

struction of diphtheria toxin. Larson and associates⁴⁻⁶ found that the sodium salt of ricinoleic acid destroys the toxic properties of diphtheria toxin and Schmidt⁷ reported that this substance changes its antigenic properties as well. According to Schmidt⁷ not all unsaturated fatty acids destroy bacterial exotoxins; for instance, fumaric acid and maleic acid do not have this effect.

From a practical point of view it seems desirable to have available for the prevention or treatment of infections caused by toxin-producing bacteria antimicrobial compounds of marked effectiveness and low toxicity which, moreover, may be destructive to tox-

ins. Zephiran chloride in concentrations which are bacteriostatic or bactericidal *in vitro* and *in vivo* inhibits the toxic effects of tetanus toxin. The inactivation of this toxin takes place almost immediately. However, it must be emphasized that presence of serum definitely interferes with this inactivation. The possible effects of antimicrobial agents upon the activity of toxins deserves further study.

Summary. (1) Alkyl-dimethyl-benzyl-ammonium chlorides (Zephiran chloride) in a concentration of 1:2,000 inactivates tetanus toxin. This effect takes place almost immediately. Zephiran chloride in a dilution of 1:20,000 fails to do so. (2) Human serum mixed with Zephiran chloride prior to the addition of tetanus toxin markedly interferes with the inactivation of toxin by this compound. Addition of serum to a mixture of Zephiran chloride and tetanus toxin does not reactivate the toxin.

⁴ Larson, W. P., and Nelson, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1923-24, **21**, 278.

⁵ Larson, W. P., Evans, R. D., and Nelson, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1924-25, **22**, 194.

⁶ Larson, W. P., and Halvorson, H. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1924-25, **22**, 550.

⁷ Schmidt, S., *Bioch. Z.*, 1932, **256**, 158.

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Effects of Alkyl-Dimethyl-Benzyl-Ammonium Chlorides upon Plasma-Coagulation by Staphylococcus and Fibrinolysis by Streptococcus.

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Certain surgical antiseptics and chemotherapeutic substances inhibit *in vitro* streptococcal fibrinolysis and coagulation of plasma by staphylococcus. Madison and Snow¹ showed that tincture of iodine (1:3,000) renders fibrin but one-eighth its normal susceptibility to liquefaction by streptococci. Other substances such as merthiolate (1%), phenol (2%), and mercuric chloride (1%) did not affect fibrinolysis, nor did sulfanilamide (10%) and pron-tosil (2.5%). Spink and Vivino² observed that sulfadiazine inhibits the plasma-clotting

effect of staphylocoagulase; in contrast, sulfathiazole and, to a slightly lesser extent, sulfanilamide accelerated clot formation. Recently it was shown³ that two antimicrobial agents of biological origin, namely, tyrothricin and actinomycin A, inhibit both coagulation of plasma by staphylococcus and lysis of plasma clots by group A *beta hemolytic streptococcus*. Alkyl-dimethyl-benzyl-ammonium chlorides (Zephiran chloride), a disinfectant of marked antimicrobial activity, inhibits the toxic effects of tetanus toxin.⁴ It seemed of interest to determine what

¹ Madison, R. R., and Snow, J. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 522.

² Spink, W. W., and Vivino, J. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **50**, 37.

³ Neter, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **49**, 163, 167.

⁴ Neter, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **51**, 254.

effects, if any, this compound has upon plasma-coagulation by staphylococcus and fibrinolysis by streptococcus.

Material and Methods. An aqueous solution of alkyl-dimethyl-benzyl-ammonium chlorides (Zephiran chloride) was used in the experiments. In the tests on fibrinolysis the technic of Tillett and Garner⁵ was followed. A strain of *beta hemolytic streptococcus* (Group A) obtained from man was used as a source of fibrinolysin. The technic of the staphylococcal coagulase test was essentially the same as that described by Fisk.⁶ A strain of *Staphylococcus aureus hemolyticus* obtained from a skin lesion was employed as a source of coagulase.

TABLE I.

Effect of Alkyl-dimethyl-benzylammonium Chlorides (Zephiran) upon Plasma-Coagulation by *Staphylococcus*.

Time of observation hr	0.1 ml of <i>Staphylococcus</i> culture 0.9 ml of Zephiran in dilutions of			
	1/10 ⁴	1/10 ⁵	1/10 ⁶	0
1	—	—	++++	++++
5	—	++++	++++	++++
18	—	++++	++++	++++

Results read after the addition of 0.2 ml of oxalated human plasma.

— = No clot formation.

++++ = Complete clot formation.

Plasma was obtained from human sources. One ml of a 2% potassium oxalate solution was evaporated in small bottles. Five ml of human blood were added and shaken well. Plasma was removed following centrifugalization of the blood.

Results. In the first experiment, the action of alkyl-dimethyl-benzyl-ammonium-chlorides (Zephiran chloride) in various concentrations upon plasma-coagulation by staphylococcus was determined. To 0.1 ml of an 18-hr infusion broth culture of staphylococcus were added, respectively, 0.9 ml of Zephiran chloride in dilutions of 1:10,000, 1:100,000, 1:1,000,000, and 0.9 ml of physiological salt solution as control. Then, 0.2 ml of oxalated human plasma was placed in each tube. The tubes were incubated at

37°C. Clot formation was noted at various intervals. The results of this experiment are summarized in Table I.

It may be seen from Table I that Zephiran chloride in a dilution of 1:10,000 completely prevented clotting of human plasma by staphylococcus for 18 hr. In a dilution of 1:100,000 it caused a delay in the clot-formation. Essentially the same results were obtained in several experiments. In some tests, Zephiran chloride even in a dilution of 1:1,000,000 caused a transitory inhibition of plasma-coagulation by staphylococcus. The question arises as to whether this inhibitory action is due to effects upon the staphylococcal coagulase or upon the plasma itself. Zephiran chloride in a dilution of 1:1,000 inhibits the clotting of oxalated plasma after the addition of minimal amounts of calcium chloride; in dilutions of 1:100,000 and above, however, its anti-coagulating effects are minimal. Furthermore, it was found that the addition of calcium chloride or rabbit clotting globulin* to a mixture of staphylococcal culture, Zephiran chloride, and plasma caused clot-formation. Although these experiments do not allow of any final conclusions as to the mode of action of Zephiran chloride upon plasma-coagulation by staphylococcus, nevertheless, they seem to indicate that the inhibitory effect of this compound is not directed against the mechanism of clot formation and therefore by exclusion may be due to its action upon the staphylococcus culture or the staphylocoagulase.

A second series of experiments was carried out to determine the effects of Zephiran chloride upon fibrinolysis by *beta hemolytic streptococcus*. The experiment was set up as follows: To 0.1 ml of an 18-hr infusion broth culture of *streptococcus hemolyticus* was added, respectively, 0.9 ml of Zephiran chloride in dilutions of 1:10,000, 1:100,000, 1:1,000,000 and 0.9 ml of physiological salt solution. As control, 0.1 ml of infusion broth was mixed with 0.9 ml of Zephiran chloride in a dilution of 1:10,000. Follow-

⁵ Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

⁶ Fisk, A., *Brit. J. Exp. Path.*, 1940, **21**, 311.

* The rabbit-clotting globulin was obtained from Lederle Laboratories through the courtesy of Dr. A. L. Joyner.

TABLE II.
Effect of Alkyl-dimethyl-benzyl-ammonium Chlorides (Zephiran) upon Fibrinolysis by
Hemolytic Streptococcus.

Time of observation	0.1 ml of Streptococcus culture 0.9 ml of Zephiran in dilutions of				0.1 ml broth 0.9 ml Zephiran (1/10,000)
	1/10 ⁴	1/10 ⁵	1/10 ⁶	0	
15 min	—	++++	++++	++++	—
30 "	—	++++	++++	++++	—
2 hr	++	++++	++++	++++	—
3 "	++++	++++	+	—	+
5 "	++++	++++	—	—	++++
18 "	++++	—	—	—	++++

Results read after the addition of 0.2 ml of oxalated human plasma and 0.35 ml of 0.25% CaCl₂.

— = No clot formation.

+ to ++++ = Various degrees of clot formation.

ing incubation for 90 min at 37°C, 0.2 ml of oxalated human plasma and 0.35 ml of 0.25% calcium chloride solution were added to all tubes. The tubes were incubated at 37°C. The results are presented in Table II.

Table II reveals that Zephiran chloride in a dilution of 1:10,000 prevented completely the lysis of fibrin clots by streptococcus. A marked delay in fibrinolysis resulted from the presence of Zephiran chloride in a dilution of 1:100,000. It is also evident that Zephiran chloride in a dilution of 1:10,000 caused a retardation in the clot-formation following the addition of calcium chloride solution. In other experiments this effect was less pronounced.

In order to determine whether or not inhibition of fibrinolysis necessitates incubation of Zephiran chloride and streptococcus culture, the following experiment was carried out. In part I, Zephiran chloride was incubated with the streptococcus broth culture for 1 hr at 37°C prior to the addition of plasma and calcium chloride solution; in part II, Zephiran chloride, streptococcus cul-

ture, plasma, and chloride solution were mixed without previous incubation. The experiment revealed that in part I Zephiran chloride in dilutions up to 1:160,000 inhibited fibrinolysis, whereas in part II Zephiran chloride in dilutions up to only 1:40,000 caused this effect. Similar results were obtained in repeated experiments. It may be concluded that the action of Zephiran chloride upon plasma alone does not account for the above described effect upon streptococcal fibrinolysis. The significance of the inhibitory effects of Zephiran chloride upon plasma-coagulation by staphylococcus and fibrinolysis by *hemolytic streptococcus* and their possible applications, remain to be determined.

Summary. (1) Zephiran chloride in a dilution of 1:10,000 inhibits and in higher dilutions (up to 1:1,000,000) delays the clotting of oxalated human plasma by staphylococcal cultures. (2) Zephiran chloride in dilutions up to 1:100,000 inhibits fibrinolysis by *hemolytic streptococcus*.