THE EFFECT OF SERUM UPON THE GERMICIDAL ACTION OF SOAPS.

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That serum inhibits the germicidal action of soaps was shown by Noguchi (1907) and by Landsteiner and Ehrlich (1908). Further observations were later made by Lamar (1911) and by Walker (1924), Walker being the first to show that the effect of serum upon soaps varied both with the soap used and with the organism.

In all these investigations, the effect of the pH was neglected. Serum that has come to equilibrium with the CO_2 of the air has a pH of 8.6 to 8.8. As the soaps having twelve or more carbon atoms are strongly hydrolyzed, their solutions are more or less alkaline. Hence, with no adjustment of the pH, the test fluid will have a reaction of pH 8.6 to 9.5 or more.[1] As a previous investigation (Eggerth, 1926) had shown that the germicidal action of soaps in salt solutions was greatly modified by the pH, it was determined to study the action of serum on soaps over a wide pH range.

EXPERIMENTAL.

Soaps of the following normal fatty acids were used: caprylic, capric, lauric, myristic, palmitic, and oleic. The soaps were prepared by adding the theoretical quantity of fatty acid (Eastman's) to N/5 KOH. Kahlbaum's sodium oleate was used. Just before each experiment, serial dilutions of the soap used were prepared in sterile N/10 NaCl solution.

The test organisms were: *Streptococcus pyogenes* ("Gay" strain); *B. diphtheriæ* ("Park-Williams No. 8"); *B. typhosus* ("Pfeiffer" strain); and *Staphylococcus aureus* (old laboratory culture). These are the same organisms that were used in the previous investigation (Eggerth, 1926).

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[1] The effect of the addition of boric acid to serum-soap mixtures, first described by von Liebermann and von Fenyvessy (1909), is due to the shift in the pH.

Fresh, sterile sheep serum was used. To every 10 cc. of serum, 1.0 cc. of N/5 HCl was added, to neutralize the $NaHCO_3$ present. The serum then stood overnight to allow the escape of the liberated CO_2; the pH was then about 7.2 to 7.6. It was then divided into five or more portions, and each portion adjusted with acid or alkali to the desired pH, the colorimetric method of Cullen (1922) being used to determine the end-point. The volumes of the different portions were then equalized with sterile N/10 NaCl. When it was desired to dilute the serum, this was done with sterile phosphate-glycine buffer mixtures of the corresponding pH. These buffers have a salt content of N/10; their composition is given in the first article of this series (Eggerth, 1926).

The serum mixtures were then inoculated with the test organism. The inocula

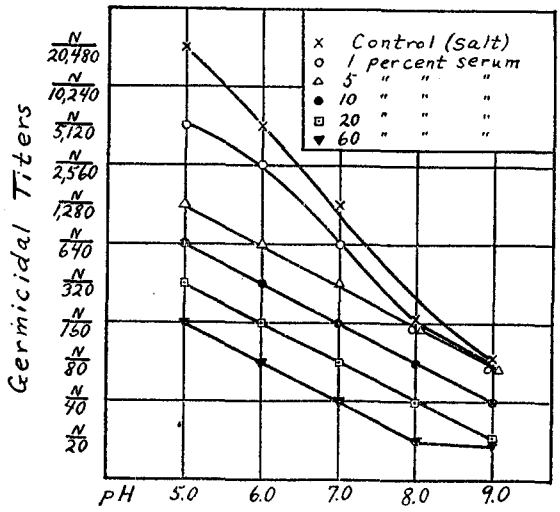

FIG. 1. The germicidal titers of laurate for *Staphylococcus*. The incubation period was 2 hours at 37°C.

were such that each 0.5 cc. of test fluid contained 0.04 cc. of broth culture of *Streptococcus pyogenes* or *B. diphtheriæ*, or 0.02 cc. of *Staphylococcus aureus* or *B. typhosus*. Then 0.5 cc. quantities were pipetted into series of small test-tubes. To each tube, 0.5 cc. quantities of soap dilutions were then added. (When a final serum concentration of 60 per cent was desired, 0.75 cc. of adjusted serum and 0.25 cc. of soap dilution were used.) Finally each tube was 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* and *B. diphtheriæ*) or plain agar (*Staphylococcus* and *B. typhosus*). At the close of the experiment, the pH of each tube was tested with the appropriate indicator. When substances other than serum (egg white, gela-

tin, etc.) were tested, the same general procedure was followed. The final concentration of salt in the tubes was N/10 or a little greater.

The results of some typical experiments are shown in Figs. 1 to 3. In each case, the curve for a particular concentration of serum bears a definite relationship to the curve obtained for salt solution (buffer) alone. Thus, wherever the titer in salt solution alone is high, even small percentages of serum greatly diminish that titer. Wherever the titer in salt solution is low (less than N/320), small percentages of added serum leave the titer practically unchanged.

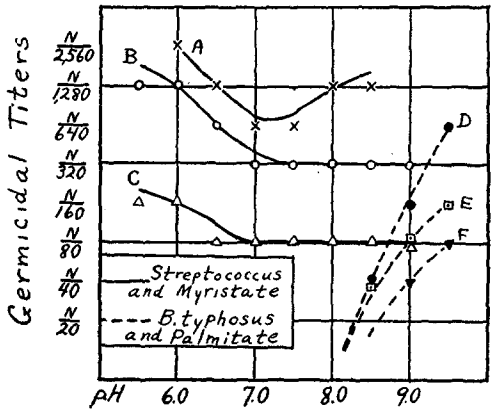

FIG. 2. The germicidal titers of myristate for *Streptococcus: A*, salt control; *B*, 10 per cent serum; *C*, 60 per cent serum. The germicidal titers of palmitate for *B. typhosus: D*, salt control; *E*, 10 per cent serum; *F*, 60 per cent serum. The incubation period was 2 hours at 37°C.

Thus small additions of serum flatten the curves and make them more nearly horizontal. If further larger amounts of serum are added, the titers are reduced all along the line.

In the combination of *Staphylococcus* with laurate, shown in Fig. 1, the salt solution control makes a steep curve; the ratio of the titer at pH 5.0 to the titer at pH 9.0 is 256:1. The addition of 5 per cent of serum to the soap diminishes the titer at pH 5.0 from N/20,480 to N/1,280, while the titer at pH 8.0 to 9.0 is unchanged, and the ratio is now only 16:1. Further additions of serum now diminish the titer equally at all pH; the curves for 5, 10, 20, and 60 per cent

of serum are parallel. Other combinations that, in the presence of serum, gave curves similar to those of Fig. 1 were: *Staphylococcus* with caprylate, caprate, and myristate; *B. typhosus* with caprylate and caprate; *B. diphtheriæ* with caprylate, caprate, and laurate; *Streptococcus* with caprylate, caprate, and laurate.

In the combination of *Streptococcus* with myristate, shown in Fig. 2, the control curve for salt solution is comparatively horizontal.

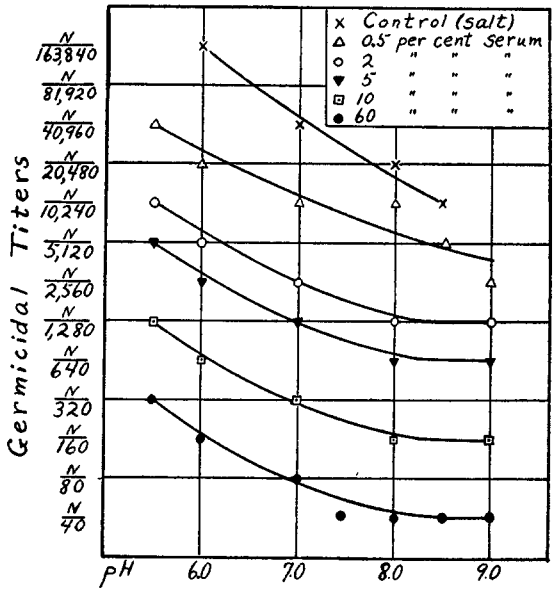

FIG. 3. The germicidal titers of oleate for *Streptococcus*. The incubation period was 2 hours at 37°C.

The addition of serum reduces the titer approximately equally at every pH, so that the serum-soap curves have about the same shape and inclination as the control. The following combinations behaved similarly to this one in the presence of serum: *Streptococcus* with palmitate, and *B. diphtheriæ* with myristate and palmitate.

In Fig. 2 are also shown the curves for *B. typhosus* and palmitate. Here the curve of the control rises steeply toward the alkaline side; the addition of serum inclines the curve toward the horizontal. Similar results were obtained with *B. typhosus* and laurate, myristate, and oleate.

Fig. 3 gives the effect of serum upon *Streptococcus* and oleate. Here the titer in salt solution is high at every pH, though highest in the acid range. Even 0.5 per cent of serum diminishes the titer from N/163,840 to N/10,240 at pH 6.0, and from N/10,240 to N/5,120 at pH 8.0 and 8.5. Further additions of serum diminish the titer equally at every pH.

It is an interesting fact that serum increases both the acid and the alkali tolerance of bacteria. Thus, in salt solution (phosphate-glycine buffer) this strain of *Streptococcus* invariably dies within 30 minutes at pH 5.5 and pH 9.0 at 37°C., while in 0.5 per cent serum it survives for 18 hours at the same reactions. (This organism has become more sensitive to acid and alkali during the past year, hence the tolerance is now different from that reported previously.)

DISCUSSION.

Several investigators have attempted to explain the mechanism of the inhibiting effect of serum upon soap. Von Liebermann (1907) ascribed the inhibitory action to the calcium of the serum, and von Liebermann and von Fenyvessy (1909) suggested both calcium and cholesterol as active constituents of the serum. Sachs (1908), on the other hand, believed the serum proteins to be responsible. Von Korányi (1908) showed that the addition of serum to soap solution caused a slow increase of the surface tension of the soap solution, and suggested this as the cause of the inhibitory effect of the serum. Walker (1924) again suggested calcium as the effective substance, although Lamar (1911) had denied this, and Clark, Zinck, and Evans (1921) stated that calcium played only a minor part in the inhibitory action of serum.

Inasmuch as the mechanism of soap inhibition by serum is of considerable interest, this problem was taken up. Preliminary experiments showed that the active principle was heat-stable; when diluted and boiled, serum is still inhibitory. The active principle does not diffuse through collodion membranes whose permeability is such that they hold protein back. When serum is fractionated by dialysis or by ammonium sulfate precipitation, each fraction is active, and the activity varies approximately with the protein content of that fraction.

The calcium of the serum undoubtedly plays some part, but a minor one, as was also found by Clark, Zinck, and Evans (1921) in their studies on soap hemolysis. When oxalated serum was compared with normal serum, the germicidal titers with the two sera were exactly the same at every pH in 6 experiments, as follows: *Streptococcus* with laurate and myristate; *Staphylococcus* with laurate and myristate; *B. typhosus* with laurate and oleate. In 3 other experiments, the oxalated serum gave a soap titer twice as high as the normal serum at every pH, as follows: *Streptococcus* with oleate (2 experiments) and *Streptococcus* with laurate. A 10 per cent concentration of serum was used for these experiments.

TABLE I.

Germicidal Titers of Potassium Laurate for Staphylococcus in the Presence of Calcium Chloride.

pH	Acetate-asparagine buffer			Phosphate-glycine buffer		
	Control	1–5,000 $CaCl_2$	1–1,250 $CaCl_2$	Control	1–10,000 $CaCl_2$	1–2,000 $CaCl_2$
5.0	N/20,480	N/20,480	N/20,480	N/20,480	N/20,480	N/20,480
6.0	N/5,120	N/5,120	N/320	N/5,120	N/5,120	N/160
7.0	N/1,280	N/320	N/80	N/1,280	N/320	N/40
8.0	N/320	N/160	N/20	N/320	N/160	N/40
9.0	N/160	N/80	N/20	N/160	N/80	N/40

The period of incubation was 2 hours; the temperature, 37°C.

In another series of experiments, varying quantities of $CaCl_2$ were added to buffer solutions without serum, and the soap titers determined. As the phosphate-glycine buffer precipitates the calcium in neutral and alkaline reactions, parallel experiments were undertaken with a special buffer mixture containing N/10 potassium acetate and N/10 asparagine. This buffer was adjusted to the desired pH by means of acetic acid or potassium hydroxide. As is shown in Table I, concentrations of 1–5,000 to 1–10,000 of $CaCl_2$ definitely lower the titer, but only in the neutral and alkaline ranges. In this respect the effect is quite different from that of serum. Larger amounts of calcium produce an even more decided lowering of the titer, except at pH 5.0.

Table I shows that it does not make any difference whether the buffer precipitates the calcium or not. In the case of the phosphate-glycine buffer, either a double decomposition occurs, and calcium laurate is formed, or the precipitated calcium phosphate adsorbs the laurate; in either case the soap is removed from solution.

The amount of calcium in human blood serum is given as 10 to 12 mg. per 100 cc. If it is assumed that sheep serum contains the same amount, then a 60 per cent serum would contain as much calcium as a 1–6,000 solution of $CaCl_2$; while 10 per cent serum should contain as much as a 1–36,000 solution. From Table I it is apparent that such small amounts of calcium can play only a very minor part in the serum inhibition of soaps, and then only in the neutral and alkaline reactions.

The serum protein is undoubtedly more important. Unfortunately this cannot be conclusively proved directly, as no method is known for completely freeing serum protein from lipoid without at the same time denaturating the protein and making it water-insoluble. Extraction with ether, as is well known (Maclean, 1918), removes only a small part of the lipoid. A method which removes a large part of the serum lipoid without denaturating the protein is that of Hardy and Gardiner (1910). This method, modified somewhat to serve the present purpose, is as follows:

To 1 volume of serum at 0°C., 12 volumes of absolute alcohol at 0°C. are added. The mixture is held at 0°C. for 1 hour, with occasional shaking; it is then filtered with suction. The precipitate is washed, first with a mixture of cold alcohol and ether, then with pure ether. In all these operations, the temperature is kept at or near 0°C. A dry powder is obtained which is readily and completely soluble in N/10 NaCl solution.

In Figs. 4 and 5 are shown the titers given by such a "defatted" serum having a concentration of 0.7 per cent dry protein (representing 10 per cent of original serum). The acid titers are somewhat higher in both cases than in 10 per cent normal serum, while the alkaline titers are only a little higher. It should be remembered that this method does not remove all the serum lipoid; repeated extraction with hot alcohol is necessary for complete defatting. Hence it is impossible to tell whether the activity of this partially defatted serum

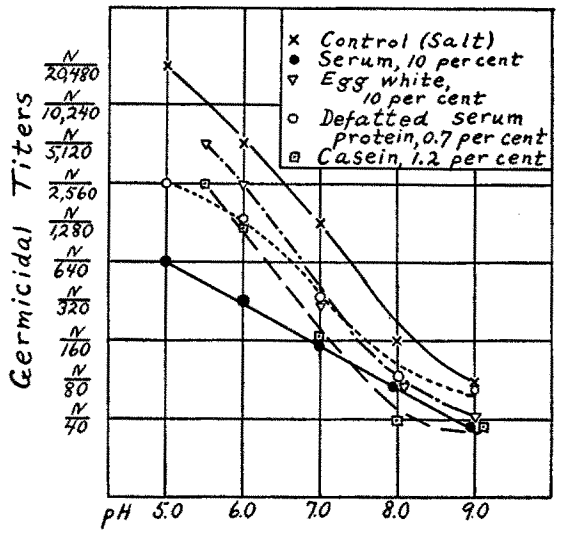

FIG. 4. The germicidal titers of laurate for *Staphylococcus* in the presence of various proteins. The incubation period was 2 hours at 37°C. Crystalline egg albumin in a concentration of 1.5 per cent; gelatin, 2 per cent; and peptone, 2 per cent, each gave a curve exactly like that of the salt control.

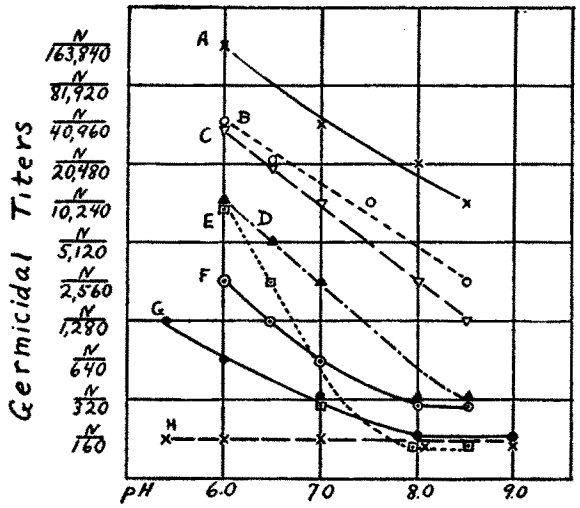

FIG. 5. The germicidal titers of oleate for *Streptococcus* in the presence of various proteins. *A*, salt control; *B*, crystalline egg albumin, 1.5 per cent; also peptone, 2 per cent; *C*, gelatin, 2 per cent; *D*, egg white, 10 per cent; *E*, casein, 1.2 per cent; *F*, defatted serum protein, 0.7 per cent; *G*, serum, 10 per cent; *H*, egg yolk, 1 per cent. The period of incubation was 2 hours at 37°C.

represents the true activity of the protein or that of the residual lipoids.

A number of other protein substances were tested (Figs. 4 and 5). Unaltered egg white, in a concentration of 10 per cent, gave quite different curves from 10 per cent serum, the titers in acid reactions being very much higher than with serum. Crystalline egg white was prepared by the method of Hopkins and Pinkus (1898), recrystallized three times, and dialyzed. In a concentration of 1.5 per cent, it showed no inhibitory action whatever for laurate with *Staphylococcus*, and only slight inhibition of oleate with *Streptococcus* (Fig. 5). Clark, Zinck, and Evans (1921), using crystalline egg albumin in hemolytic experiments with sodium oleate, found that this substance, instead of inhibiting, augmented hemolysis by the soap; this was probably a pH effect, as the crystalline albumin is acid.

Gelatin (freed from calcium by the method of Loeb (1919)), in 2 per cent concentration, and peptone (Parke, Davis and Co.), also in 2 per cent concentration, behaved like the crystallized egg albumin; no effect of these substances was noticed on the action of laurate with *Staphylococcus*, while they only slightly inhibited oleate with *Streptococcus* (Fig. 5).

Casein was prepared from fresh milk by the method of Van Slyke and Baker (1918), and was carefully freed from lipoid by repeated extraction with warm alcohol and ether. In a concentration of 0.75 to 2 per cent, it proved to be an actively inhibitory substance, but the curves are decidedly different from those of serum (Figs. 4 and 5). The alcohol-soluble protein associated with casein (see Osborne and Wakeman, 1918), when suspended in the buffer solutions, proved to be very strongly inhibitory to the germicidal action of soaps.

Egg yolk also was found to be a powerfully inhibitory substance (Fig. 5). With only 1 per cent egg yolk, the titer of laurate for *Staphylococcus* was reduced to N/80 at every pH between 5.0 and 9.0 (not shown in the figure). The activity of egg yolk is probably mostly due to its high lipoid content.

Washed red cells, added to the soap solutions, gave curves almost identical with those of serum.

From these experiments it will be seen that every protein substance tested was more or less inhibitory to the oleate-*Streptococcus* combina-

tion; crystalline egg albumin, gelatin, and peptone, however, failed to inhibit the action of laurate upon *Staphylococcus*. It is undoubtedly a significant fact that all the curves obtained for protein substances run more nearly parallel to the curve for salt solution than they do to the curves for serum.

The lipoids of the serum have hitherto not been definitely related to the soap-inhibiting property. Von Liebermann and Fenyvessy (1909), it is true, reported that cholesterol diminished the hemolytic power of sodium oleate, but Clark and Evans (1921), with more accurate titrations, failed to confirm this. Sachs (1908) extracted lipoids from serum by means of ether, and found that the lipoids alone had no effect on soap hemolysis. Clark and Evans (1921) found the petroleum ether extractives of serum to be inactive, as likewise commercial lecithin.

The experiments shown in Figs. 6 to 8, however, indicate that the lipoids are actively inhibitory to the action of soaps. In the experiments of Figs. 6 and 7, the lipoids were tested in the form of aqueous emulsions, which were prepared as follows:

1. Cholesterol, cholesteryl oleate, and olive oil were made up in a 0.5 per cent solution in acetone and added to the soap dilutions. The acetone was then driven off by heat, and the lipoid-soap emulsions added to the inoculated buffers. The cholesteryl oleate was prepared by the method of Hürthle (1895).

2. Lecithin was prepared from egg yolks, as commercial lecithin was found to be very impure. The yolks were dried, by two extractions with acetone; then they were extracted twice with alcohol at 40°C. The alcoholic extracts were united and evaporated at 45°C.; the residue was dissolved in ether, filtered, and precipitated with acetone. This precipitate was then twice dissolved in ether and precipitated with acetone. The final product was very pale yellow in color and dissolved completely in alcohol. It probably contained considerable cephalin; but as cephalin is also found in serum, there seemed to be no reason for removing it. This lecithin was readily emulsified by stirring in water.

3. Oleic acid emulsion was prepared by adding HCl to sodium oleate solution. Oleic acid and sodium oleate are practically non-toxic for *Staphylococcus* (Walker, 1924; Eggerth, 1926).

4. The serum lipoids were prepared by extracting serum several times with 5 volumes of warm alcohol and then ether: the filtrates were evaporated and the residue dissolved in alcohol and filtered. The alcoholic solution was evaporated, with additions of water from time to time. When free from alcohol, the volume was made up to that of the original serum.

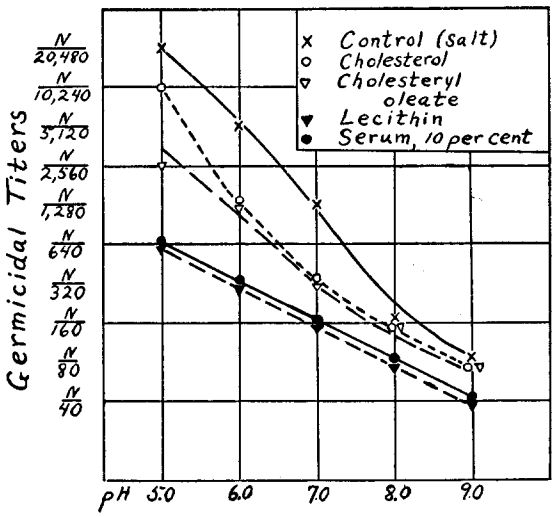

FIG. 6. The germicidal titers of laurate for *Staphylococcus* in the presence of lipoid emulsions. The lipoid emulsions shown are all in a concentration of 1–1,000. An oleic acid emulsion of the same concentration gave a curve identical with that shown for cholesteryl oleate, as did also olive oil. Serum lipoids, in a concentration equivalent to 10 per cent serum, also gave a curve identical with that for cholesteryl oleate. The incubation period was 2 hours at 37°C.

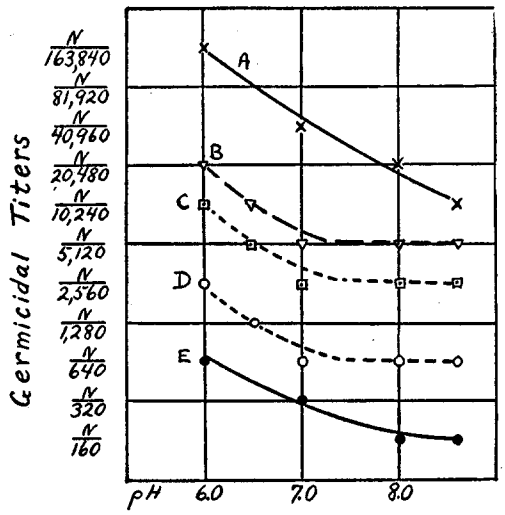

FIG. 7. The germicidal titers of oleate for *Streptococcus* in the presence of lipoid emulsions. *A*, salt control; *B*, cholesteryl oleate, 1–1,000; also olive oil, 1-1,000; *C*, lecithin, 1-5,000; *D*, lecithin, 1-1,000; *E*, serum, 10 per cent. The incubation period was 2 hours at 37°C.

Figs. 6 and 7 show that the lipoids in aqueous emulsions all inhibit the action of soaps. This is especially true of lecithin. A 1–1,000 lecithin emulsion gave a curve identical with that of 10 per cent serum (Fig. 6). Even very dilute emulsions of lecithin were appreciably active; thus, a 1–8,000 emulsion gave a curve identical with that of 1–1,000 cholesteryl oleate (Fig. 6). This outstanding activity of lecithin is undoubtedly due to the basic nature of its molecule, which favors union with the fatty acids.

As the lipoids in serum are present as a highly dispersed colloidal solution, it seemed desirable to test them in aqueous solution. Two such preparations could be obtained. (1) Oleic acid is highly soluble in casein solutions. A 5 per cent casein solution containing N/40 sodium oleate remains perfectly clear when brought to pH 5.5, whereas the solubility of oleic acid in buffer solutions at pH 5.5 is N/5,120. In the experiments shown in Fig. 8, the test fluids contained 0.75 per cent of casein and N/320 of oleate; they remained water-clear at all reactions indicated. (2) Lecithin is highly soluble in bile salts, as shown by Long and Gephart (1908). A sample of sodium taurocholate (Eimer and Amend, "pure") was found to contain an ether-soluble impurity which interfered with the solution of lecithin and was also toxic for *Streptococcus*. The commercial preparation was purified by dissolving it in alcohol containing 2 per cent of sulfuric acid, then precipitating with ether. The precipitate was twice dissolved in alcohol and precipitated with ether, then dried over sulfuric acid. A concentrated solution of taurocholic acid or its salt will dissolve a large quantity of lecithin. Long and Gephart (1908) state that 5 gm. of "bile salt" will dissolve 4.2 gm. of lecithin; with the preparations used in these experiments, even larger amounts of lecithin were readily dissolved. The solutions are perfectly clear and stable at every pH tested and at all dilutions.

The results of these experiments are shown in Fig. 8. The solution of oleic acid-sodium oleate in casein is more inhibitory to the germicidal action of laurate upon *Staphylococcus* than casein alone or the oleic acid-sodium oleate alone in emulsion form (Fig. 6). The taurocholate alone is quite indifferent to the *Staphylococcus*-laurate combination in the concentration tested (1–500); but when lecithin, in a concentration of 1–750, is dissolved in the taurocholate, the

inhibition is pronounced. The lecithin in solution is more active than the lecithin in emulsion (Fig. 6).

The curve for *Streptococcus* and oleate with taurocholate is rather peculiar. This is because the taurocholate, in a concentration of 1–500, is itself germicidal to this *Streptococcus* at pH 6.0. At pH 6.5 it is not germicidal, but its toxicity is added to that of the soap,

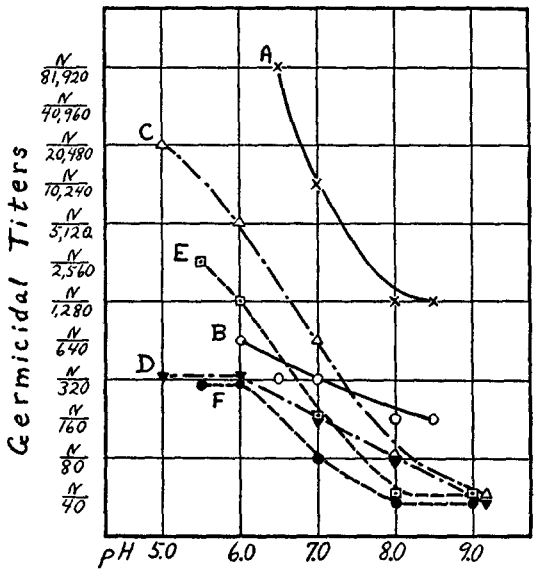

FIG. 8. The germicidal titers of soaps in the presence of lipoids in solution. *A*, titers of oleate for *Streptococcus* in the presence of 1–500 taurocholate (control); *B*, titers of oleate for *Streptococcus* in the presence of lecithin, 1–750, dissolved in 1–500 taurocholate. *C*, titers of laurate for *Staphylococcus* in the presence of 1-500 taurocholate (control); *D*, titers of laurate for *Staphylococcus* in the presence of lecithin, 1–750, dissolved in 1-500 taurocholate. *E*, titers of laurate for *Staphylococcus* in the presence of 0.75 per cent casein (control); *F*, titers of laurate for *Stapylococcus* in the presence of 1–1,000 oleic acid dissolved in 0.75 per cent casein. The incubation period was 2 hours at 37°C.

giving a very high soap titer. At pH 8.0 and 8.5, the taurocholate definitely inhibits the germicidal action of the oleate. But when lecithin was dissolved in the taurocholate solution, a curve was obtained that was identical with that of 10 per cent serum. The lecithin inhibited not only the germicidal action of the oleate, but that of the taurocholate as well.

It was noticed, early in the course of the experiments, that those substances that lower the germicidal titer of the soap, usually increase the solubility of that soap; this increase in solubility was often most marked in the acid reactions. It was often possible to predict the effect of a test substance upon the germicidal titer from the solubility of the soap in that substance. These relationships are shown in Table II.

In determining the solubilities of soaps in serum (Table II) it was found necessary to oxalate the serum to determine solubility at pH 7.0 and more alkaline reactions, otherwise a part of the soap is precipitated by the serum calcium and the results obscured.

A number of interesting facts are brought out by Table II. In the presence of serum at different dilutions and different pH, the ratio of the solubility of the soap to its germicidal titer is remarkably constant, indicating that these phenomena are in some way connected. The corresponding ratios for other test substances vary a great deal; yet it will be observed that every substance that increased the solubility of the soap (or fatty acid) diminished its germicidal titer more or less. The high solubility of oleic acid in casein solution, and of both oleic and lauric acids in lecithin-taurocholate solutions, is noteworthy.

The experiments described above indicate that the serum lipoids and serum proteins both participate in the action of serum upon soap. In unaltered serum it is probable that they largely exist as protein-lipoid conpounds. The question still remains, what is the mechanics of their action upon soaps?

Du Noüy (1922, 1926), in his surface tension studies, interpreted the action of serum upon soaps as being due to an adsorption of soap molecules on the surfaces of the huge serum molecules. In this way the soap is bound so that it can no longer concentrate on other surfaces, and the surface tension of the mixture becomes, in a short time, the same as the surface tension of the serum alone. Even very small quantities of serum, 5 per cent or less, suffice to bring this about.

This explanation might be taken as it stands to account for the effect of serum upon the germicidal action of soaps, were it not for two facts: (1) Under certain conditions, serum inhibits the germicidal action very little or not at all (*e.g.*, the effect of 5 per cent serum on

TABLE II.

The Relation of Solubility to Germicidal Titer.

Test substance	Laurate with *Staphylococcus*				Oleate with *Streptococcus*			
	pH	Solubility	Germicidal titer	Ratio	pH	Solubility	Germicidal titer	Ratio
Phosphate-glycine buffer	5.5	N/5,120	N/10,240	1–2	6.0	N/5,120	N/163,840	1–32
" "	7.0	N/1,280	N/1,280	1–1	7.0	N/5,120	N/40,960	1–8
" "	9.0	N/320	N/80	4–1	8.5	N/640	N/10,240	1–16
Serum, 10 per cent	5.5	N/2,560	N/640	4–1	6.0	N/2,560	N/640	4–1
" 60 " "	5.5	N/640	N/160	4–1	6.0	N/640	N/160	4–1
Oxalated serum, 10 per cent	7.0	N/640	N/160	4–1	7.0	N/1,280	N/640	2–1
" " 60 " "	7.0	N/320	N/40	8–1	7.0	N/640	N/160	4–1
" " 10 " "	9.0	N/160	N/40	4–1	9.0	N/640	N/320	2–1
" " 60 " "	9.0	N/80	N/20	4–1				
Egg white, 10 per cent	5.5	N/5,120	N/5,120	1–1	6.0	N/5,120	N/10,240	1–2
Crystalline egg albumin, 1.5 per cent	5.5	N/5,120	N/10,240	1–2	6.0	N/5,120	N/40,960	1–8
Gelatin, 2 per cent	5.5	N/5,120	N/10,240	1–2	6.0	N/5,120	N/40,960	1–8
Taurocholate, 1–500	5.5	N/5,120	N/10,240	1–2				
Lecithin, 1–750 (in taurocholate)	5.5	N/160	N/320	1–2	6.0	N/320	N/640	1–2
Casein, 1.5 per cent	5.5	N/640	N/2,560	1–4	6.0	N/40	N/2,560	1–64

The solubilities and germicidal titers were determined after 2 hours at 37°C. All test substances were made up in the standard phosphate-glycine buffer. The oxalated serum was prepared by adding 0.5 cc. of 2 per cent ammonium oxalate to 10 cc. of serum and centrifuging until clear.

laurate with *Staphylococcus* at pH 8.0 and 9.0, Fig. 1); and (2) certain substances found by du Noüy to be as active as serum in raising the surface tension of soap solutions, such as gelatin and crystalline egg albumin, have little or no effect upon the germicidal action of soap (Figs. 4 and 5).

In the case where lipoid emulsions are added to soaps (Figs. 6 and 7), it is obvious that the soap will be distributed between three phases: the suspended lipoid, the water, and the bacteria. The concentration of soap in each of these phases will vary, in this particular case, with the solubility of the soap in the respective phases. As the solubility of fatty acids in lipoids is high, then, in acid reactions, the greater proportion of the soap (fatty acid) will be in the suspended lipoid: the concentration of germicide in the bacterial protoplasm will consequently be low, and germicidal action will be greatly diminished. On the other hand, salts of the fatty acids (soaps) are not very soluble in lipoids; hence in alkaline reactions the lipoid phase will contain only a small part of the soap, and the concentration of germicide in the bacteria will be as great or nearly as great as in the salt control; hence germicidal action will not be appreciably inhibited. Figs. 6 and 7 show that this actually occurs. When lipoids in aqueous solution are the test substances (Fig. 8) the results are the same, and it seems likely that the mechanism also is the same.

When serum and bacteria are added to soap solutions the same thing probably occurs. The soap divides itself between three phases: bacterial protoplasm, water, and serum molecules: the final concentration in each phase will vary with the combination attraction of that phase for the soap. Whether the combination with the serum molecules is one of solution of the soap in colloidal micellæ (as with lipoid emulsions) or of chemical combination, or of adsorption in the sense of du Noüy, is immaterial. Crystalline egg albumin and gelatin are less effective than serum because their attraction for soap molecules is less; this is also shown by the fact that neither of them increases the solubility of soap in water.

SUMMARY AND CONCLUSIONS.

1. Far more information about the effect of serum or other substances upon the germicidal action of soaps can be obtained by deter-

mining the germicidal titers over a wide range of pH than by determining the titer at a single pH. In this way a characteristic curve for each test substance is obtained.

2. The curve for a particular concentration of serum bears a definite relationship to the curve for salt solution (buffer) alone. Wherever the titer in salt solution is high, very small amounts of serum greatly diminish that titer. Wherever the titer in salt solution is low, small amounts of serum leave the titer unchanged. Thus small additions of serum flatten the curves and make them more nearly horizontal. If further large amounts of serum are added, a further reduction in titer takes place at all reactions.

3. The calcium of serum has only a very slight effect upon the soap titer.

4. The protein of serum is probably inhibitory to soaps; but the curve for partially defatted serum, and the curves for other protein substances tested, do not run parallel to the serum-soap curves.

5. The various lipoids that are known to be present in serum are inhibitory to the action of soaps, both as emulsions and as clear solutions.

6. The action of serum upon soaps may be regarded as a complex reaction, in which lipoids, protein, and, to a lesser extent, calcium salts take part. Their effect is due to the fact that these substances, by combining with the soaps, remove them from the field of germicidal action.

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