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Detection of Type E Botulinal Toxin in Cultures by Fluorescent-Antibody Microscopy

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Vegetative cells of toxigenic *Clostridium botulinum* type E cultures were stained with fluorescent antitoxin prepared against purified toxin. The staining seems to be specific.

Lack of specificity has been observed in attempts to identify *Clostridium botulinum* by staining with fluorescent antibodies prepared against whole cells (1, 7, 9).

Inukai and Riemann (3) attempted to overcome the problem of unspecific fluorescent-antibody staining by developing a method which would specifically stain the intracellular botulinal toxin. They used crude antitoxin for indirect fluorescent staining after absorption to remove somatic antibodies. The results were promising and have been reproduced in a direct staining technique using antiserum prepared against purified toxin, thus eliminating the need for absorption. Horse serum containing 900 immunizing units of antitoxin E per ml was obtained after immunization with highly purified type E progenitor toxin (12S) (4), and γ-globulins from 10 ml of serum were isolated on Sephadex G-200 with 0.1 M phosphate buffer, *p*H 8.0, containing 0.1 M NaCl. The γ-globulins, after dialysis against phosphate-buffered saline (0.15 M NaCl, 0.01 M phosphate, *p*H 7.1) and adjustment of the protein concentration to 2 mg/ml, were conjugated with fluorescein isothiocyanate (BBL, lot no. 8011468) by the method of Nairn (8). The conjugate isolated on Sephadex G-25 was dialyzed against 0.05 M phosphate buffer, *p*H 7.2, and eluted from a diethylaminoethyl Sephadex with the same buffer containing increasing NaCl concentration from 0.1 M to 1 M. The first fraction, containing approximately 40 immunizing units per ml, was used for staining by using the method of Georgala and Boothroyd (2). Cell preparations from agar colonies or liquid cul-

TABLE 1. *Fluorescent staining of cells from 24-hr blood agar colonies*

Group[a]	Strains[b]	Staining intensity[c]
	Toxic	
I	A62	0
I	B32	0
I	F Langeland	0
III *Clostridium*	C573	0
III *botulinum*	D8265	0
II	B17	0
II	E Minnesota	+++
II	E Saratoga	+++
II	E Detroit	+++
	Nontoxic	
II	5i	+
II	S-5	0
II	S-9[d]	0
II	GB3[d]	0
II	14APL3[e]	0
II	13BPL	0
II	17CPL	0
II	7BPL2 (24)	+
II	15FPL	0
II	20FPL (26)	0
II	1957/61	0
I	PA3679	0
Unrelated	OS Minnesota	+

[a] According to genetic relationship (6).

[b] Strains are those listed by Lee and Riemann (6) (except the ones listed below).

[c] Symbols: 0, no staining; +, very weak staining; +++, distinct staining.

[d] S9 and GB3 were obtained from D. Kautter, Food and Drug Administration Laboratory, Washington, D.C.

[e] 14APL3-1957/61 was obtained from I. Batty, Wellcome Research Laboratories, England.

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tures were air-dried but not fixed. A Zeiss fluorescent microscope was used with an Osram HBO 200 high-pressure lamp, exciter filter UG 2 and barrier filter 41. The results of staining are shown in Table 1.

Only *C. botulinum* type E strains were stained. Cells of the genetically related strains of *C. botulinum* B17 or nontoxigenic E-like organisms could not be stained. The nontoxigenic 5i and OS Minnesota which showed weak staining were isolated from a toxic E culture, and there is no proof that these cultures are completely pure. Heating to 100 C abolished staining of toxic E cells, and blocking with nonconjugated E antitoxin prevented staining of nonheated cells. Staining of cells from liquid cultures was less satisfactory than staining of cell suspensions from colonies because of lysis evident already after 12 hr of incubation. Free spores of toxic E cultures stained brightly, even after heating, not only with E antitoxin but also with antitoxin against *C. botulinum* B 32. The explanation of this phenomenon, which was not shown by nontoxic E cultures, is unknown. However, the technique seems to be specific for vegetative cells of toxic *C. botulinum*, and could be useful in rapid identification of toxic colonies and in testing purity of clones, e.g., in genetic studies.

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