Inhibition of L-Forms of Staphylococci by Coccal Forms of Staphylococci[1] (34313)

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It was observed that coccal colonies of one strain of staphylococcus growing in the presence of a confluent growth of the L-forms of another strain of staphylococcus were surrounded by circular areas devoid of L-form growth. The purpose of this study was to determine to what extent coccal forms of one strain of staphylococcus inhibit L-forms or coccal forms of other strains of staphylococcus. The data indicate that coccal strains of *Staphylococcus aureus* and of *Staphylococcus albus* show varying degrees of inhibition of growth of staphylococcal L-forms. This phenomenon appears to be related either to some toxic substance or to loss from the media of some unidentified nutritional factor. Alpha-hemolysin was shown not to be responsible for this effect.

Materials and Methods. The term L-form has been defined in detail in previous reports from this department (1–3).

Nine strains of staphylococcus (six *S. aureus* and three *S.* albus) and their derived L-forms were studied. The L-forms were induced with methicillin except No. 316, which was isolated directly from sputum of a patient with cystic fibrosis of the pancreas. Eight of the strains were from patients and one from the American Type Culture Collection (6538P).

The coccal forms were maintained on brain heart infusion (BHI) agar slants (Difco). The L-colonies were induced and maintained on the same medium, to which 10% inactivated horse serum, 0.1% $MgSO_4 \cdot 7H_2O$, 3.5% NaCl, and 250 μg/ml of sodium methicillin were added (SSM, salt serum methicillin). Streaks of L-form colonies were transferred from the SSM plates to an L-form maintenance medium without methicillin (SS) (1). Streaks of parent colony growth were made at right angles across streaks of L-forms. On other plates L-forms of one strain were streaked across streaks of L-forms of other strains. The plates were inverted and maintained under aerobic conditions at 37°. When zones of inhibition were clearly apparent, the plates were refrigerated and examined daily thereafter. The areas of inhibition between the growth of one strip and that of the other remained relatively constant. The zones of inhibition were recorded in millimeters (Table I).

This effect was strikingly demonstrated using Millipore filters (0.45 μ). The filters were placed on the center of SS agar plates. Colonies of cocci were grown on top of the filters for 24 hrs. The filters were then removed and streaks of coccal forms and their derived L-forms were placed radially to the center of the plates (Fig. 1).

That the coccal forms studied were producing alpha-hemolysin was demonstrated using anti-alpha-hemolysin (4). Alpha-hemolysin was, therefore, tested as a possible cause of the inhibition. Extracts from beneath filters, which had been placed on BHI and SS plates and on which coccal colonies had been grown, were placed on freshly planted plates of L-forms. Isolated alpha-hemolysin was also placed on similar plates.

Results. Inhibition. The L-forms were inhibited to varying degrees by coccal colonies (Table I). Coccal colonies of one strain did not inhibit coccal colonies of another strain.

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TABLE I. The Influence of Coccal Colony Growth Upon the Growth of L-Forms.

	Coccal form[a]								
L-Form	212	ATCC 6538P	342	Georgio	964	502A	292	325	316
212	++	±	++	++	++	+	+	+	+
ATCC 6538P	++	—	+	++	++	—	+	+	+
342	++	—	+	++	++	+	+	+	+
Georgio	++	±	+	+	++	+	+	+	+
964	++	—	+	+	+	—	+	+	+
502A	+	±	+	++	+	+	+	—	+
292	++	±	++	++	++	+	+	+	+
325	++	—	+	+	++	+	+	+	+
316	+	—	++	++	+	—	+	+	+

[a] Key: ++, inhibition of 2 mm; +, inhibition of 1 mm; and —, no inhibition.

The L-form colonies similarly did not inhibit L-form colonies. That inoculation size played a role was suggested by the observation that the inhibiting effect was more pronounced with heavier growths of coccal forms. The experiment using Millipore filters showed that L-forms were inhibited where the filters had been, whereas the coccal forms were not (Fig. 1).

Filtration of inhibiting factor(s). When supernatant aqueous extracts were prepared from agar cut from beneath the filters and when these extracts were placed on freshly planted plates of L-forms, they did not inhibit the growth of the L-form colonies.

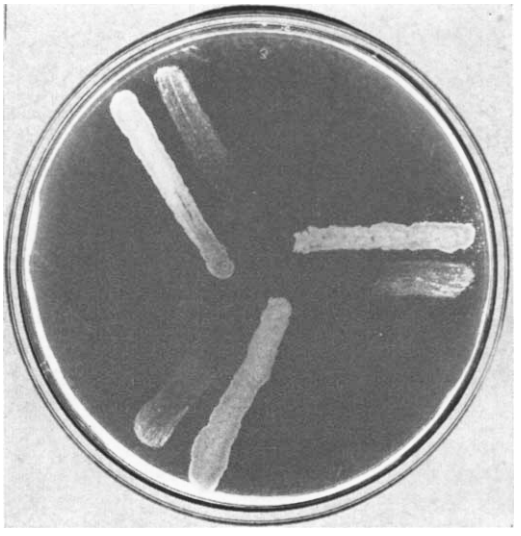

FIG. 1. Filtration of inhibiting factors; coccal strain 212 grown on Millipore filter which has been removed; each pair represents streaks of coccal and of L-forms of the same strain (upper left, 502A, middle right, 342. lower left, 212).

It was found that the same coccal strains which produced alpha-hemolysin in BHI media produced very little or no demonstrable alpha-hemolysin in media containing 3.5% NaCl (SS). When the NaCl concentration in these BHI extracts was raised to 3.5% to avoid inhibition due to osmotic shock and the extracts were tested for inhibitory effect, no inhibition was found. Isolated alpha-hemolysin placed on SS agar likewise did not inhibit L-form growth.

Thus it appears that alpha-hemolysin is not responsible for the observed inhibition of L-forms by coccal forms. No water-soluble inhibitory substance was demonstrated.

Discussion. Coccal forms inhibited L-forms. The L-forms did not inhibit either other L-forms or coccal forms. This suggests that the cause of inhibition is related to the production, presence, and/or maintenance of the cell wall. That the cause of the inhibition is elated to the cell wall, however, is not proven since it is possible that the inhibitory action is related to some metabolic difference, independent of the cell wall, which may exist between L-forms and coccal forms.

A variety of mechanisms might be responsible for the inhibition: (i) reduction or exhaustion of some nutrient(s) necessary for the growth of L-forms but the reduction of which does not noticeably interfere with the growth of coccal forms; (ii) accumulation of

some nonwater-soluble waste product(s) which interfere(s) with L-form growth; or (iii) the active production by the coccal forms of some nonwater-soluble toxic substance.

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