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Effects of Tyrothricin and Actinomycin A Upon Bacterial Fibrinolysis and Plasma-Coagulation.

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Antagonism between various microörganisms has been known for many years. Recently, these phenomena have again attained attention in an effort to determine their mechanisms and possible applications for the prevention and treatment of infectious diseases. Antagonistic interrelations of microörganisms have been admirably reviewed by Waksman.[1] Of particular interest are antagonistic phenomena due to the production and liberation of bacteriostatic or bactericidal substances. Fleming,[2] Abraham and associates[3] obtained a substance from *Penicillium notatum,* referred to as penicillin, that is markedly antagonistic to certain microörganisms. Waksman[4] succeeded in isolating from *A. antibioticus 2* such substances, that were designated as actinomycin A and B. The former exerts highly selected bacteriostatic effects, whereas the latter is largely bactericidal. Dubos and Hotchkiss[5] isolated from *Bacillus brevis 2* substances with marked antimicrobial activity: Gramicidin, acting upon gram-positive bacteria, and tyrocidin, which affects both gram-positive as well as gram-negative bacteria. Tyrothricin is the product obtained from *Bacillus brevis* that contains both gramicidin and tyrocidin.

In view of the marked antagonistic activity of these substances of microbial origin toward many bacteria and fungi the question arises as to whether or not they act also upon bacterial toxins and toxin-like substances. In the following communication experiments are presented dealing with the effects of tyrothricin and actinomycin A upon fibrinolysis by *beta hemolytic streptococcus* and plasma-coagulation by staphylococcus.

Tyrothricin was obtained through the courtesy of Dr. D. F.

1 Waksman, S. A., *Bact. Rev.*, 1941, **5**, 231.

2 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

3 Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 609.

4 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Florey, H. W., Heatley, N. G., and Jennings, M. A., *Lancet*, 1941, 177.

5 Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, **73**, 629.

Robertson, Associate Medical Director, Merck & Company. A stock solution was prepared by dissolving 200 mg of tyrothricin in 10 cc of 95% ethyl alcohol. Appropriate amounts of this alcoholic solution were added to physiological saline solution to give the desired concentrations of the drug.

Actinomycin A was made available through the kindness of Dr. Selman A. Waksman, State of New Jersey Agricultural Experiment Station, New Brunswick, N. J. A stock solution was prepared by dissolving 10 mg of actinomycin A in 10 cc of 95% ethyl alcohol. Adequate amounts of this stock solution were dissolved in physiological saline solution to obtain the desired concentrations.

In the tests on fibrinolysis the technic of Tillett and Garner[6] was followed. Two strains of *beta hemolytic streptococcus* (Group A) obtained from man were used as source of fibrinolysin. Partially purified fibrinolysin was obtained through the courtesy of Dr. W. S. Tillett and Dr. L. R. Christensen. The technic of the staphylococcal coagulase test was essentially the same as that described by Fisk.[7] A strain of *Staphylococcus aureus hemolyticus* obtained from a skin lesion was used as source of coagulase.

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

In order to determine the effects of tyrothricin and actinomycin A upon the fibrinolytic activity of *beta hemolytic streptococcus* (Group A Lancefield) the following experiment was carried out. The supernatant of an 18-hour infusion broth culture of the streptococcus (volume 0.5 cc) in a dilution of 1:10 was mixed with various amounts (volume 0.5 cc) of tyrothricin (0.01 mg to 0.000001 mg) and actinomycin A (0.0005 mg to 0.0000005 mg), respectively. As controls, the supernatant of the streptococcus culture was mixed with physiological saline solution and saline solution containing 0.95% alcohol, respectively. The latter control was included in order to determine whether small amounts of alcohol, as present in the tyrothricin and actinomycin A solutions, affect fibrinolysis. To these mixtures 0.2 cc of human plasma and 0.25 cc of an 0.25% calcium chloride solution were added. The tubes were incubated at 37°C. The resulting clot formation and secondary lysis of the plasma clots were noted. The results of this experiment are summarized in Table I.

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

[7] Fisk, A., *Brit. J. Exp. Path.*, 1940, **21**, 311.

TABLE I.
Effects of Tyrothricin and Actinomycin A upon Fibrinolysis by *Streptococcus hemolyticus.*

	Supernatant of streptococcal culture (volume 0.5 cc) diluted 1:10 Plasma (volume 0.2 cc) $CaCl_2$ 0.25% (volume 0.25 cc) Fibrinolysis following incubation at 37° C for		
	10 min.	3 hr	24 hr
Tyrothricin in mg (vol. 0.5 cc)			
1. .01	—	—	—
2. .001	—	—	—
3. .0001	—	++++	++++
4. .000001	—	++++	++++
5.	—	++++	++++
Actinomycin A in mg (vol. 0.5 cc)			
1. .0005	—	—	—
2. .00005	—	—	—
3. .000005	—	++++	++++
4. .0000005	—	++++	++++
5.	—	++++	++++
Alcohol Control	—	++++	++++

— = No fibrinolysis (clot formation).
+ to ++++ = Various degrees of fibrinolysis.

It may be seen from Table I that (1) fibrinolysis occurred in the controls after an incubation period of 3 hours and that (2) fibrinolysis was completely and continuously prevented by both tyrothricin and actinomycin A. Essentially the same results were obtained in several experiments employing two different strains of streptococci. It may be mentioned, however, that the degree of inhibition of fibrinolysis varied somewhat with different plasmas employed. In some experiments inhibition of fibrinolysis was only transitory.

The inhibitory effects of tyrothricin and actinomycin A upon fibrinolysis by streptococcus was noted also when tyrothricin was allowed to act for several hours at 4°C upon the streptococcal culture or supernatant thereof. No marked increase in the inhibitory effects were observed.

Controls indicated that neither tyrothricin nor actinomycin A in the amounts used caused either clotting of plasma or lysis of plasma clots.

The problem arises as to whether or not this inhibition of streptococcal fibrinolysis is due to a direct action of tyrothricin and actinomycin A upon the fibrinolysin itself. No final answer can as yet be given. A preliminary experiment with partially purified fibrinolysin failed to reveal any definite inhibitory effects.

It is interesting to mention that according to Doudoroff[8] certain bacteria exert antifibrinolytic activity, among them, certain streptococci, *Proteus vulgaris, Ps. fluorescens, Ps. aeruginosa, B. anthracis, Cl. sporogenes,* and *Cl. histolyticum.* The question presents itself as to whether this antifibrinolytic activity is due to chemical substances produced and liberated by these microörganisms, and, furthermore, whether other microörganisms, pathogenic or nonpathogenic, also interfere with streptococcal fibrinolysis.

The effects of tyrothricin and actinomycin A upon plasma-coagulation by *Staphylococcus aureus hemolyticus* were investigated in the following experiment. Tyrothricin in amounts ranging from 0.01 mg to 0.00001 mg and actinomycin A in amounts of 0.0005 mg to 0.0000005 mg (volume 0.5 cc) were mixed with 0.2 cc of a 1:10 diluted 18-hour broth culture of the staphylococcus. To this mixture 0.5 cc of 1:5 diluted human plasma was added. The tubes were shaken and incubated at 37°C for 18 hours. The resulting clot formation was noted at various intervals. The results of this experiment are summarized in Table II.

It may be seen from Table II that (1) *Staphylococcus aureus hemolyticus* culture caused coagulation of human plasma after an

TABLE II.
Effects of Tyrothricin and Actinomycin A upon Plasma Coagulation by *Staphylococcus aureus hemolyticus.*

	Staphylococcal broth culture (volume 0.2 cc) dilution of 1:10 Plasma (volume 0.5 cc) in dilution of 1:5 Clot formation after incubation at 37°C for			
	1½ hr	2½ hr	4 hr	18 hr
Tyrothricin in mg (vol. 0.5 cc)				
1. .01	—	—	—	—
2. .001	—	—	—	+
3. .0001	—	—	+	+
4. .00001	+	+	+	+
5.	+	+	+	+
Actinomycin A in mg (vol. 0.5 cc)				
1. .0005	—	—	—	—
2. .00005	+	+	+	+
3. .000005	+	+	+	+
4. .0000005	+	+	+	+
5.	+	+	+	+
Alcohol control	+	+	+	+

+ = Clot formation.
— = No clot formation.

[8] Doudoroff, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1467.

incubation period of 90 minutes; (2) in contrast, clot formation did not occur in the presence of tyrothricin and actinomycin A. The inhibitory effect of tyrothricin and actinomycin A upon plasma-coagulation by *Staphylococcus aureus hemolyticus* could be demonstrated in repeated experiments. These substances also prevented clot formation by supernatants of staphylococcal cultures. Furthermore, this inhibitory effect of tyrothricin and actinomycin A upon plasma-coagulation by staphylococci was observed also when the mixtures were incubated for 18 hours at either 37°C or at 4°C prior to the addition of plasma.

It may be mentioned that neither tyrothricin nor actinomycin A in the above amounts prevented coagulation of human plasma following the addition of calcium chloride; furthermore, that the addition of calcium chloride to mixtures of staphylococcal culture, human plasma and tyrothricin or actinomycin A, that failed to show coagulation of plasma, resulted in prompt clot formation. As yet, it cannot be stated whether or not this inhibitory effect of tyrothricin and actinomycin A upon plasma-coagulation of staphylococcus is due to a direct action upon the coagulase.

There is experimental evidence indicating that fibrinolysin and coagulase play a rôle in the pathogenesis of streptococcal and staphylococcal infections. It remains to be determined whether tyrothricin and actinomycin A inhibit fibrinolysis and plasma-coagulation also *in vivo* and what significance, if any, this inhibitory effect has upon the course of the infection.

Summary. (1) Tyrothricin and actinomycin A inhibit fibrinolysis by *beta hemolytic streptococcus* cultures or supernatants thereof. (2) These substances also interfere with coagulation of plasma by pathogenic staphylococcus.

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Improved Mechanical Microtome Knife Sharpener.

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For more than ten years the automatic microtome knife sharpener described by Fanz[1] has been standard equipment in most large

[1] Fanz, J. I., *J. Lab. and Clin. Med.*, 1929, **14**, 1194.