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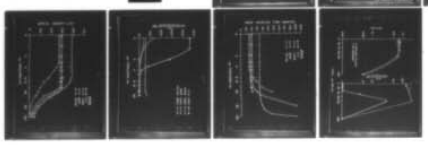

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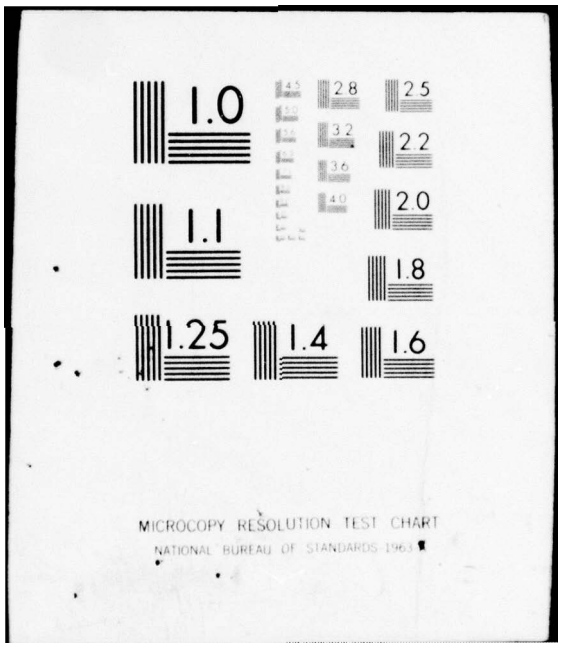
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NATIONAL BUREAU OF STANDARDS 1963

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Extreme Sensitivity of Staphylococcal Enterotoxin B and C Production to Inhibition by Cerulenin

ROBERT A. ALTENBERN

U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21701

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Production of staphylococcal enterotoxin B and C was completely inhibited by concentrations of cerulenin far too low to affect either growth rate or final growth density. Type A toxin formation was not similarly inhibited.

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ABSTRACT

Production of staphylococcal enterotoxins B and C was completely inhibited by concentrations of cerulenin far too low to affect either growth rate or final growth density. Type A toxin formation was not similarly inhibited.

The secretion of bacterial exoproteins has been most extensively investigated by Lampen and collaborators (5,7) in the case of penicillinase production by Bacillus licheniformis. From these studies, it appears that lipid of the cytoplasmic membrane participates in the synthesis and transport of penicillinase to the exterior medium. Kimura and Izui (4) have recently reported that membrane fluidity, which is controlled by the fatty acids in the membrane, plays an important role in induction of alkaline phosphatase in Escherichia coli. In this laboratory, there is an ongoing investigation of the role of membrane fatty acids in production of enterotoxin B by Staphylococcus aureus. Fatty acid nutrition in S. aureus can be directed by exposing cells to the minimal inhibitory concentration of cerulenin, an antibiotic which inhibits fatty acid biosynthesis (6), and restoring growth by supplementation with appropriate saturated and unsaturated fatty acids. An unexpected result of these studies was the observation that production of staphylococcal enterotoxins B and C is inhibited by concentrations of cerulenin far too low to affect either the growth rate or final growth density of the strains employed. The present report presents details of this phenomenon.

The strains of S. aureus employed were as follows: ATCC 14458, S-6, 137-H2, and 2909. Both strains 14458 and S-6 are wild type strains which produce enterotoxin B (SEB). Strain 137-H2 is a wild type that elaborates enterotoxin C_1 (SEC). Strain 2909 produces relatively large amounts of enterotoxin A (SEA) and was derived from strain 100 by a multistep mutagenesis procedure (3). Cells were grown in 18 x 150 mm tubes containing 5 ml of NAK medium (2) prepared in 0.067 M phosphate

buffer, pH 7.0, and containing graded concentrations of cerulenin. The tubes were incubated at an angle on a shaker at 37 C. Growth rate was monitored by measuring optical density (absorbance) at 600 nm in a Coleman Jr. spectrophotometer. Final growth density (after 21 h incubation) was assessed by measurement of absorbance at 600 nm of a 1:5 dilution (into water) of the culture. A portion of the final culture was centrifuged to remove cells and the supernatant was assayed for enterotoxin by the Oudin tube method. Assays for α-toxin and coagulase were conducted by microtitration and by conventional serial dilution, respectively.

The minimal inhibitory concentration (MIC) of cerulenin for strains 14458, S-6, and 2909 was approximately 100 μg of antibiotic per ml; strain 137-H2 was somewhat more sensitive, with an MIC of about 50 μg/ml (Fig. 1). Concentrations of cerulenin up to at least 10 μg/ml had no demonstrable effect on the final growth density for strains 14458, S-6, or 2909. The final growth density of strain 137-H2 declined gradually above 3 μg cerulenin/ml.

Pronounced suppression of SEB production by cerulenin concentrations above 1 μg/ml was noted for strains 14458 and S-6 (Fig. 2). Formation of SEC by strain 137-H2 was completely inhibited by a cerulenin concentration (3 μg/ml) which had no effect on final growth density. In contrast, there appeared to be no suppression of SEA production by strain 2909 by concentration of cerulenin that did not inhibit growth.

Addition of cerulenin up to 20 μg/ml to preformed enterotoxin B in NAK medium did not alter the assay value by the Oudin method, thus ruling out possible inactivation of SEB or inhibition of the antigen-antibody reaction by the antibiotic. Similar tests on SEA and SEC were not performed.

Determinations of mass doubling times from optical density data during exponential growth revealed there was no increase in doubling time for strains 14458, S-6, and 2909 up to at least 10 µg cerulenin/ml (Fig. 3). These data parallel the effects on final growth density depicted in Fig. 1. There was also agreement for strain 137-H2 between the concentration of cerulenin required to induce suppression of final growth density and extension of the mass doubling time (5 µg/ml and above).

Cerulenin is an unstaurated fatty acid amide (6) and it might be expected that other saturated or unsaturated fatty acids would antagonize the inhibition of production of enterotoxin B by cerulenin. Accordingly, two sets of tubes of NAK medium were prepared, one set without cerulenin and the other containing 10 µg cerulenin/ml. Graded concentrations of a mixture of saturated fatty acids were added to each series. The saturated fatty acid mixture (SFA) was composed of equal concentrations (w/v) of lauric, myristic, palmitic, stearic, arachidic, behenic, and lignoceric acids. Figure 4 shows that the final growth density of the cultures was affected to the same degree by SFA regardless of the presence or absence of cerulenin in the medium. There was no suppression of SEB formation by the SFA mixture in cerulenin-free medium until final growth density was depressed by the added fatty acids. In contrast, in tubes containing cerulenin at 10 µg/ml, a narrow concentration range of SFA mixture partially reversed the total suppression of SEB formation by cerulenin. A mixture of 10 unsaturated fatty acids from C_{16} to C_{24} was unable to reverse cerulenin-induced suppression of SEB production without concomitant severe inhibition of growth (data not shown).

Employing strain 14458, it was found that low concentrations of cerulenin, which were noninhibitory for growth rate or final growth density, also strongly suppressed production of α-toxin and coagulase. It appears that production of SEB,α-toxin and coagulase is severely inhibited by the same concentration of cerulenin (Table 1). It is noteworthy that the inhibition is a general effect, altering production of all three exoproteins in the same manner.

The mechanism of this effect is unknown. The concentration of cerulenin required to suppress production of enterotoxins, α-hemolysin and coagulase is only a small fraction of the minimal inhibitory concentration. Since neither the final growth density nor the growth rate is demonstrably affected by these low antibiotic concentrations, it is suggested that interference with fatty acid biosynthesis does not occur, although subtle modifications of synthesis of fatty acids cannot be excluded by these data. The effects of cerulenin reported here resemble the inhibition of coagulase release by very low, subinhibitory concentrations of chloramphenicol (1). The observation that strain 2909 is resistant to inhibition of SEA production when growth rate and final density are unaffected is not surprising. Strain 2909 was derived from wild type strain 100 by a 13-step mutagenesis procedure and is far removed in many of its growth properties from the original wild type. The very low production of SEA by wild type strains such as strain 100 (less than 3 μg/ml) precluded determination by Oudin assay of the effect of cerulenin in subinhibitory concentrations on formation of this toxin type.

ACKNOWLEDGEMENTS

I am grateful to Allen R. Knott for performing Oudin assays for the enterotoxins and to Anna Johnson for conducting α-hemolysin titrations.

LEGEND TO FIGURES

FIG. 1. Effect of graded concentrations of cerulenin on final growth density of S. aureus strains.

FIG. 2. Suppression of enterotoxin formation by graded concentrations of cerulenin. Enterotoxin in µg/ml divided by absorbance of 1:5 dilution yields a measure of toxin production per unit cell mass. Strain number in parentheses. The lower limit of determination of enterotoxin amounts of all types by Oudin technique is 3 µg/ml.

FIG. 3. Alteration of mass doubling times of S. aureus strains by cerulenin.

FIG. 4. Saturated fatty acid-induced reversal of cerulenin suppression of enterotoxin B formation by 14458.

TABLE 1. Suppression of production of α-hemolysin and coagulase by strain 14458 by graded concentrations of cerulenin.

µg Cerulenin/ml	Titers α-toxin[a]	Titers Coagulase[b]
0	256	4
1	128	2
2	64	2
3	32	0
4	8	0
5	4	0
8	0	0
10	0	0
15	0	0
20	0	0

[a]Reciprocal of highest dilution showing 50% lysis.

[b]Reciprocal of highest dilution displaying any clot.

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Fig 1

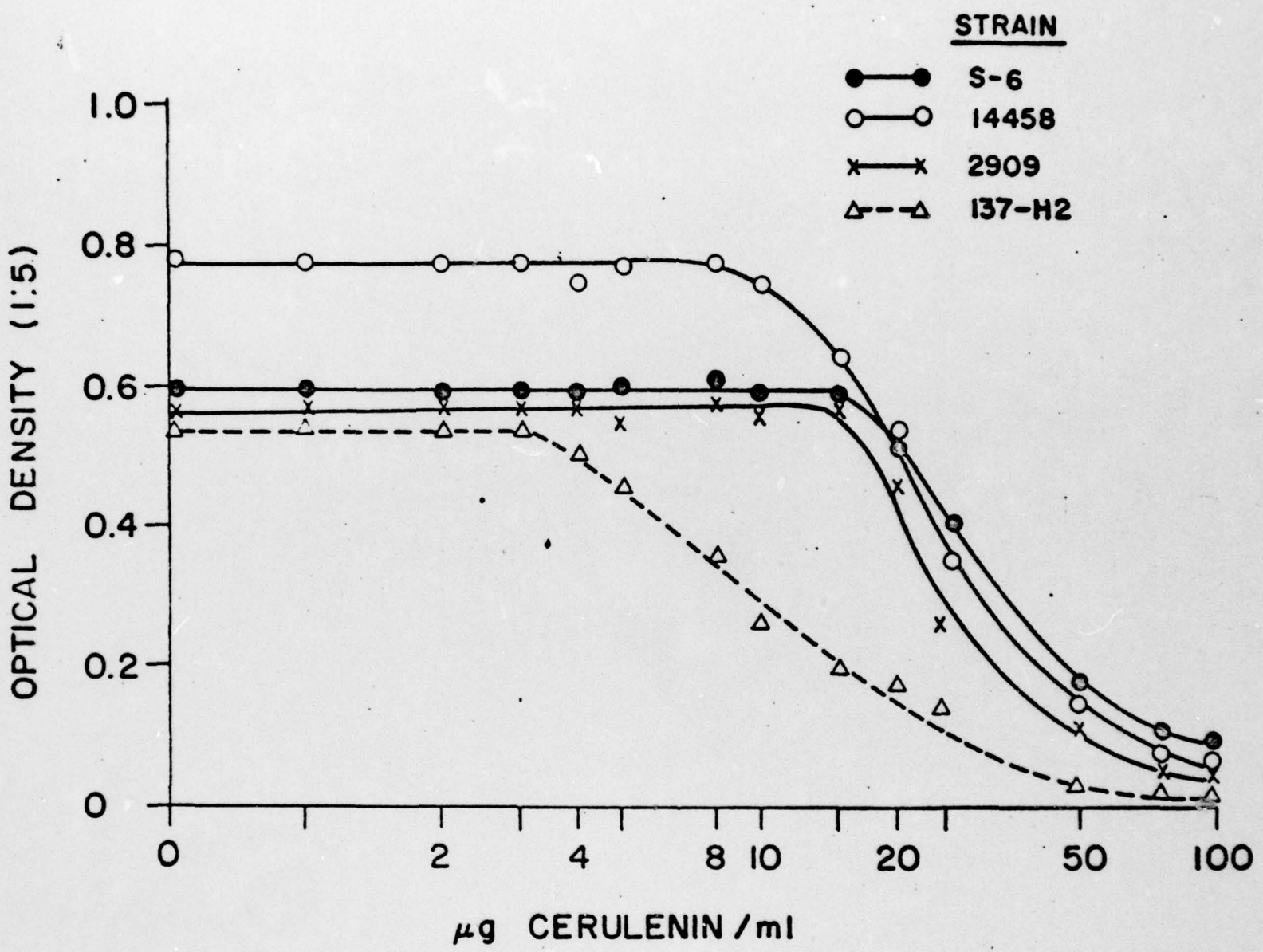

STRAIN
S-6
14458
2909
137-H2
OPTICAL DENSITY (1:5)
1.0
0.8
0.6
0.4
0.2
0
0
2
4
8
10
20
50
100
μg CERULENIN / ml

Fig 2

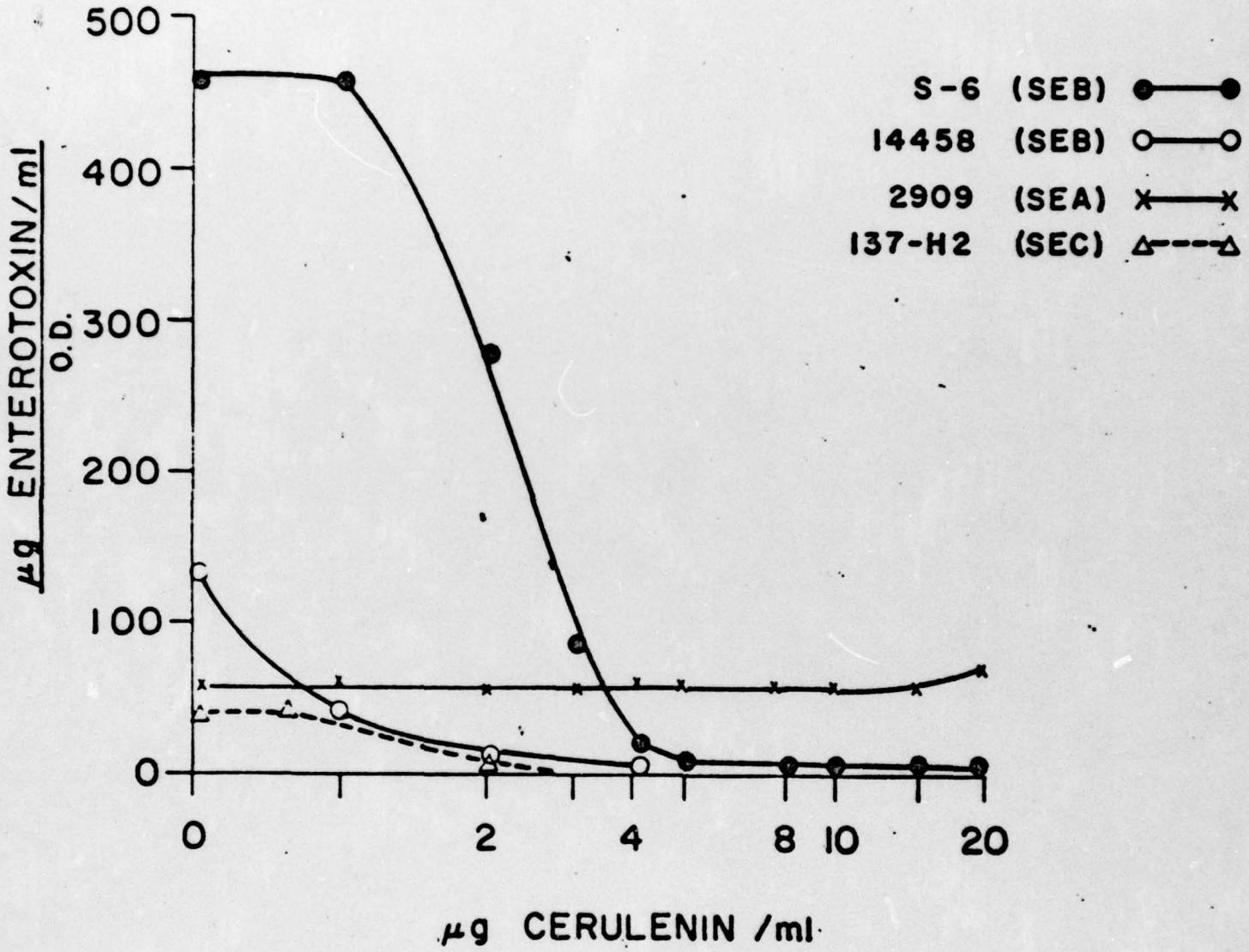
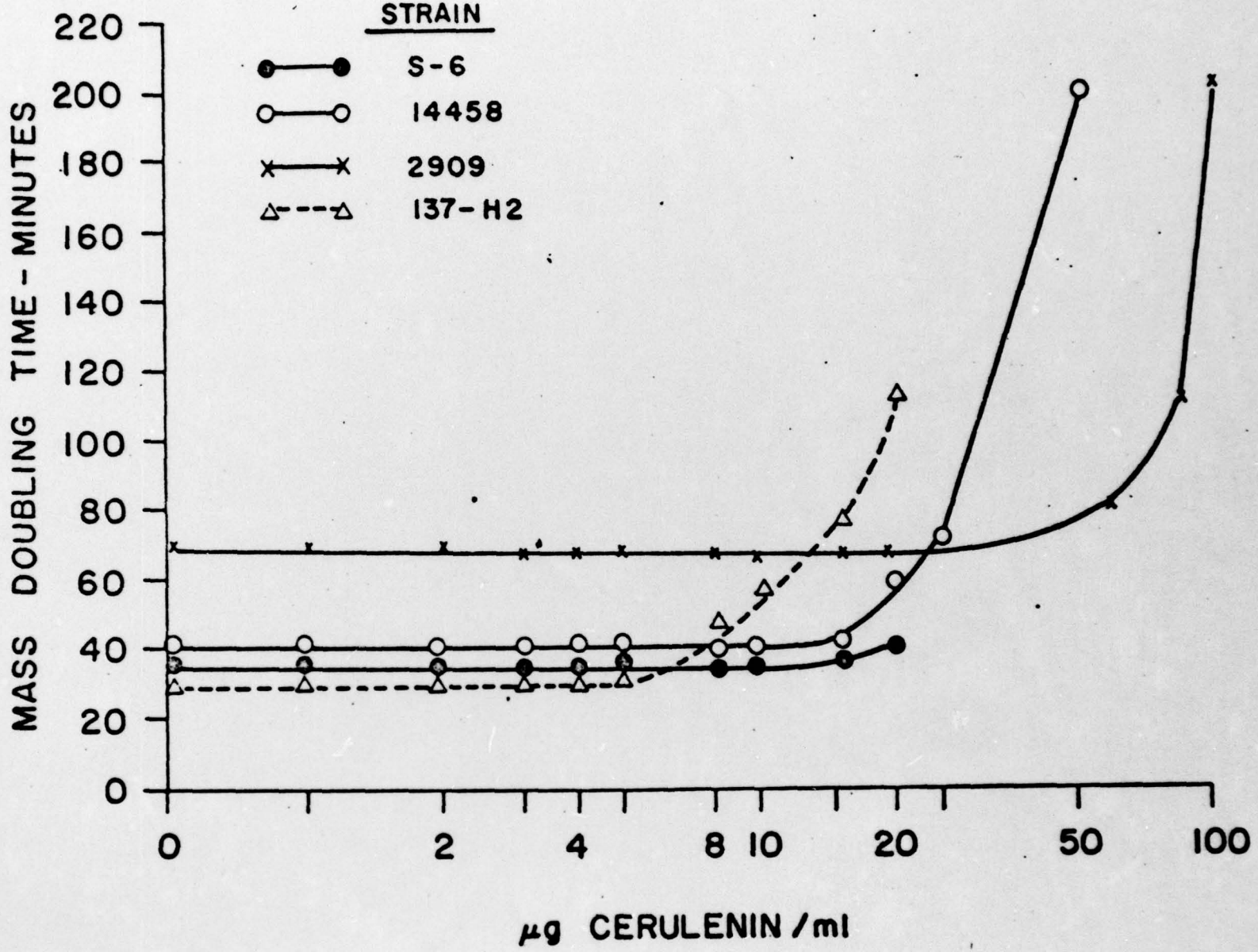
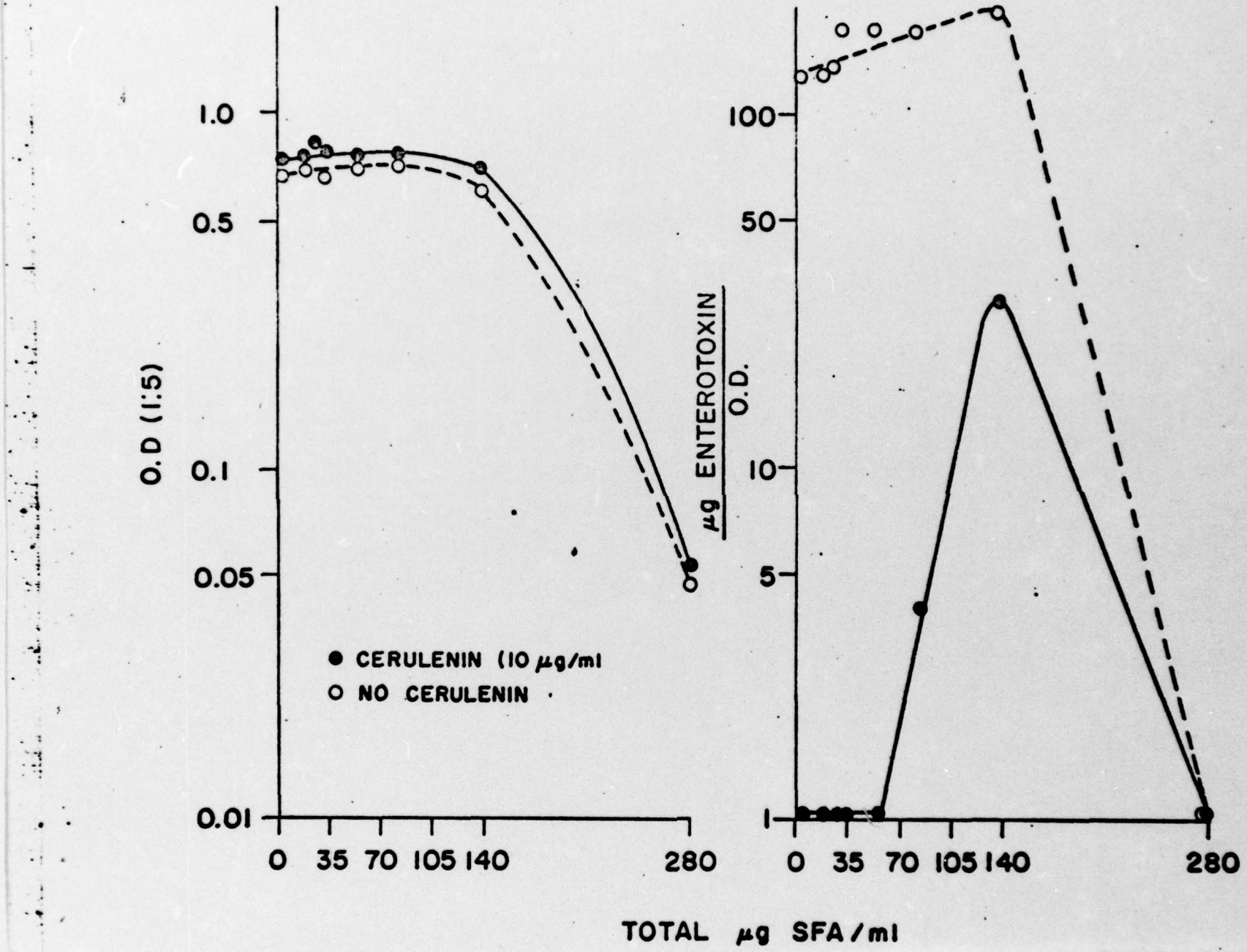

500
400
300
200
100
0
μg ENTEROTOXIN/ml
O.D.
0
2
4
8
10
20
μg CERULENIN /ml
S-6 (SEB)
14458 (SEB)
2909 (SEA)
137-H2 (SEC)

Fig 3


STRAIN
S-6
14458
2909
137-H2
MASS DOUBLING TIME - MINUTES
220
200
180
160
140
120
100
80
60
40
20
0
0
2
4
8
10
20
50
100
µg CERULENIN / ml


1.0
0.5
0.1
0.05
0.01
O.D (1:5)
0 35 70 105 140 280
● CERULENIN (10 µg/ml
○ NO CERULENIN
100
50
10
5
1
µg ENTEROTOXIN / O.D.
0 35 70 105 140 280
TOTAL µg SFA/ml