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# NOTES

## Immunofluorescent Demonstration of Cell-Associated Staphylococcal Enterotoxin B

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Enterotoxin B can be demonstrated readily in culture filtrates of *Staphylococcus aureus* S6. The failure to demonstrate an accumulation of enterotoxin B in cells of this organism by examination of variously produced lysates led to a morphological investigation of the cellular location of the toxin. The technique for immunofluorescent identification of antigens (Coons and Kaplan, J. Exptl. Med. **91**:1, 1950) was applied to this problem. This method has been used extensively for microscopic demonstration of a wide variety of antigens (Beutner, Bacteriol. Rev. **25**:49, 1961).

A purified enterotoxin (Schantz et al., Army Sci. Conf. 31, 1964) was used to immunize a burro. The globulin fraction of this antiserum was labeled with fluorescein isothiocyanate (BBL) by use of a modification of the procedure described by Riggs et al. (Am. J. Pathol. **34**:1087, 1958). Before use, the reagent was adsorbed three times with cells of a nonenterotoxic strain of *S. aureus* and then passed through a column of Sephadex G-25. Either *S. aureus* no. 1 or a mutant derived from strain S6 was used for the adsorption. The reagent treated in this manner no longer stained smears of these nonenterotoxic strains.

Smears prepared from cultures of strain S6 in the early log phase (3 hr) contained cells with minimal green-fluorescence. If the cultures were washed twice by centrifugation and resuspension in distilled water, the washed cells displayed little or no fluorescence. Filtrates from these cultures were negative for enterotoxin when assayed by the single agar-diffusion technique described by Surgalla, Bergdoll, and Dack (J. Immunol. **69**:357, 1952).

A brilliant wide band of peripheral fluorescence was seen in unwashed cells from 8-hr cultures (Fig. 1A). Washed 8-hr cells also reacted with the labeled antibody; however, the fluorescence was restricted to the interstitial spaces between packed cells (Fig. 1B). Very little fluorescence was present on the exposed, peripheral surface of the packed cells. This indicates a loose binding of enterotoxin to the surface of the bacterial cells. Filtrates of the 8-hr cultures contained 14 $\mu$g/ml of enterotoxin B.

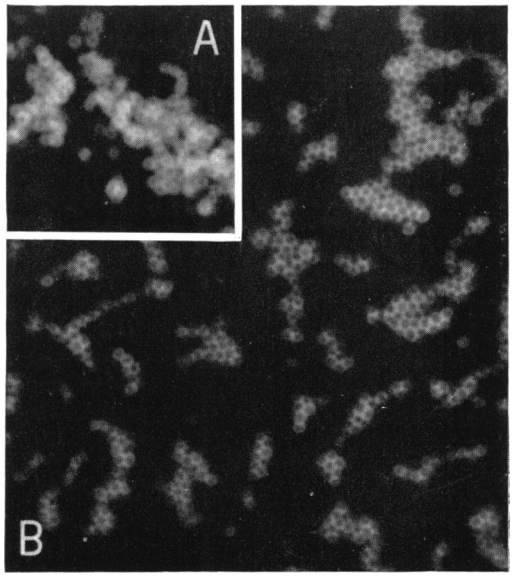


FIG. 1. *Smears of Staphylococcus aureus S6 prepared from an 8-hr culture. (A) With unwashed cells, a peripheral band of green fluorescence is prominent on each cell; (B) with washed cells, the green fluorescence is limited to the interstices formed by the packed cells. 1,500 ×.*

After Bergdoll (Ann. N.Y. Acad. Sci. **65**:139, 1956) reported that staphylococcal enterotoxin was a water-soluble protein, rich in lysine and resistant to trypsin, Hartman and Goodgal (Ann. Rev. Microbiol. **13**:465, 1959) suggested that enterotoxin has the characteristics of a cell surface constituent of bacteria. Our results with immunofluorescence further support this view.