THE EFFECT OF THE pH ON THE GERMICIDAL ACTION OF SOAPS.

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That soaps possess a marked germicidal action has been known since the time of Robert Koch (1881). This valuable property finds every-day application in the sterilization of the skin, dishes, and soiled clothing. Soaps are furthermore of interest to the bacteriologist because they, or substances similar to them, very probably have something to do with the body's defence against infections (see Flexner's introduction to the paper of Lamar, 1911, a).

Solutions of soaps having 12 or more carbon atoms in the molecule are alkaline in reaction, because of hydrolysis. The longer the chain of carbon atoms, the greater is the hydrolysis and the more alkaline is the solution. Most investigators of the germicidal action of soaps are agreed that this alkaline reaction favors the destructive effect on the bacteria. Reichenbach (1908), working with *B. coli* as test organism, found that the more strongly hydrolyzed and therefore more alkaline soaps were most germicidal; addition of alkali increased the germicidal titer. Lamar (1911, b) found sodium oleate to be more hemolytic than oleic acid; this difference he ascribed to the lower solubility of the acid. Nichols (1919-20) found that the soaps commonly used in washing dishes (the resinates, stearates, and palmitates) were precipitated and lost their germicidal action when the reaction was brought to pH 7.0 or more acid. Further than this, there seem to be no observations recorded in the literature on the effect of the pH on the germicidal action of the soaps.

EXPERIMENTAL.

Soaps of the following normal fatty acids were investigated: butyric, caproic, caprylic, capric, undecylic, lauric, tridecylic, myristic,

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pentadecylic, palmitic, stearic, oleic, and ricinoleic. The butyric, caproic, and oleic acids were Kahlbaum's; the remainder were obtained from the Eastman Kodak Company. The soaps were prepared by adding the theoretical quantity of fatty acid to N/5 KOH. Potassium soaps were chosen because of their greater solubility. Walker (1924) has shown that the sodium and potassium soaps have prac-

### TABLE I.

*Composition of Buffer Mixtures.*

<table>
<thead>
<tr>
<th>pH</th>
<th>N/5 KH₂PO₄</th>
<th>N/5 KOH</th>
<th>N/10 glycine</th>
<th>N/10 KOH</th>
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<tbody>
<tr>
<td>5.0</td>
<td>9.90</td>
<td>0.10</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>5.5</td>
<td>9.71</td>
<td>0.29</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>6.0</td>
<td>8.97</td>
<td>1.03</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>6.5</td>
<td>7.67</td>
<td>2.33</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>7.0</td>
<td>6.28</td>
<td>3.72</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>7.5</td>
<td>5.50</td>
<td>4.50</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>8.0</td>
<td>5.17</td>
<td>4.83</td>
<td>9.75</td>
<td>0.25</td>
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<tr>
<td>8.5</td>
<td>5.10</td>
<td>4.90</td>
<td>9.0</td>
<td>1.0</td>
</tr>
<tr>
<td>9.0</td>
<td>5.10</td>
<td>4.90</td>
<td>8.0</td>
<td>2.0</td>
</tr>
<tr>
<td>9.5</td>
<td>5.10</td>
<td>4.90</td>
<td>6.5</td>
<td>3.5</td>
</tr>
<tr>
<td>10.0</td>
<td>5.10</td>
<td>4.90</td>
<td>5.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>

### TABLE II.

*Composition of Lactic Acid-Potassium Lactate Buffer Mixtures.*

<table>
<thead>
<tr>
<th>pH</th>
<th>N/10 potassium lactate</th>
<th>N/1 lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>10.0</td>
<td>0.0625</td>
</tr>
<tr>
<td>4.7</td>
<td>10.0</td>
<td>0.125</td>
</tr>
<tr>
<td>4.4</td>
<td>10.0</td>
<td>0.25</td>
</tr>
<tr>
<td>4.1</td>
<td>10.0</td>
<td>0.50</td>
</tr>
<tr>
<td>3.8</td>
<td>10.0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

tically the same germicidal value. Before each experiment, serial dilutions of the soaps required were made with sterile distilled water. The pH of the soap solutions was controlled by the addition of buffer mixtures, whose composition is given in Tables I and II. The composition of the phosphate and glycine buffers was calculated from the data given in Clark (1922). The pH of the glycine-
KOH mixtures is for 37°C. The phosphate mixtures were made up in larger quantities; the glycine-KOH mixtures were made up fresh for each experiment and added to the phosphate.

Five different species of test organisms were used. These were:

1. *Streptococcus pyogenes*. The strain used was the "H" strain first described by Gay and Stone (1920); its extremely high virulence for rabbits has been maintained by constant animal passage. This strain is hemolytic; it grows vigorously and diffusely in infusion broth.

2. *B. diphtheriae*. The "Park-Williams No. 8" was the strain used.

3. *Staphylococcus aureus*. An old laboratory stock culture was employed.

4. *B. typhosus*. The "Pfeiffer" strain was used.

5. *Vibrio cholerae*. The strain used was an old stock culture of unknown origin.

The germicidal tests were performed in the following manner:

The required buffer solutions were sterilized; when cool, they were inoculated with the test organisms and well mixed. Then 0.5 cc. quantities were pipetted into series of small sterile test-tubes. The inocula were such that each 0.5 cc. of the buffer fluid contained 0.04 cc. of an 18 to 24 hour broth culture of *Streptococcus pyogenes*, *B. diphtheriae*, or *V. cholerae*; or 0.02 cc. of *Staphylococcus aureus* or *B. typhosus*. Then 0.5 cc. quantities of the soap solutions (serial dilutions of which were prepared in advance) were added to each tube. Finally each tube was gently rotated while in a slanting position and placed in the water bath at 37°C. At the end of 30 minutes, 2 hours, and 18 hours, a 4 mm. loopful from each tube was subcultured on plates of blood agar (*Streptococcus*, *B. diphtheriae*, and *V. cholerae*) or plain agar (*Staphylococcus* and *B. typhosus*). In case the tube contained a precipitate of insoluble fatty acid, the precipitate was well stirred with the platinum loop on subculture. The plates were incubated at 37°C. for 48 hours and read. In several experiments duplicate subcultures were made: the first set on agar plates, the second set in tubes of infusion broth. No essential differences were observed.

The soap solutions added were in certain instances strongly alka-
line. The question thus arises, how adequate are these buffer mix-
tures to resist change of pH on the addition of this alkali? The alka-
line end of the series—from pH 8.0 on—is very well buffered against
weak alkalies such as the soaps; the acid end, on the other hand, is
but poorly buffered against any but very dilute solutions. It was
found necessary to test the final pH of the solutions with indicators
to determine where any serious shifting of pH had occurred. The
less hydrolyzed soaps gave very little trouble. Those with fewer than
10 carbon atoms could easily be adjusted with acid before being added
to the buffer mixtures. The potassium laurate, in concentrations

<table>
<thead>
<tr>
<th></th>
<th>Acid limit of tolerance</th>
<th>Alkaline limit of tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hrs.</td>
<td>18 hrs.</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>5.5</td>
<td>6.0</td>
</tr>
<tr>
<td><em>B. diphtheriae</em></td>
<td>4.4</td>
<td>4.7</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>5.5</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>3.8</td>
<td>4.4</td>
</tr>
<tr>
<td><em>B. typhosus</em></td>
<td>4.4</td>
<td>5.0</td>
</tr>
</tbody>
</table>

The pH values given indicate the most acid or alkaline tube that gave growth on
subculture. The tests were conducted in the same manner as the germicidal tests
with soap, except that distilled water instead of soap solution was added to the
buffer fluids.

of N/160 or less, caused no serious shifting of pH except in the most
acid of the phosphate mixtures. The same was true of the soaps of
higher molecular weight when the concentration was N/320 or less.
When any shift in the pH of a significant tube occurred, the ob-
served pH (colorimetric) was recorded. As a matter of fact, correc-
tions of this nature were not often required.

Inasmuch as an excess of either acid or alkali is destructive to
bacteria, repeated tests were made to determine the acid and alka-
line limits of pH tolerated by the five organisms here studied. The
results are given in Table III.

The results of the germicidal experiments with soaps are given
in Figs. 1 to 7. Attention must be called to the fact that the ger-
micidal titers given in these figures refer, not to the amount of soap or fatty acid in solution, but to the total amount added, irrespective of whether a precipitate formed or not. Thus, when \( \frac{n}{10} \) potassium stearate was brought to pH 6.0, practically all of it was precipitated out. When such a mixture was found to be without germicidal properties, it was recorded as having a titer of \(<\frac{n}{10}\). Again, when \( \frac{n}{640} \) potassium myristate was brought to pH 6.0 (as in Fig. 1) a precipitate formed, and the actual concentration of myristate in solution became much less, yet it was recorded as \( \frac{n}{640} \). This procedure was adopted for two reasons: first the difficulty of estimating the amount of fatty acid actually in solution; and second, the fact that the substances in solution are in equilibrium with the precipitate, so that the latter cannot be regarded as wholly inert.

A study of the experimental data brings out the following facts.

1. Potassium butyrate is non-germicidal in a concentration of \( \frac{n}{10} \) at all pH values within the limits given in Table III.

2. The lower members of the saturated series of soaps are most germicidal in an acid reaction. The most striking example of this was found in the case of potassium caprate and Staphylococcus: the titer here was 1000 times as great at pH 4.4 to 4.7 as at pH 9.0 to 10.0.

3. The curves for the higher members of the saturated series are the reverse of those for the lower members, showing greater germicidal action when the pH is alkaline. Certain soaps (such as the myristate and the palmitate with Streptococcus) are intermediate in their behavior, showing a definite minimum in their activity near the neutral point.

4. With increasing molecular weight of the soap, the germicidal titer increases to a maximum and then diminishes. The point at which this inflection occurs varies with the pH and the organisms (Figs. 5 and 6). Thus, with B. typhosus at pH 5.5, the titer rises from caproic to capric acid, and then falls off very rapidly through undecylic acid to lauric acid, where no germicidal action could be demonstrated. With the other four organisms tested, the maximum for the acid range was reached at lauric and tridecylic acids, with a somewhat slower falling off of the titer with increasing molecular weight. In the alkaline pH range, the germicidal action toward all five organ-
isms increases with the molecular weight to the palmitate and then diminishes.

5. The oleate and ricinoleate were not tested against all five organisms. Fig. 7 shows that the oleate with *Streptococcus* and the ricinoleate with *B. diphtheriae* are far more effective in acid than in neutral or alkaline ranges. The oleate with *B. diphtheriae* gave a curve quite unlike any other that was obtained, with maximal germicidal action at pH 6.5 and 7.0. The oleate showed no action on *Staphylococcus* and very little on *B. typhosus*, and then only at the most alkaline reactions. This confirms the observations of Reichenbach (1908) and Walker (1924).

From this data it is clear that the soaps have a germicidal power that varies with their structure and the species of germ acted upon; and that the corresponding fatty acid may likewise be germicidal, often far more than the soap.

The question arises, what is the cause of the sudden drop in the curves (Figs. 5 and 6) after a maximum has been reached? Why are the curves in Figs. 1 to 4 reversed as we pass from soaps of lower to higher molecular weight?

One reason for this is the diminishing solubility of the soap or fatty acid as the molecular weight increases. Thus, the most concentrated solution of potassium laurate that remains clear at pH 6.0 is N/5120. More concentrated solutions contain insoluble matter in suspension. It is impossible to determine whether N/160 dissolved laurate or palmitate would kill the typhoid bacillus at pH 6.0, because that amount cannot be obtained in solution.

There is also evidence to show that the germicidal power of the fatty acids also actually diminishes with increasing molecular weight, after a maximum has been reached. It is suggested that this may be due to diminished solubility of the germicide in the bacterial protoplasm. In the case of *B. typhosus* (Fig. 6), the sudden drop from capric to undecylic acid can be explained only by a diminution in germicidal power.

A comparison of the germicidal titers for the three different time intervals gave some rather interesting results. The following combinations ran practically the same titers for the 30 minute, 2 hour, and 18 hour periods: *Staphylococcus* with soaps of 12 or fewer carbon
atoms; *B. typhosus*, *B. diphtheriae*, and *V. cholerae* with 11 or fewer carbon atoms. With soaps of higher molecular weight, the titers were increased by lengthening the incubation period. This increase, however, was not always uniform over the whole pH range. Thus, for the 18 hour period, the curve for the myristate and *Staphylococcus* (Fig. 1) was flattened out without being raised at the ends, while the palmitate and stearate curves were shifted to the left without any change in their shape. On the other hand, the myristate curve for *V. cholerae* (Fig. 3) instead of being flattened, was strongly raised at both ends when the incubation period was increased to 18 hours.

The tests described were carried out in a salt concentration of N/20. A detailed study of the effect of salt on the germicidal action of soaps was not undertaken, but a few experiments were made which indicated that the addition of salts increases the germicidal action. One such experiment is shown in Table IV.

This effect of salt upon the germicidal action of soaps and fatty acids is similar to the effect of salt upon phenol, another organic acid. In the latter case, the effect of salt was explained by Spiro and Bruns (1898) in this manner: When bacteria are added to a solution containing phenol, the equilibrium concentrations of phenol in bacteria phenol in water will depend upon the relative solubility of the phenol in the two phases. If anything is added to the water phase (such as alcohol) that increases the solubility of the phenol in it, then the concentration of phenol in the bacteria is diminished, and likewise germicidal action. Con-
versely, as salt diminishes the solubility of phenol in water, it increases the phenol concentration in the bacteria, causing increased germicidal action. As salts diminish the solubility of soaps and fatty acids in water, the reasoning of Spiro and Bruns would equally well explain the salt effect here. It should be noted, however, that where the added salt actually precipitates the soap out of solution such enhancement of germicidal action is not to be expected.

If we consider the germicidal titer of soaps for *Streptococcus,* we see that with only one exception (potassium stearate) the different soaps tried were more active in acid than in neutral or alkaline solutions (Figs. 2 and 7). It seems probable that the stearate failed to show this phenomenon because its acid is too insoluble. With *B. diphtheriae,* *Staphylococcus,* and *V. cholerae,* the palmitate, pentadecylate, and myristate likewise fail to be effective in acid ranges; and with *B. typhosus,* which is the most resistant organism of the group, the tridecylate, laurate, and undecylate are added to the above. In each case it seems likely that the germicidal activity of the fatty acid fails to become manifest because it is not soluble in the necessary concentration. Certainly, in all cases where the acid is sufficiently soluble to show its true germicidal action it is more active than the corresponding soap. In this there is again an analogy with phenol, as Scheurlen and Spiro (1897) showed that phenol is a more powerful germicide than sodium phenolate.

Several factors suggest themselves as being involved in this increased activity in acid solutions:

1. The primary effect of the pH may be on the bacterium rather than on the germicide; an acid reaction may favor germicidal action by rendering the bacterium more sensitive to the soap or fatty acid.
2. An acid reaction may diminish the surface tension of the soap solution, and hence greatly increase the concentration of the germicide at the surface of the bacterium. For soaps containing 12 or fewer carbon atoms this is actually the case. For soaps containing more than 12 carbon atoms the reverse is true (Jarisch, 1922–23). The extreme insolubility of the latter in acid buffer fluids, however, makes surface tension measurements rather unsatisfactory.
3. The effect of the pH may be due to alterations of the solubility of the soaps in the aqueous phase or in the bacterial protoplasm.
This would affect the distribution coefficient of the germicide between germs and test fluid. We do not know what changes occur in the pH of living bacterial protoplasm when the pH of the outside fluid is altered. However, Coulter (1924-25) has shown that when acid is added to a suspension of erythrocytes, the cell contents do not become as acid as the outside fluid. If this is the case with bacteria, an acid outside pH would be accompanied by a much less acid pH of the bacterial protoplasm. As acids diminish the solubility of soaps, an acid reaction of the outside fluid should, therefore, increase the concentration of soap (or fatty acid) in the bacteria. If, however, the added acid precipitates most of the soap out of solution, the actual concentration within the bacteria would be diminished, even though the relative concentration is still greater than that in solution in the outside fluid.

4. The greater germicidal activity of the fatty acid may be due to the fact that the acid is less dissociated than the soap. There is a good deal of evidence to show that undissociated molecules penetrate more readily into protoplasm than do ions (Osterhout, 1925-26). Michaelis and Denby (1922) believe that the germicidal effect of the organic bases studied by them is due entirely to the undissociated molecules; they show that the curve for the germicidal titer closely parallels the dissociation residue curve for a weak base. Scudder (1914) gives the value of $K_a$ for caprylic acid as $1.44 \times 10^{-5}$; the dissociation residue for this acid corresponds very closely with the germicidal titer curves for the same acid (Figs. 1 to 4). The latter, however, are logarithmic curves; hence it would appear that the logarithm of the germicidal titer varies with the dissociation residue of this acid. This means either that other variables enter into the germicidal effect, or that the dissociation residue affects the germicidal titer in two or more different ways (e.g. by affecting the surface tension or the distribution coefficient as well as the ability to penetrate).

**SUMMARY.**

1. The effect of the pH on the germicidal action of soaps has been studied. The lower members of the series were found to be most active in acid solution; the higher members, in alkaline. The point of transition varied with the test organism used.
2. This is probably due to the effect of the pH on the dissociation residue and on the solubility of the soap. The dissociation residue may affect the germicidal titer by modifying the surface tension, the penetration into the bacteria, and the partition coefficient of the germicide between bacteria and water.

![Graph showing germicidal titers of saturated soaps for Staphylococcus aureus. The incubation period was 2 hours at 37°C. The numbers at the right of the curves designate the carbon atoms in the molecule. The curves for the undecylate, tridecylate, and pentadecylate are omitted from the figure for simplification; they would occupy an intermediate position between the adjacent soaps with an even number of carbon atoms.](image-url)
Fig. 2. Germicidal titers of saturated soaps for *Streptococcus pyogenes*. The incubation period was 2 hours at 37°C. The numbers at the right of the curves designate the carbon atoms in the molecule. The curves for the undecylate, tridecylate, and pentadecylate are omitted; they would occupy an intermediate position between the adjacent soaps with an even number of carbon atoms.

Fig. 3. Germicidal titers of saturated soaps for *V. cholerae*. The incubation period was 2 hours at 37°C. The numbers at the right of the curves designate the carbon atoms in the molecule. The curves for the undecylate, tridecylate, and pentadecylate are omitted; they would occupy an intermediate position between the adjacent soaps with an even number of carbon atoms.
Fig. 4. Germicidal titers of saturated soaps for B. typhosus. The incubation period was 2 hours at 37°C. The numbers at the right of the curves designate the carbon atoms in the molecule. The curves for the soaps with 13, 14, and 15 carbon atoms were omitted to simplify the figure.

Fig. 5. The germicidal titers of saturated soaps for B. diptheriae. The incubation period was 2 hours at 37°C. The soaps are designated by the number of carbon atoms in their molecule.
Fig. 6. The germicidal titers of saturated soaps for *B. typhosus*. The incubation period was 2 hours at 37°C. The soaps are designated by the number of carbon atoms in their molecule.

Fig. 7. The germicidal titers of two unsaturated soaps. The incubation period was 2 hours at 37°C. A. Potassium oleate and *Streptococcus pyogenes*. B. Potassium oleate and *B. diphtheriae*. C. Potassium ricinoleate and *B. diphtheriae*. D. Potassium oleate and *B. typhosus*. E. Potassium oleate and *Staphylococcus aureus*.
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