ENDURACIDIN, AN INHIBITOR OF CELL WALL SYNTHESIS

Sir:

The antibiotic, enduracidin, which is a basic polypeptide[1] isolated[2] from *Streptomyces fungicidicus* No. B-5477, demonstrated[3] a strong *in vitro* and *in vivo* activity against Gram-positive microorganisms as well as against strains resistant to other known antibiotics. This communication reports the effec- of enduracidin on the *Staphylococcus aureus* cell wall synthesis.

Staphylococcus aureus 209P was cultivated in Trypticase soy broth at 37°C in shaking culture, and the growth inhibiting effect of enduracidin added at the logarithmic growth phase was observed by optical density measurement and viable cell count. As indicated in Fig. 1, a concentration of 1 mcg/ml had little influence on the growth, 2 mcg/ml inhibited the growth of microorganism and 4 mcg/ml or higher concentration caused a progressive decline in the turbidity as well as viable cell count owing to the lytic action of the drug upon the microorganism.

The culture of the microorganism at the point of half maximum growth was divided into several portions; one was used as a control; the antibiotic (in various concentrations) was added to the other portions, and the cells were harvested 0.5, 1, 2 and 3 hours after the addition. Aliquots of 35 ml of culture were centrifuged and the cells were washed twice with water. Extraction of the cells with cold trichloroacetic acid, determination of the N-acetylamino sugars and separation of the nucleotides by anion-exchange chromatography using Dowex-1 was performed as described by STROMINGER[4], and ultraviolet absorption were determined

Fig. 1. Influence of enduracidin upon the growth of *Staphylococcus aureus* 209P

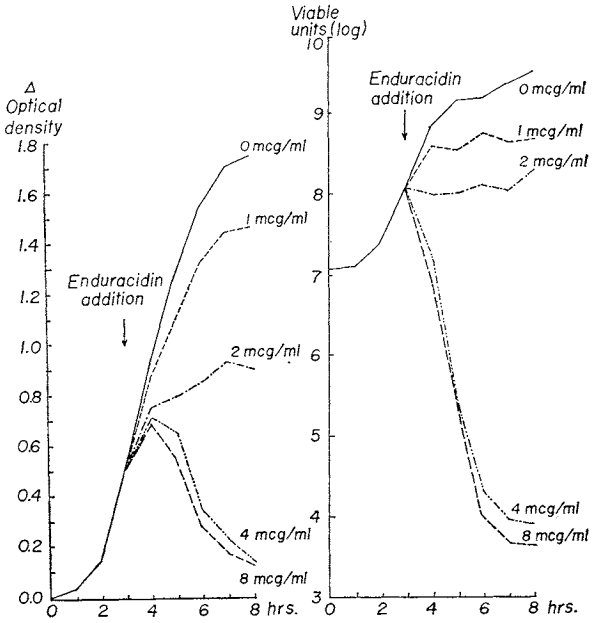


Fig. 2. Time course of accumulation of N-acetylamino sugar in *Staphylococcus aureus* 209P in the presence of varying amounts (0, 2 and 4 mcg/ml) of enduracidin.

The data are expressed as micrograms of N-acetylamino sugar esters in 1 ml of the cold 10 % trichloroacetic acid extract.

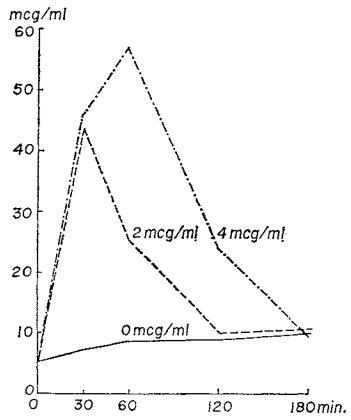


Fig. 3. Relationship of the various concentrations of enduracidin (0~4 mcg/ml) and the accumulation of N-acetylamino sugar at 37°C for 30 minutes harvesting.

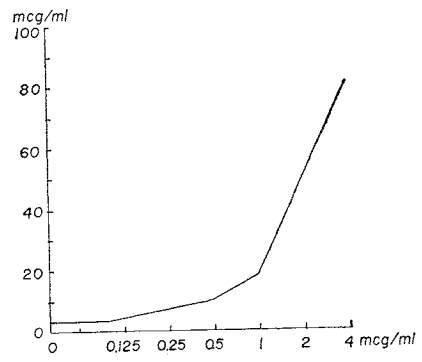


Fig. 4. Anion exchange chromatogram of enduracidin-treated *Staphylococcus aureus* 209P.

The data are expressed as the 260 mμ absorption, the letters with arrow refer to the eluents employed in accordance with STROMINGER[4].

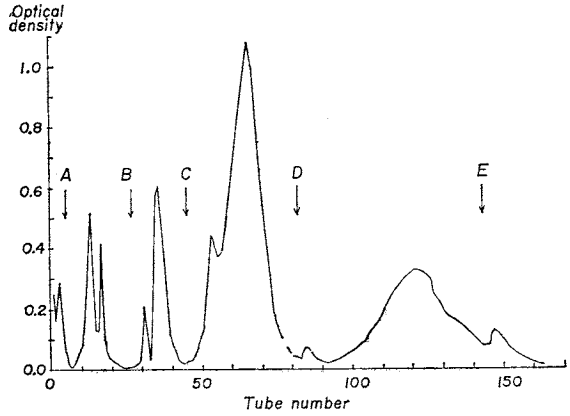


Table 1. Analysis of fraction C

Compound isolated	Micromoles found	Mol per mol glutamic acid
Amino sugar	0.52	0.69
Glutamic acid	0.75	(1.0)
Alanine	1.96	2.61
Lysine	0.62	0.83

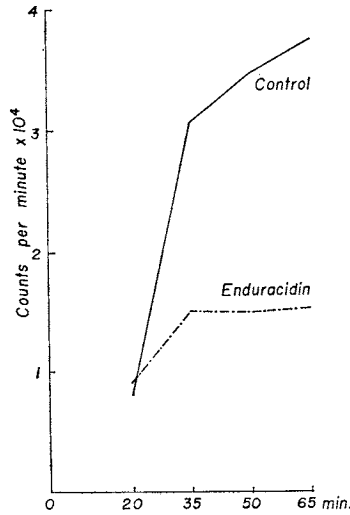
After hydrolysis of sample in 6 N HCl at 105°C overnight, the componenents were separated on a column of Dowex-50. Amino sugar was measured after hydrolysis in 0.1 N HCl.

during the course of chromatography.

As shown in Fig. 2, with 2 mcg/ml of enduracidin the maximum sugar was found after 30 minutes incubation; with 4 mcg/ml after 60 minutes. The relationship between the concentration of the antibiotic and the accumulation of the sugar at 30 minutes incubation is shown in Fig. 3, and it is noted that the minimum concentration required for the accumulation was the same as the minimum growth inhibitory concentration.

The elution diagram using anion-exchange Dowex-1 is indicated in Fig. 4; the Fraction C (eluent C-eluted fraction) contained hexosamine and its ultraviolet absorption maximum was at 262 mμ which indicates uridine nucleotide. The nucleotide was absorbed on charcoal and recovered by elution using ammoniacal ethanol. The result of the analysis of Fraction C showed that per mole of glutamic acid, there is one mole each of hexosamine and lysine, as well as three

Fig. 5. Inhibitory effect of enduracidin on the incorporation of ^{14}C-glutamic acid into the cell wall mucopeptide of *Staphylococcus aureus* 209P.



moles of alanine as shown in Table 1. These results indicate that the nucleotide which accumulated after the addition of enduracidin was identical with PARK's nucleotide[5], UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala, which is concerned with a precursor of the cell wall peptidoglycan, and which is reported to accumulate when the biosynthesis of the cell wall is inhibited by antibiotics such as penicillin[4], bacitracin[6], novobiocin[7], vancomycin[8], ristocetin[9] and moenomycin[10].

It was further observed that the antibiotic had no inhibitory influence upon the incorporation of ^{14}C-L-glutamic acid into the trypsine solubilyzed protein (separated by the method of PARK and HANCOCK[11]) and that the antibiotic showed a pronounced inhibition of the incorporation of glutamic acid into the cell wall mucopeptide as shown in Fig. 5.

These results suggest that the primary site of action of enduracidin on *Staphylococcus aureus* is the inhibition of the synthesis of cell wall mucopeptide. However, it remains to be clarified whether enduracidin inhibits the utilization of UDP-MurNAc-pentapeptide to form linear peptidoglycan strands like vancomycin[12], ristocetin[12] and bacitracin[13] or disturbs the

cross-linking of the linear strands like penicillin[14].

Inhibition of bacterial cell wall synthesis is an important mechanism of action of antibiotics. Bacterial cell walls are chemically and morphologically distinct from any other structures found in animal cells. Therefore, the inhibition of the bacterial cell walls synthesis results in the selective toxicity of the new antibiotic, enduracidin.

The authors are grateful to Dr. K. KAZIWARA for his revision of this report, and to Dr. J. UEYANAGI for amino acid analysis.

KANJI TSUCHIYA
YOKO TAKEUCHI

Biological Research Laboratories,
Research and Development Division,
Takeda Chemical Industries, Ltd.,
Higashiyodogawa-ku, Osaka, Japan

(Received April 18, 1968)

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