; B. pyocyaneus*. With the exception of the last, these organisms are the same strains as those used in previous investigations with soaps (Eggerth, 1926, 1927, 1929).

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, B. typhosus*, and *B. pyocyaneus*, where the inoculum was 0.01 cc.

DISCUSSION

As with other soaps previously reported, the germicidal titers of the α-hydroxy soaps increase with increasing molecular weight to a

TABLE I

The Germicidal Titers of α- Hydroxy-Soaps

		α-hydroxy-capry-late	α-hydroxy-caprate	α-hydroxy-laurate	α-hydroxy-myristate	α-hydroxy-palmitate	α-hydroxy-stearate	α-hydroxy-behenate
No. of carbon atoms		8	10	12	14	16	18	22
Diplococcus pneumoniæ								
Time	pH							
2 hrs.	6.5	0	N/160	N/2560	N/20,480	N/163,840	N/163,840	
	7.5	0	N/40	N/320	N/2560	N/10,240	N/5120	0
	8.5	0	N/40	N/320	N/2560	N/10,240	N/2560	
18 hrs.	6.5	N/20	N/640	N/5120	N/81,920	N/327,680	N/327,680	
	7.5	N/20	N/80	N/1280	N/5120	N/40,960	N/40,960	N/20,480
	8.5	N/20	N/80	N/1280	N/2560	N/40,960	N/20,480	
Streptococcus hæmolyticus								
2 hrs.	6.5	0	N/20	N/320	0	0	0	
	7.5	0	N/20	N/160	N/320	N/160	N/80	0
	8.5	0	0	N/160	N/640	N/160	N/160	
18 hrs.	6.5	0	N/320	N/640	N/2560	N/5120	N/640	
	7.5	0	N/40	N/640	N/1280	N/1280	N/640	N/640
	8.5	0	N/40	N/640	N/1280	N/1280	N/640	
Staphylococcus aureus								
2 hrs.	6.5	0	0	N/2560	N/10,240	N520	N/640	
	7.5	0	N/10	N/160	N/80 N/20,480	N/80	N/80	0
	8.5	0	0	N/40	N/320	N/160	N/80	
18 hrs.	6.5	0	N/40	N/5120	N/40,960	N/20,480	N/1280	
	7.5	0	N/20	N/640	N/160 N/20,480	N/160 N/10,240	N/1280	0
	8.5	0	0	N/160	N/640	N/1280	N/640	
B. diphtheriæ								
2 hrs.	6.5	0	N/160	N/2560	N/20,480	N/20,480	N/320	
	7.5	0	N/20	N/160	N/80	N/80	N/80	0
	8.5	0	N/20	N/160	N/320	N/640	N/320	
18 hrs.	6.5	0	N/640	N/10,240	N/81,920	N/81,920	N/40,960	
	7.5	0	N/80	N/1280	N/20,480	N/40,960	N/20,480	0
	8.5	0	N/20	N/320	N/2560	N/5120	N/1280	

TABLE I—*Continued*

		α-hydroxy-caprylate	α-hydroxy-caprate	α-hydroxy-laurate	α-hydroxy-myristate	α-hydroxy-palmitate	α-hydroxy-stearate	α-hydroxy-behenate
No. of carbon atoms		8	10	12	14	16	18	22
Micrococcus ovalis								
Time	pH							
2 hrs.	6.5	0	0	N/160	0	0	0	
	7.5	0	0	N/80	N/80	0	0	0
	8.5	0	0	N/80	N/160	N/320	0	
18 hrs.	6.5	0	N/40	N/320	N/1280	N/1280	0	
	7.5	0	N/20	N/160	N/160	N/320	0	0
	8.5	0	0	N/160	N/640	N/1280	0	
Vibro choleræ								
2 hrs.	6.5	0	N/160	N/2560	N/5120	N/320	0	
	7.5	0	N/40	N/320	N/1280	N/640	0	0
	8.5	0	N/40	N/160	N/1280	N/640	N/160	
18 hrs.	6.5	N/20	N/640	N/5120	N/5120	N/640	0	0
	7.5	0	N/40	N/640	N/2560	N/640	N/160	0
	8.5	0	N/40	N/640	N/2560	N/640	N/160	
B. lepisepticus								
2 hrs.	6.5	0	N/320	N/5120	N/20,480	N/5120	N/320	
	7.5	0	N/80	N/320	N/1280	N/2560	N/640	N/160
	8.5	0	N/80	N/160	N/1280	N/5120	N/640	
18 hrs.	6.5	N/40	N/640	N/10,240	N/81,920	N/10,240	N/2560	
	7.5	N/20	N/320	N/1280	N/2560	N/2560	N/1280	N/640
	8.5	N/10	N/160	N/640	N/2560	N/5120	N/2560	
B. melitensis								
2 hrs.	6.5	0	N/80	N/320	N/2560	0	0	
	7.5	0	N/40	N/160	N/1280	N/80	0	0
	8.5	0	N/40	N/160	N/1280	N/320	0	
18 hrs.	6.5	N/20	N/160	N/2560	N/5120	N/5120	0	
	7.5	N/10	N/160	N/640	N/1280	N/160	0	0
	8.5	N/10	N/80	N/320	N/1280	N/1280	0	

TABLE I—*Concluded*

		α-hydroxy-capry-late	α-hydroxy-caprate	α-hydroxy-laurate	α-hydroxy-myristate	α-hydroxy-palmitate	α-hydroxy-stearate	α-hydroxy-behenate
No. of carbon atoms		8	10	12	14	16	18	22
B. pyocyaneus								
Time	pH							
2 hrs.	6.5	0	0	0	0	0	0	
	7.5	0	0	N/80	N/160	N/80	0	0
	8.5	0	0	N/80	N/640	N/1280	0	
18 hrs.	6.5	0	0	0	0	0	0	
	7.5	0	0	N/80	N/160	N/80	0	0
	8.5	0	0	N/80	N/2560	N/1280	0	
B. typhosus								
2 hrs.	6.5	0	N/40	N/160	0	0	0	
	7.5	0	N/10	N/160	N/640	N/80	0	0
	8.5	0	N/40	N/160	N/1280	N/320	0	
18 hrs.	6.5	0	N/40	N/320	0	0	0	
	7.5	0	N/40	N/160	N/640	N/160	N/80	0
	8.5	0	N/40	N/320	N/2560	N/1280	N/160	

The lowest dilutions tested were N/10 for the hydroxycaprylate and hydroxycaprate, N/40 for the hydroxylaurate, and N/80 for the others. Temperature, 37°C.

given maximum, and then diminish. (Table I and Figs. 1 and 2.) With all organisms, this maximum occurs either with the a-hydroxymyristate or the -palmitate; there is no sharp differentiation between Gram positive and Gram negative bacteria as was the case with the α-brom soaps (Eggerth, 1929).

The germicidal action of the α-hydroxy soaps varies a great deal with the different test organisms (see Table I). The α-hydroxycaprylate does not kill any organism in a concentration of N/10 in 2 hours; in 18 hours, slight germicidal action is manifested towards *Diplococcus pneumoniæ*, *B. melitensis*, and *B. lepisepticus*. The α-hydroxycaprate is more active, especially toward *Diplococcus pneumoniæ*, *B. melitensis*, *B. lepisepticus*, *Vibrio choleræ*, and *B. typhosus;* there is no action upon *B. pyocyaneus*, and very little upon *Staphyloccus aureus* and *Micrococcus ovalis*. The α-hydroxylaurate acts more

uniformly toward the ten test organisms than the other soaps of this series; the highest titers at pH 7.5 for 2 hours being N/320 and the lowest, N/80. Germicidal titers increase with the α-hydroxy-myristate, though very unequally. As we pass to the α-hydroxypalmitate, we find that the titers are increased with *Diplococcus pneumoniæ* and *B. lepisepticus*, but diminished with *Streptococcus hæmolytics*, *Vibrio choleræ*, *B. melitensis; B. pyocyaneus*, and *B. typhosus*. The α-hydroxystearate is still highly germicidal for *Diplococcus pneumoniæ*, *B. diphtheriæ*, and *B. lepisepticus;* its titer for the other organisms is

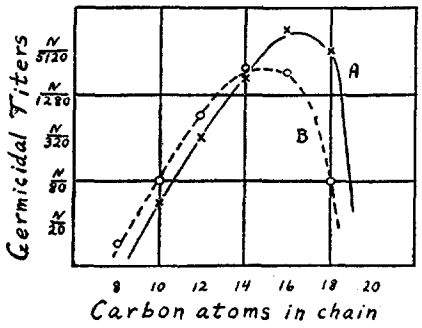

FIG. 1. The germicidal titers of α-hydroxy soaps (*A*) and of unsubstituted saturated soaps (*B*) for *Diplococcus pneumoniæ*, at pH 7.5. The soaps are designated by the number of carbon atoms in their molecule. Time of test, 2 hours; temperature, 37°C.

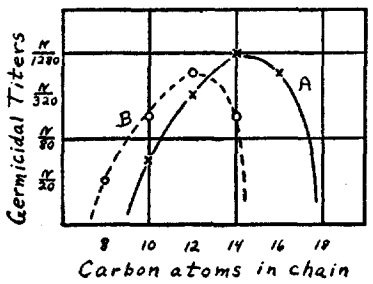

FIG. 2. The germicidal titers of α-hydroxy soaps (*A*) and of unsubstituted saturated soaps (*B*) for *Vibrio choleræ*, at pH 7.5. The soaps are designated by the number of carbon atoms in their molecule. Time of test, 2 hours; temperature, 37°C.

very much diminished. Curiously enough, the only germ to be killed by the α-hydroxybehenate in 2 hours was *B. lepisepticus;* in 18 hours, this soap was toxic for three species: *Diplococcus pneumoniæ*, *Streptococcus hæmolyticus*, and *B. lepisepticus*.

The specific effect of the hydroxyl group upon the germicidal titer may be seen on comparing Table I with Table II, and in Figs. 1 and 2. In many cases, though by no means all, the germicidal titers of an α-hydroxy soap are very nearly the same as that of an unsubstituted soap having two less carbon atoms. This is particularly true of the titers for *Vibrio choleræ*, *B. melitensis*, *B. lepisepticus*, and *Strepto-*

TABLE II

The Germicidal Titers of Unsubstituted Soaps, at pH 7.5

	Caproate	Caprylate	Caprate	Laurate	Myristate	Palmitate	Stearate
No. of carbon atoms	6	8	10	12	14	16	18
				Diplococcus pneumoniæ			
2 hrs.	0	N/10	N/80	N/640	N/2560	N/2560	N/80
18 "	N/10	N/20	N/160	N/1280	N/10,240	N/10,240	N/160
				Streptococcus hæmolyticus			
2 hrs.	0	N/10	N/40	N/320	N/640	N/160	0
18 "	0	N/20	N/80	N/640	N/1280	N/640	0
				Staphylococcus aureus			
2 hrs.	0	0	N/40	N/320	N/160	0	0
18 "	0	0	N/40	N/320	N/640	0	0
				B. diphtheriæ			
2 hrs.	0	0	N/40	N/640	N/320	N/80	0
18 "	0	N/10	N/160	N/1280	N/5120	N/80	N/80
				Micrococcus ovalis			
2 hrs.	0	0	N/20	N/80	N/40	0	0
18 "	0	0	N/80	N/320	N/40	0	0
				Vibrio choleræ			
2 hrs.	0	N/20	N/160	N/640	N/160	0	0
18 "	N/20	N/20	N/320	N/640	N/320	0	0
				B. lepisepticus			
2 hrs.	0	N/10	N/160	N/1280	N/2560	N/160	0
18 "	0	N/40	N/640	N/10,240	N/10,240	N/320	0
				B. melitensis			
2 hrs.	0	N/10	N/160	N/1280	N/320	0	0
18 "	0	N/20	N/160	N/2560	N/1280	0	0

TABLE II—*Concluded*

	Caproate	Caprylate	Caprate	Laurate	Myristate	Palmitate	Stearate
No. of carbon atoms	6	8	10	12	14	16	18
				B. pyocyaneus			
2 hrs.	0	0	0	N/20	N/40	0	0
18 "	0	0	0	N/20	N/40	0	0
				B. typhosus			
2 hrs.	0	N/10	N/40	N/160	0	0	0
18 "	0	N/10	N/80	N/160	0	0	0

The lowest dilutions tested were N/10 for the caproate, caprylate, and caprate, N/20 for the laurate, N/40 for the myristate and N/80 for the palmitate and stearate. Temperature, 37°C.

coccus hæmolyticus. If the germicidal titers are plotted against the number of carbon atoms in the soap molecule, as in Figs. 1 and 2, it will be seen that the effect of hydroxylation is to shift the curve to the right.

In addition to this general effect, specific effects varying a great deal with the test organisms may be observed. This is best brought out by computing the ratios between the titers of an unsubstituted soap, such as potassium myristate, and the corresponding α-hydroxymyristate. This is done in Table III, which is condensed from Tables I and II. For comparison, the titers for potassium α-bromomyristate from a previous paper (Eggerth, 1929) are included. The last column in Table III shows that the effect of introducing a hydroxyl group (or bromine atom) is not uniform, but varies widely with the species of organism. Table III shows that the hydroxylation of myristic acid greatly increases the germicidal action for *B. typhosus*, *B. pyocyaneus*, *B. melitensis*, *Vibrio choleræ*, and *Staphylococcus aureus* (second value); it diminishes the 2 hour titer for *B. diphtheriæ* (but, as shown in Table I, it greatly increases the 18 hour titer); the titers for the other organisms are practically unchanged. It is to be noticed that the effects of hydroxylation are often quite different from those of bromination (Table III).

With the unsubstituted soaps, the 18 hour titer is in most cases double that of the 2 hour titer (Table II); with the hydroxy soaps, the 18 hour titer is often much greater than that for 2 hours. The most striking example of this is *B. diphtheriæ* with the α-hydroxymyristate, -palmitate, and -stearate, where, at pH 7.5 the 18 hour titer is 256 times as great as the 2 hour titer (Table I).

With one organism, *Staphylococcus aureus*, a very peculiar result was quite consistently obtained. With the α-hydroxymyristate and -palmitate, this germ gave two distinct zones of germicidal action at

TABLE III

The Germicidal Titers of Potassium Myristate, Potassium α-Hydroxymyristate, and Potassium α-Bromomyristate

Test organism	Myristate	α-hydroxy-myristate	α-bromo-myristate	Ratio
Diplococcus pneumoniæ	N/2560	N/2560	N/40,960	1:1:16
Streptococcus hæmolyticus	N/640	N/320	N/10,240	2:1:32
Staphylococcus aureus	N/160	N/80 N/20,480	N/5120	2:1:64 1:128:32
B. diphtheriæ	N/320	N/80	N/5120	4:1:64
Micrococcus ovalis	N/40	N/80	N/2560	1:2:64
Vibrio choleræ	N/160	N/1280	N/1280	1:8:8
B. melitensis	N/320	N/1280	N/2560	1:4:8
B. lepisepticus	N/2560	N/1280	N/5120	2:1:4
B. pyocyaneus	N/40	N/160	N/20	2:8:1
B. typhosus	0	N/640	N/20	0:16:1

All tests were made at pH 7.5; temperature, 37°C.

pH 7.5 (Table I). Thus, with the hydroxymyristate, concentrations of N/40 and N/80 were germicidal in 2 hours; concentrations of N/160 to N/2560 gave growth on subculture (though often with a much diminished number of colonies); concentrations of N/5120, N/10,240, and N/20,480 were again usually germicidal, though occasionally one to five colonies appeared on the subculture. (A loopful of the control, when diluted and plated out, was shown to give at least 15,000 colonies). Higher dilutions showed no germicidal action. When the time of test was increased to 18 hours, the results were usually still the same, though occasionally all concentrations up to N/20,480 were found to be germicidal. These two zones appeared likewise with the α-

hydroxypalmitate, but not with any other soap. They appeared only at pH 7.5. With *B. diphtheriæ*, a similar phenomenon was occasionally observed with the same soaps, but it appeared so irregularly that it was not indicated in Table I.

The above phenomenon appeared so extraordinary that a number of experiments were undertaken to verify and explain it. These may be summarized as follows:

1. Two other strains of *Staphylococcus aureus*, recently isolated from pus, showed the same phenomenon.

2. Colonies from *Staphylococci* that survived an α-hydroxymyristate concentration of N/320 for 18 hours were fished and tested. They gave practically the same titers as the original stock culture. The resisting forms are, therefore, not mutants.

3. Mixed inoculations of *Staphylococcus aureus* and *Streptococcus hæmolyticus* were tested with α-hydroxymyristate at pH 7.5. Concentrations of N/160 and N/320 gave, on subculture, pure cultures of *Staphylococcus aureus;* concentrations of N/5120, N/10,240, and N/20,480 gave pure cultures of *Streptococcus hæmolyticus*. Mixed inoculations of *Staphylococcus aureus* with *Diplococcus pneumoniæ*, *Vibrio choleræ*, *B. lepisepticus*, *B. melitensis*, and *B. typhosus* gave similar results.

4. *Staphylococci* were grown on agar and emulsified in sterile buffer in such quantity that the fluid was faintly turbid. Dilutions of α-hydroxymyristate were added as usual. With this large inoculum, organisms could be found with ease in stained films. Subcultures of all dilutions were still positive after 24 hours. After 48 hours, subcultures from soap dilutions of N/160 to N/5120 were negative; films made at the same time showed large numbers of well staining, unagglutinated *Staphylococci*. This experiment rules out agglutination of the organisms by higher dilutions of the soap as a possible cause of the phenomenon.

5. Bacteriostatic tests were made in fluid media. These tests were complicated by the fact that both peptone and meat infusion markedly inhibit the germicidal and growth inhibiting action of the α-hydroxy soaps. In this respect, these soaps differ decidedly from the unsubstituted soaps and sodium oleate (Eggerth, 1927), upon which peptone has no inhibiting action. However, it was found that *Staphylococcus aureus* grows well in a medium having the following composition: N/40 potassium phosphate at pH 7.5; 0.03% Parke Davis peptone, and 0.3% glucose. This small amount of peptone is only slightly inhibitory to these soaps. After 3 days at 37°C. stained films were made from the sediment of each tube, and phenol red was added to determine acid production; these two tests for growth always confirmed one another. The bacteriostatic concentrations of these soaps for *Staphylococcus aureus* were found to be: N/640 for α-hydroxylaurate; N/10,240 for α-hydroxymyristate and -palmitate; N/2560 for α-hydroxystearate. Two other recently isolated *Staphylococci* gave the same results.

6. Instead of subculturing one loopful in the usual way, quantities of 0.1, 0.01, and 0.001 cc. of test fluids were plated out and colony counts made. The results are given in Table IV. This experiment shows that concentrations of the α-hydroxymyristate of N/20,480 or more, rapidly kill off the great majority of the *Staphylococci* present. There are, however, a few survivors in concentrations less than N/80, and the survivors are more numerous in the zone from N/160 to N/1280 than in zone from N/2560 to N/20,480.

It is remarkable that some organisms in a culture can tolerate a concentration of germicide that is 128 times as great as that which

TABLE IV

Colony Counts of Staphylococcus aureus Treated with Potassium α-Hydroxymyristate

Concentration of soap	Number of colonies per cubic centimeter	
	After 2 hrs.	After 18 hrs.
N/80	0	0
N/160	5000	0
N/320	12,000	650
N/640	30,000	3500
N/1280	80,000	5000
N/2560	50	10
N/5120	80	0
N/10,240	30	0
N/20,480	200	250
N/40,960	Innumerable	Innumerable
N/81,920	"	"
Control	"	"

This test was made at pH 7.5; temperature, 37°C.

is lethal to the great bulk of the bacteria in the culture. When the number of survivors is reduced to 100 or less per cubic centimeter a loopful, when cultured, is likely not to give any growth at all, thus giving the second germicidal zone.

The experiment shown in Table IV was repeated several times; the actual colony counts varied a great deal in different experiments, showing that the number of soap resistant organisms in a culture varied greatly. The comparative results, however, remained essentially the same. Attempts were made to determine what factors influenced the proportion of resistant organisms in a culture, but without suc-

TABLE V

The Germicidal Titers of Oleates and Ricinoleates

pH	Oleate		Ricinoleate	
	2 hrs.	18 hrs.	2 hrs.	18 hrs.
		Diplococcus pneumoniæ		
6.5	N/327,680	N/327,680	N/20,480	N/81,920
7.5	N/40,960	N/327,680	N/5120	N/20,480
8.5	N/20,480	N/163,840	N/2560	N/20,480
		Streptococcus hæmolyticus		
6.5	N/163,840	N/327,680	N/10,240	N/20,480
7.5	N/20,480	N/40,960	N/2560	N/10,240
8.5	N/10,240	N/40,960	N/1280	N/5120
		B. diphtheriæ		
6.5	N/81,920	N/163,840	N/10,240	N/20,480
7.5	N/20,480	N/40,960	N/1280	N/2560
8.5	N/10,240	N/20,480	N/320	N/1280
		Staphylococcus aureus		
6.5	0	0	N/2560	N/5120
7.5	0	0	N/640	N/1280
8.5	N/40	N/80	N/640	N/1280
		Micrococcus ovalis		
6.5	0	0	0	0
7.5	0	N/40	N/160	N/320
8.5	N/40	N/160	N/80	N/80
		Vibrio choleræ		
6.5	0	0	N/80	N/320
7.5	0	N/80	N/320	N/320
8.5	0	N/640	N/160	N/320
		B. lepisepticus		
6.5	0	0	N/5120	N/20,480
7.5	N/40	N/80	N/2560	N/10,240
8.5	N/640	N/2560	N/1280	N/5120

TABLE V—*Concluded*

pH	Oleate		Ricinoleate	
	2 hrs.	18 hrs.	2 hrs.	18 hrs.
		B. melitensis		
6.5	0	0	N/2560	N/5120
7.5	0	0	N/1280	N/1280
8.5	N/80	N/160	N/640	N/640
		B. pyocyaneus		
6.5	0	0	0	0
7.5	0	0	0	0
8.5	0	0	0	0
		B. typhosus		
6.5	0	0	0	0
7.5	0	0	0	N/40
8.5	0	0	0	N/80

The lowest concentrations tested were N/40. Temperature, 37°C.

cess; such factors as the age or previous history of the culture, or even the composition of the culture medium, seemed to be without influence.

The effect of the pH upon the germicidal action of the α-hydroxy soaps is, in general, the same as upon other soaps (Eggerth, 1926, 1929). Two main types of pH effect may be observed. First, what might be called the "normal pH effect" is illustrated by all of these soaps with *Diplococcus pneumoniæ;* the titer is highest in acid reactions, and diminishes with increasing alkalinity. Second, with might be called the "reversed pH effect," as in the case of *B. typhosus* with the α-hydroxymyristate; germicidal action is lacking at pH 6.5, but appears and increases with increasing alkalinity. In some cases a combination of the two effects is evident. The "reversed pH effect" is to be ascribed to the fact that the soaps are too insoluble at the acid pH to get a germicidal concentration into solution. In certain cases, the pH effect is of the "reversed" type for the 2 hour period, and of the "normal" type for the 18 hours period, as for *Streptococcus hæmolyticus* with α-hydroxymyristate and -palmitate (Table I). In the

latter cases it is obvious that enough soap went into solution at pH 6.5 to kill in 18 hours, but not enough to kill in 2 hours.

The effect of introducing a hydroxyl radical into an unsaturated soap is shown in Table V. One is struck by the fact that where the oleate titers are high, those for the ricinoleate are much lower; where the oleate titers are low, the corresponding ones for the ricinoleate are higher (except for *B. pyocyaneus*). The hydroxyl group in this unsaturated soap, therefore diminishes *selective* germicidal action; whereas the hydroxyl group in saturated soaps rather increases selective germicidal action.

It will be noticed that the titers of the oleate for *Streptococcus hæmolyticus* and *B. diphtheriæ* are somewhat higher than those reported for the same two organisms several years ago (Eggerth, 1926 and 1927). This seems to be due to an actual increased susceptibility of these two cultures, as several samples of Kahlbaum's oleic acid and sodium oleate were tried, and all gave the same high titers.

The Selective Germicidal Action of Soaps

It has been known for some time that the unsaturated soaps, such as the oleates, will destroy certain organisms such as *Diplococcus pneumoniæ* and *Streptococcus* in high dilutions, and yet have very little toxicity for other species, as *Staphylococcus* (Avery, 1918). Walker (1924) has more recently shown that the saturated soaps, especially the laurate, likewise show considerable selective germicidal action. The writer has tested ten organisms against three complete series of soaps, and has been repeatedly impressed by the fact that all of these soaps show selective germicidal action, though no two of them in exactly the same way. That being the case, it should be possible, by selecting the right soap, to kill at will any one of a mixture of organisms. This was actually done in a number of instances. Thus, in a mixture of *Streptococcus hæmolyticus*, *Staphylococcus aureus*, *B. diphtheriæ*, and *B. typhosus*, the soap potassium α-bromostearate in concentrations of N/40,960 and N/81,920 killed only the *Streptococcus* in 2 hours at pH 7.5; the α-hydroxymyristate in concentrations of N/5120, N/10,240, and N/20,480 killed only the *Staphylococcus* at pH 7.5; the α-hydroxystearate in concentrations of N/2560 to N/40,960 killed only *B. diphtheriæ* in 18 hours at pH 7.5; while the α-hydroxy-

myristate at pH 8.5 killed only *B. typhosus* in a concentration of N/1280. Other combinations of four organisms could not be arranged so successfully, but numerous two-organism combinations were tested with the desired results. Thus N/5120 and N/10,240 potassium laurate kills *B. lepisepticus* at pH 7.5 in 18 hours, but not *Diplococcus pneumoniæ;* whereas potassium oleate kills the latter in high dilutions, but not the former. In a mixture of *Streptococcus* and *Vibrio choleræ*, oleate or α-bromostearate destroys the *Streptococcus;* α-hydroxymyristate, the *Vibrio*. In a mixture of *Micrococcus ovalis* and *B. pyocyaneus*, α-bromostearate kills the former; α-hydroxymyristate at pH 8.5, the latter. Other similar combinations are possible.

As soon as data on other soaps and other organisms is obtained, it is likely that even more striking examples of selective germicidal action will be encountered. The possibilities are interesting.

SUMMARY AND CONCLUSIONS

1. The α-hydroxy soaps exhibit a high germicidal action toward certain organisms. As with other soaps, the germicidal action increases with molecular weight to a maximum, then diminishes. The pH effects the germicidal action as it does other soaps.

2. Certain α-hydroxy soaps give two distinct germicidal zones with *Staphylococcus aureus.*

3. The effect of the hydroxyl group in saturated soaps is to increase selective germicidal action; the effect of the hydroxyl group in an unsaturated soap is to diminish it.

4. The soaps offer a means of separating mixtures of organisms by selective germicidal action.

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