THE GERMICIDAL ACTION OF α-MERCAPTO AND α-DISULFO SOAPS

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The soaps are in many respects the most interesting of all germicides. Some of them, such as the oleate and the bromopalmitate, are among the most powerful and rapid germicides known. It is possible that certain of the soaps play an important part in the body defence against bacteria (see Flexner's introduction to Lamar, 1911). In at least one disease—leprosy—a soap has been found to have a specific chemotherapeutic value. The chief interest of the soaps, however, lies in the fact that they demonstrate to an unusual degree the phenomenon of selective germicidal action; they are, therefor, ideally suited to a study of the effect of chemical structure on such selective action. The selective action of the oleates has been known for some time (Avery, 1918). In previous studies reported by the writer (Eggerth 1926, 1929 a and b) numerous instances have been given in which one soap was highly toxic for one species of bacteria and of low toxicity for a second species, while another soap acted in just the reverse manner on the same two species. One interesting experiment may be recalled here: a mixture was made of four species of bacteria; then by employing four different soaps, any one of the four species could be killed at will without destroying the remaining three (Eggerth, 1929 b). Such a high selective action is unique among germicides.

In the hope of extending our knowledge of this interesting phenomenon, the writer has prepared and tested two series of sulfur-containing soaps: the α-mercapto and the α-disulfo soaps, which are the subject of this paper.

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Preparation of the Mercapto and Disulfo Soaps

Three methods of preparing the α-mercapto fatty acids were tried: the method of Eckert and Halla (1913), which consists of refluxing the α-brom soaps with alcoholic NaSH; the method of Nicolet and Bate (1927) in which the brom fatty acids are converted to the pseudothiohydantoins by thiourea and then broken down by boiling with alkali; and the method of Lovén and Johansson (1916), in which the brom soaps are treated with potassium xanthogenate and the resulting compound broken down by ammonia. The best yields were obtained by the method of Lovén and Johansson. The mercapto acids were purified by crystallization from petroleum ether; the acid with eight carbon atoms required the use of solid CO_2.

The disulfo acids are easily prepared from the mercapto acids by oxidizing the latter with iodine in alcoholic solution, as follows:

$$\begin{array}{l} 2\ \mathrm{R\ \underset{\underset{H}{|}}{\underset{S}{|}}{CH}\ COOH} + 2\ \mathrm{I} = \mathrm{R\ CH\ COOH} + 2\ \mathrm{HI} \\ \qquad\qquad\qquad\qquad\quad\ \mathrm{S} \\ \qquad\qquad\qquad\qquad\quad\ \mathrm{S} \\ \qquad\qquad\qquad\qquad\ \mathrm{R\ CH\ COOH} \end{array}$$

The calculated amount of iodine is added to the mercapto acid in alcohol. The proper quantity is checked by testing with starch-potassium iodide. The disulfo acids are also crystallized from petroleum ether.

The melting points of these acids were found to be as follows:

	Mercapto acids	Disulfo acids
Caprylic	oil	oil
Capric	47°C.	37°C.
Lauric	59°C.	48°C.
Myristic	66°C.	56°C.
Palmitic	72°C.	63°C.
Stearic	80°C.	72°C.

The mercapto and disulfo acids are much stronger acids than the parent unsubstituted acids. Their soaps are therefor very little hydrolyzed by water; both sodium and potassium soaps, even of the long chained acids, are very soluble.

The technic of doing the germicidal tests has been fully described in previous papers and need not be repeated here.

DISCUSSION

The number of carbon atoms in the molecule of these soaps markedly influences germicidal action (Tables 1 and 2, and Figs. 1 and 2). The germicidal titers increase rapidly with the length of the chain until a maximum is reached, then the titers fall off. The most active soap in the series is not always the same for the different test organ-

TABLE 1

The Germicidal Titers of α-Mercapto Soaps

	α-Mercapto-caprylate	α-Mercapto-caprate	α-Mercapto-laurate	α-Mercapto-myristate	α-Mercapto-palmitate	α-Mercapto-stearate
No. of carbon atoms.......	8	10	12	14	16	18
			Diplococcus pneumoniae			
pH						
6.5	N/320	N/5120	N/81,920	N/327,680	N/10,240	N/2560
7.5	N/160	N/2560	N/20,480	N/81,920	N/10,240	N/5120
8.5	N/80	N/1280	N/10,240	N/40,960	N/20,480	N/5120
			Streptococcus haemolyticus			
6.5	N/320	N/2560	N/20,480	N/5120	N/1280	N/640
7.5	N/80	N/1280	N/10,240	N/20,480	N/1280	N/640
8.5	N/80	N/640	N/10,240	N/10,240	N/2560	N/1280
			B. diphtheriae			
6.5	N/320	N/1280	N/10,240	N/10,240	N/1280	N/640
7.5	N/80	N/640	N/10,240	N/10,240	N/1280	N/640
8.5	N/40	N/640	N/5120	N/10,240	N/2560	N/1280
			Staphylococcus aureus			
6.5	N/40	N/320	N/1280	N/2560	N/80	0
7.5	N/20	N/80	N/640	N/1280	N/40	0
8.5	0	N/20	N/640	N/1280	N/20	0
			Micrococcus ovalis			
6.5	N/40	N/320	N/1280	N/640	0	0
7.5	N/20	N/80	N/1280	N/640	N/40	0
8.5	N/10	N/80	N/1280	N/640	N/160	0
			B. lepisepticus			
6.5	N/320	N/2560	N/5120	N/5120	0	0
7.5	N/80	N/640	N/2560	N/2560	N/40	0
8.5	N/80	N/640	N/1280	N/2560	N/160	N/80
			B. melitensis			
6.5	N/80	N/640	N/10,240	N/10,240	N/160	0
7.5	N/80	N/320	N/5120	N/5120	N/160	0
8.5	N/20	N/80	N/2560	N/5120	N/320	0

TABLE 1—*Concluded*

	α-Mercapto-caprylate	α-Mercapto-caprate	α-Mercapto-laurate	α-Mercapto-myristate	α-Mercapto-palmitate	α-Mercapto-stearate
No. of carbon atoms.......	8	10	12	14	16	18
			Vibrio cholerae			
pH						
6.5	N/320	N/1280	N/10,240	N/5120	N/320	0
7.5	N/80	N/320	N/2560	N/2560	N/320	N/80
8.5	N/20	N/160	N/1280	N/1280	N/640	N/160
			B. typhosus			
6.5	N/40	N/40	N/40	0	0	0
7.5	N/20	N/40	N/80	N/80	0	0
8.5	N/10	N/40	N/80	N/160	0	0
			B. pyocyaneus			
6.5	0	0	N/40	0	0	0
7.5	0	0	N/40	0	0	0
8.5	0	0	N/40	0	0	0

Time of tests, 2 hours; temperature, 37°C.

isms. Thus, at pH 7.5, in the α-mercapto series (Table 1), the mercaptolaurate is the most active soap against *Micr. ovalis* and *B. pyocyaneus*; the mercaptolaurate and mercaptomyristate are equally active against *B. diphtheriae*, *B. lepisepticus*, *B. melitensis*, *Vibrio cholerae*, and *B. typhosus*; while the mercaptomyristate is the most active against *Diplococcus pneumoniae*, *Strep. haemolyticus*, and *Staph. aureus*. In the disulfo series of soaps (Table 2), the dicaprate is most germicidal for *B. lepisepticus*, *Vibrio cholerae*, and *B. typhosus*; the dilaurate is most germicidal for *B. diphtheriae*, *Staph. aureus*, *Micr. ovalis*, and *B. melitensis*; and the dimyristate is most germicidal for *Diplococcus pneumoniae* and *Strep. haemolyticus*.

With other series of soaps a similar close relationship between germicidal action and number of carbon atoms in the molecule has been shown. Thus, in the unsubstituted normal aliphatic series, the soaps with 12 and 14 carbon atoms are most germicidal; in the α-brom series, those of 12, 14, 16, and 18 carbon atoms are most active; in the α-hydroxy series, it is those with 14 and 16 carbon atoms (Eggerth

TABLE 2

The Germicidal Titers of Disulfo Soaps

	α-Disulfodi-caprylate	α-Disulfodi-caprate	α-Disulfodi-laurate	α-Disulfodi-myristate	α-Disulfodi-palmitate	α-Disulfodi-stearate
No. of carbon atoms.......	2 x 8	2 x 10	2 x 12	2 x 14	2 x 16	2 x 18
			Diplococcus pneumoniae			
pH						
6.5	N/320	N/2560	N/40,960	N/81,920	N/10,240	N/320
7.5	N/160	N/1280	N/20,480	N/81,920	N/5120	N/160
8.5	N/80	N/1280	N/10,240	N/81,920	N/5120	N/160
			Streptococcus haemolyticus			
6.5	N/320	N/2560	N/20,480	N/20,480	N/160	0
7.5	N/80	N/1280	N/10,240	N/20,480	N/640	0
8.5	N/80	N/640	N/10,240	N/10,240	N/640	0
			B. diphtheriae			
6.5	N/320	N/1280	N/20,480	N/640	0	0
7.5	N/80	N/640	N/10,240	N/1280	N/320	0
8.5	N/40	N/320	N/10,240	N/5120	N/640	0
			Staphylococcus aureus			
6.5	N/40	N/160	N/1280	0	0	0
7.5	N/20	N/80	N/640	0	0	0
8.5	N/10	N/40	N/640	0	0	0
			Micrococcus ovalis			
6.5	N/40	N/320	N/1280	0	0	0
7.5	N/20	N/160	N/1280	N/80	0	0
8.5	N/10	N/160	N/640	N/320	0	0
			B. lepisepticus			
6.5	N/320	N/2560	N/320	0	0	0
7.5	N/80	N/640	N/160	N/40	0	0
8.5	N/80	N/640	N/160	N/80	0	0
			B. melitensis			
6.5	N/80	N/640	N/2560	N/640	0	0
7.5	N/20	N/320	N/2560	N/320	0	0
8.5	N/20	N/80	N/640	N/320	0	0

TABLE 2—*Concluded*

	α-Disulfodi-caprylate	α-Disulfodi-caprate	α-Disulfodi-laurate	α-Disulfodi-myristate	α-Disulfodi-palmitate	α-Disulfodi-stearate
No. of carbon atoms.......	2 x 8	2 x 10	2 x 12	2 x 14	2 x 16	2 x 18
			Vibrio cholerae			
pH						
6.5	N/320	N/1280	N/160	0	0	0
7.5	N/40	N/160	N/40	0	0	0
8.5	N/40	N/80	N/20	0	0	0
			B. typhosus			
6.5	0	0	0	0	0	0
7.5	0	N/20	0	0	0	0
8.5	0	0	0	0	0	0
			B. pyocyaneus			
6.5	0	0	0	0	0	0
7.5	0	0	0	0	0	0
8.5	0	0	0	0	0	0

Time of tests, 2 hours; temperature, 37°C.

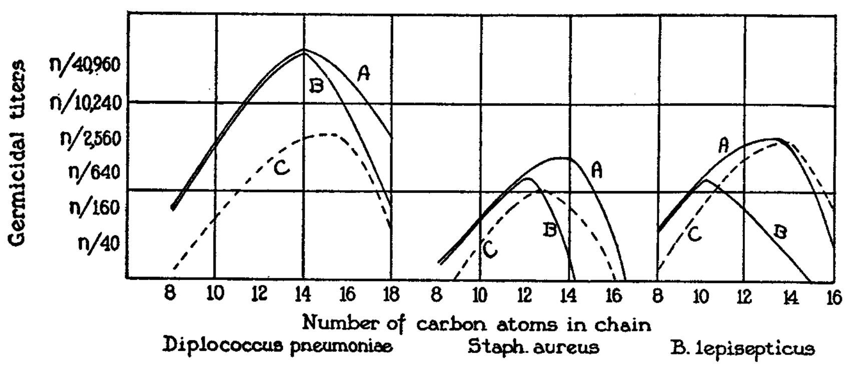

FIG. 1. The germicidal titers of α-mercapto soaps (A), α-disulfo soaps (B), and of unsubstituted saturated soaps (C). The soaps are designated by the number of carbon atoms in their molecule. Time of test, 2 hours; temperature, 37°C.; the pH is 7.5.

1926, 1929 a and b). In an extensive study of numerous cyclo alkyl acetic and dialkyl acetic soaps, Adams and his associates (numerous papers appearing since 1926 in the *Journal of the American Chemical Society*) have shown that maximal germicidal activity toward *B. leprae* occurs in soaps having 16, 17, or 18 carbon atoms.

From the forgoing it seems probable that the following general rule will apply to all soaps: in any homologous series of soaps, germicidal action increases rapidly with the number of carbon atoms in the chain, up to a maximum, after which it diminishes. The point at which this maximum occurs varies with the chemical structure of the soap and with the test organism.

As has been previously suggested by the writer (Eggerth 1929 a) it seems probable that two main factors determine the germicidal action of a soap: its ability to penetrate into the bacterial cell, and its toxic action upon the bacterial protoplasm after it has penetrated. As the number of carbon atoms in the soap molecule increases, the toxicity becomes greater but the power to penetrate into the cell diminishes. As long as molecular size does not prevent entrance of the soap into the cell, germicidal action will increase with molecular weight; eventually a point is reached where the moleucle is so large it cannot readily enter the cell, and germicidal action is diminished.

The effect of the pH upon the germicidal action of soaps has been repeatedly noted and discussed by the writer elsewhere (Eggerth 1926, 1929 a and b). Study of the present series does not add anything new to our knowledge on that subject. As the pH often modifies the germicidal titer a great deal, it is obviously necessary that strict attention be paid to it in conducting these tests. As it is impossible to predict in any particular case just what will be the effect of a change in pH upon the soap titer, it is desirable to test out several pH values.

The effect of the introduction of the SH group into the soap molecule is brought out in Table 3 and in Fig. 2. Table 3 shows that the introduction of this group into the myristate radicle markedly increases germicidal action for most of the organisms tested; with *B. lepisepticus*, the titer is not changed; with *B. pyocyaneus*, it is diminished. Compared with the corresponding brom soap, which it somewhat resembles, it is found to be twice as germicidal for *Diplococcus pneumoniae*, *Strep. haemolyticus*, *B. diphtheriae*, *Vibrio cholerae*, *B.*

melitensis, and *B. typhosus*; with the other test organisms in this series, it is less germicidal than the brom soap. Fig. 2 shows that the α-mercaptomyristate is a very powerful germicide, comparing favorably with any other soap tested by the writer. It is curious that the mercaptopalmitate and -stearate should be such feeble germicides; these soaps are readily soluble in water and titers comparable with the bromopalmitates and -stearates were expected.

The titers of the disulfo soaps bear an interesting relationship to those of the mercapto soaps. On comparing Table 2 with Table 1, it will be observed that with the lower members of this series, the

TABLE 3

The Germicidal Titers of Potassium Myristate, Potassium α-Bromomyristate, Potassium α-Mercaptomyristate, and Potassium α-Disulfodimyristate

Test organism	Myristate	α-Bromomyristate	α-Mercaptomyristate	α-Disulfodimyristate
Diplococcus pneumoniae	N/2560	N/40,960	N/81,920	N/81,920
Streptococcus haemolyticus	N/640	N/10,240	N/20,480	N/20,480
B. diphtheriae	N/320	N/5120	N/10,240	N/1280
Staphylococcus aureus	N/160	N/5120	N/1280	0
Micrococcus ovalis	N/40	N/2560	N/640	N/80
Vibrio cholerae	N/160	N/1280	N/2560	0
B. melitensis	N/320	N/2560	N/5120	N/320
B. lepisepticus	N/2560	N/5120	N/2560	N/40
B. typhosus	0	N/20	N/80	0
B. pyocyaneus	N/40	N/20	0	0

All tests at pH 7.5; time, 2 hours; temperature, 37°C.

titers are usually identical with those of the corresponding mercapto soaps; the higher members are consistently weaker germicides. In no case are the titers of the disulfo soaps higher than the corresponding mercapto soaps. This suggests that, because of their higher molecular weight, the disulfo soaps penetrate less readily; and that those that do enter the bacterial cell are promptly reduced to the mercapto form.

Examination of Fig. 2 brings out what is one of the most interesting facts among the soaps; their highly selective action. In this figure, only the most active and characteristic members of each soap series are given. It will be observed that no two bacterial species run the same titers with these soaps; conversely, no two soaps give the same titers with these ten test organisms. While certain organisms re-

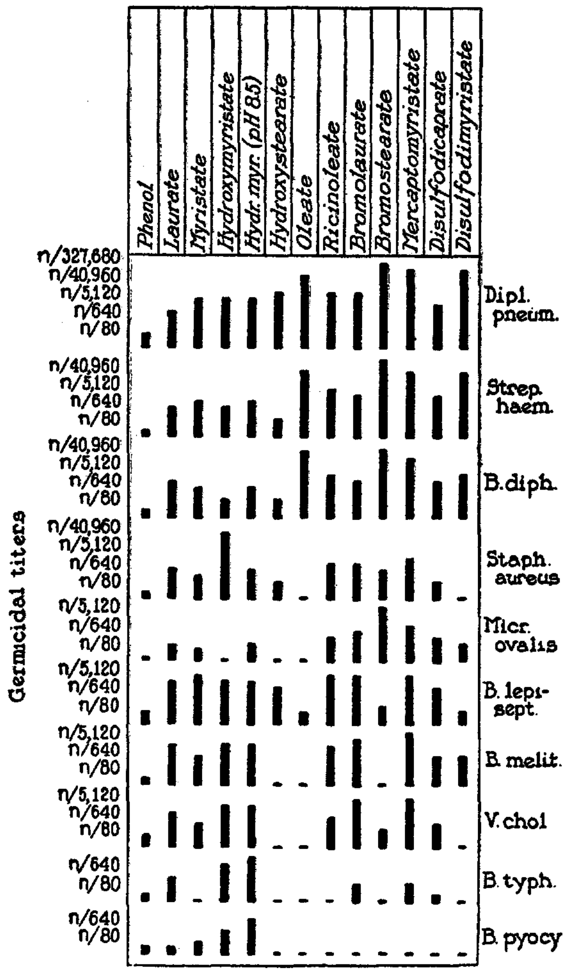

FIG. 2. The germicidal titers of various soaps, showing selective germicidal action. The titers for phenol are included for comparison. The titers of the first nine soaps are taken from previous papers (Eggerth, 1926, 1929 a and b). Time of tests, 2 hours; temperature, 37°C.; the pH is 7.5 except where otherwise indicated.

semble each other in their soap titers (*Strep. haemolyticus* and *B. diphtheriae*; *B. melitensis* and *V. cholerae*), yet certain soaps have always clearly distinguished between them. Thus, α-hydroxymyristate is 4 times, and α-disulfodimyristate is 8 times as toxic for *Strep. haemolyticus* as for *B. diphtheriae*; while α-hydroxypalmitate and -stearate, in 18 hours, are 32 times as toxic for *B. diphtheriae* as for *Strep. haemolyticus* (not shown in Fig. 2; see Eggerth, 1929 b).

Such selective reactions should be of use in classifying, isolating, and identifying bacteria. Some work in the direction has been done in this laboratory: cultures of anaerobes and Sporotrichum have been freed from contaminating cocci by using bromopalmitate agar; sodium oleate has been used to distinguish between *Micr. ovalis* (called *Strep. fecalis* by many authors) and other streptococci; a probable biological relationship between *Micr. zymogenes* and *Micr. ovalis* has been demonstrated, as they run very similar soap titers. Avery's oleate-haemoglobin agar used in isolating *B. influenzae* is, of course, widely known. Other applications of this selective germicidal action of soaps remain to be worked out.

SUMMARY AND CONCLUSIONS

1. Certain of the α-mercapto soaps and α-disulfo soaps are powerful germicides.

2. In the α-mercapto series, those soaps with 12 and 14 carbon atoms are most germicidal. In the disulfo series, the dicaprate, dilaurate, and dimyristate are most germicidal. The optimum number of carbon atoms varies with the test organism used.

3. These soaps, like others previously studied, show a markedly selective germicidal action.

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