THE LYTIC ACTION OF TYROTHRICIN AND ITS DERIVATIVES ON STAPHYLOCOCCUS AUREUS

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The antibiotic agent, tyrothricin, was first described by Dubos and his colleagues in a series of papers (1–5). Since then the *in vitro* bactericidal activity of tyrothricin against a number of bacterial species has been established. It is the purpose of the present paper to describe the lysis of staphylococci by tyrothricin and its derivatives, tyrocidine and gramicidin, both in the absence and in the presence of a staphylococcal phage.

Experimental Methods

Cultures of *Staphylococcus aureus* (the K strain) employed in the tests were freshly prepared each day by growing the organisms on tryptose agar for 18 to 24 hours at 37°C. The growth was washed off with tryptose phosphate broth, and cell suspensions of known concentrations were prepared.

The tyrothricin used in the earlier experiments was a commercial preparation (Parke Davis and Co.) made up in 92 per cent alcohol and containing 20 mg. tyrothricin per ml. Stock solutions of tyrocidine and gramicidin were prepared by dissolving in 92 per cent ethyl alcohol the purified powders of these two substances which had been supplied through the courtesy of the Western Regional Laboratories. In the experiments in which the lytic activities of all three antibiotic agents were compared, the solution of tyrothricin employed was also prepared by dissolving the purified powder obtained from the Western Regional Laboratories in 92 per cent ethyl alcohol.

The staphylococcal phage used in the experiments was the K race of phage active against staphylococci.

Cell suspensions containing 5.0×10^8 bacteria per ml. and (*a*) varying concentrations of phage, or (*b*) varying concentrations of antibiotic, or (*c*) known concentrations of phage with varying concentrations of antibiotic were prepared in tubes. The mixtures were incubated in a 37°C. water bath equipped with a mechanical shaker. Lytic curves were obtained from turbidity readings taken every 0.2 hour by direct visual comparison with staphylococcal suspensions of known density.

DISCUSSION OF EXPERIMENTAL RESULTS

Preliminary tests had indicated that concentrations of tyrothricin ranging from 0.01 to 0.001 mg. per ml. of medium proved most satisfactory for observation of the lytic effects, since concentrations greater than 0.01 mg. per

ml. of medium caused considerable precipitation of the cell suspensions, rendering turbidity readings impractical.

The data summarized in Table I illustrate the lytic activity of tyrothricin. It is evident that with a nearly optimal concentration of 0.01 mg. per ml., the lytic effect is mainly due to the content of tyrothricin, for the addition of 5.0×10^8 activity units of phage (6) does not accelerate the time of lysis

TABLE I

Lytic Effects of Tyrothricin with and without Phage

Tube No.	Tyrothricin	Phage concentration Activity units/ml. $\times 10^8$	No. bacteria/ml. $\times 10^8$	Time of half lysis
	mg./ml.			*hrs.*
1	0.01	—	5.0	0.5
2	0.001	—	5.0	1.2
3	0.0001	—	5.0	Not lysed in 4 hrs.
4	0.01	5.0	5.0	0.5
5	0.001	5.0	5.0	1.1
6	0.0001	5.0	5.0	1.6
7	—	5.0	5.0	1.4
8	—	1.0	5.0	2.4
9	0.0001	1.0	5.0	1.4

TABLE II

Lytic Effects of Tyrothricin, Tyrocidine, and Gramicidin

Tube No.	Antibiotic	Phage concentration Activity units/ml. $\times 10^8$	No bacteria/ml. $\times 10^8$	Time of half lysis
	mg./ml.			*hrs.*
1	0.01 tyrothricin	—	5.0	0.6
2	0.01 tyrothricin	1.0	5.0	0.6
3	0.01 tyrocidine	—	5.0	0.6
4	0.01 tyrocidine	1.0	5.0	0.6
5	0.01 gramicidin	—	5.0	3.2
6	0.01 gramicidin	1.0	5.0	3.5

beyond that exhibited by the antibiotic alone. When a concentration of 0.001 mg. per ml. of tyrothricin alone is employed, the time required for half lysis is considerably increased, in contrast to the results obtained with 0.01 mg. per ml. of tyrothricin. The addition of phage to this concentration of antibiotic does not appear to lessen the time necessary for half lysis.

A concentration of 0.0001 mg. tyrothricin per ml. is insufficient by itself to cause lysis. However, the presence of tyrothricin greatly accelerates the rate of phage-engendered lysis of staphylococci occurring in a concentration of 1.0×10^8 phage units per ml.

The experimental results assembled in Table II suggest that the lytic effects of tyrothricin exhibited in Table I are a function of the activity of the tyrocidine fraction, for both solutions require the same period of time to accomplish half lysis of the test suspension. The lytic action of gramicidin is considerably slower than that of tyrothricin and tyrocidine.

Repetitions of these experiments showed that the times of lysis varied from day to day, but the general trend of the data obtained from these studies was identical.

SUMMARY AND CONCLUSIONS

A tyrothricin concentration of 0.01 mg. per ml. causes rapid lysis of *Staphylococcus aureus*; this lytic action is not enhanced by phage. A concentration of 0.001 mg. per ml. of tyrothricin is likewise capable of lysing staphylococci, and the addition of phage does not appreciably alter the rate of lysis. A concentration of 0.0001 mg. per ml. of tyrothricin is unable to effect lysis though it enhances the lytic activity of phage. The tyrocidine fraction of tyrothricin appears to be responsible for the latter's lytic action.

BIBLIOGRAPHY

1. Dubos, R. J., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 311.
2. Dubos, R. J., and Cattaneo, C., *J. Exp. Med.*, 1939, **70**, 249.
3. Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1940, **132**, 791.
4. Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1941, **141**, 155.
5. Lipmann, F., Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1941, **141**, 163.
6. Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.