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Differential Characters of Two Strains of *Clostridium botulinum*, Type E: Action of Toxins in Chickens.

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The isolation and identification of 2 strains of *Clostridium botulinum*, type E, incitants of 2 fatal cases of human botulism in New York State, have been reported.[1, 2, 3] Since type E had not then been demonstrated as an incitant of human botulism in this country, a differential study of the strains was undertaken, the results of which are herein recorded. A third outbreak with which a type E strain was associated has recently been reported by Geiger.[4]

One strain of *Cl. botulinum* (No. 35396) was isolated from German-canned sprats;[1] the other strain (No. 36208) was recovered from smoked salmon that had come from Nova Scotia.[3]

Colony Formation. On 5% defibrinated horse blood agar plates[5] the colonies of the "sprat" strain vary in shape and size, have a comparatively granular surface, a slightly raised center, and a narrow, flattened, irregular periphery. The colonies of the "salmon" strain are more regularly round, have a smoother surface, and a narrow, flattened, usually entire, periphery.

Agglutinative Properties. Both strains are agglutinated to a significant degree in homologous antiserum only and the agglutinative properties are absorbed from these antisera by the homologous culture only, indicating the sprat and salmon strains to be of distinct agglutinative groups.

Toxigenic Activity. Guinea pigs, mice, rabbits, and kittens were all highly susceptible to both sprat and salmon toxins injected subcutaneously. Chickens also were highly susceptible to the salmon toxin but not to the sprat toxin. Since it had been found that the

1 Hazen, E. L., *J. Infect. Dis.*, 1937, **60**, 260.

2 Hazen, E. L., *Science*, 1938, **87**, 413.

3 Mackenzie, G. M., Three cases of botulism treated with antitoxin, *Clinical Miscellany, Mary Imogene Bassett Hospital, Cooperstown, New York*, Springfield, Ill., Thomas, 1934, Vol. I, p. 53.

4 Geiger, J. C., *J. Am. Med. Assn.*, 1941, **117**, 22.

5 Wadsworth, A. B., *Standard Methods of the Division of Laboratories and Research of the New York State Department of Health*, 2d ed., Baltimore, Williams and Wilkins Company, 1939, p. 104.

antitoxins produced against each toxin neutralized the toxins reciprocally, a further study was undertaken upon this evidence in chickens of a difference between the toxins.

White Leghorn chickens from 4 to 12 weeks old and weighing from 210 to 525 g were employed. The toxic filtrates were obtained from cultures grown in portions of the same lot of broth medium, under similar conditions. Twenty-nine chickens were tested for susceptibility to subcutaneous doses of the fluid toxins, 9 with doses ranging from approximately 300 to 4000 guinea pig M.L.D. of the sprat toxin and 20 with from approximately 4 to 300 guinea pig M.L.D. of the salmon toxin. None of the chickens that received the sprat toxin became ill, while all that had the salmon toxin showed symptoms characteristic of botulism and many died. The fatal dose for these chickens, which weighed from 230 to 315 g, was approximately 6 to 8 guinea pig M.L.D. (It is interesting that the toxin of the German sprat strain and that of a Russian strain isolated from fish and identified as type E by Gunnison, Cummings, and Meyer,[6] and transfer of which Doctor Meyer kindly supplied, acted similarly in chickens; the agglutinative properties of the two strains were also found to be similar.)

Attempt was then made to determine whether the blood of normal chickens contained a neutralizing substance for the sprat, but not for the salmon toxin. Toxin-antitoxin neutralization tests were performed in mice with (1) mixtures of sprat or salmon toxin and normal chicken serum; (2) mixtures of sprat or salmon toxin and blood of chickens immunized respectively against each toxin. (Toxoid was employed for immunization against the salmon toxin.) Briefly, from 0.5 to 1 ml of normal chicken serum afforded mice no protection against less than 2 mouse M.L.D. of either toxin, whereas 0.25 ml of sera from the immunized chickens protected mice reciprocally against from 4 to 8 M.L.D of the salmon and sprat toxins.

Since a neutralizing substance was not demonstrated in normal chicken serum, a further possibility was investigated: whether the sprat toxin was destroyed after absorption from the subcutaneous tissue; or, if not destroyed, for how long a time after injection it could be detected in the circulating blood and excreta.

Four normal chickens were bled from the veins on the ventral surface of the wings, and were then given subcutaneous injections of from approximately 2100 to 4000 guinea pig M.L.D. of the toxin. Blood was collected from 3 in one hour, from the fourth in 2 hours,

[6] Gunnison, J. B., Cummings, J. R., and Meyer, K. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 278.

and from all 4 at 24-hour intervals for 96 hours. From 0.5 to 1 ml of the whole blood was injected immediately into mice, intraperitoneally. None of those injected with normal blood became ill; all injected with blood obtained from the chickens up to 48 hours after injection of the toxin died with symptoms of botulism. Two of 4 mice injected with 72-hour specimens died. None that received 96-hour specimens were ill.

Fifteen specimens of excreta were collected at intervals of from one hour to 24 hours for the 96 hours, emulsified in physiological salt solution, and refrigerated overnight. The material was then centrifuged at high speed and from 1 to 1.5 ml of the supernatant was injected intraperitoneally into mice. Only 3 of the 15 specimens gave rise to botulism; they were from 3 different chickens and had been collected 24, 48, and 72 hours after injection of the toxin.

Summary. Differential characters of two strains of *Clostridium botulinum*, type E, are described. One strain was isolated from German-canned sprats; the other from Nova Scotian smoked salmon. Culturally they can be differentiated by colony formation on horse blood agar plates. Agglutination and absorption tests indicate them to be of distinct serologic groups.

The toxins of both strains were neutralized reciprocally by their antitoxins. Tests for toxigenic activity demonstrated, however, that the toxins were not identical. Subcutaneous injections of both toxins uniformly gave rise to symptoms characteristic of botulism in guinea pigs, mice, rabbits, and kittens. White Leghorn chickens were highly susceptible to the toxin of the salmon strain but not to that of the sprat strain. In chickens 4 to 12 weeks old, approximately 6 to 8 guinea pig M.L.D. of the salmon toxin gave rise to fatal botulism, while doses of the sprat toxin several hundred times as large failed to kill or induce symptoms of botulism. The sprat toxin was demonstrable in the blood and also in the excreta of some of the chickens 72 hours after inoculation.