

ATOXIC BOTULISM TOXIN (BOTULISM TOXOID, BOTULISM ANATOXIN).

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More than twenty years ago Lowenstein¹ pointed out that tetanus toxin treated with formalin lost its toxicity in a large measure without appreciable alteration of its antigenic or immunizing value. More recently Ramon² and his collaborators have developed the possibilities of atoxic bacterial toxins (anatoxin) as immunizing agents of merit. As the result of their investigations diphtheria toxoid has replaced diphtheria toxin-antitoxin in European countries as well as Canada in the field of public health. The same principle of detoxification has been applied to snake venom and tetanus toxin with encouraging results, while Weinberg and Goy³ demonstrated that rabbits could be actively immunized to botulism toxins A and B with formalin treated toxin. The detoxified toxin was also regarded as substitute for the unaltered toxin in repeated injections of small animals in preparing antitoxin. The results of their experiments permitted the suggestion that horses could be similarly treated.

The potential advantages of formalized atoxic botulism toxin as an immunizing agent prompted a series of experiments at the Illinois Experiment Station with Cl. botulinum cultures and filtrates A, B, and C. Varying amounts of formalin (.3 to .6 percent) were added to filtered and unfiltered botulism cultures. The formalized toxins were then incubated for varying lengths of time at 37°C to 42°C. The formalin treated toxins were tested weekly for toxicity by injecting .5 cc to 2 cc subcutaneously into guinea pigs (250 to 300 grams). If inoculated pigs remained healthy and maintained their weight, the toxoid was regarded as nontoxic. The time required for detoxification

1. Cited by Pfeiffer, R. and Lubinski, H. Ueber die Wirkung des Formalins auf Endotoxin (Anatoxinbildung?) Centralbl. F. Bakt. Parasitenk. u. Infect. L., Orig. 102, 459-470, June 8, 1927.

2. Ramon, Ann. de l. Institut Pasteur—1923 XXXVII—1001. Ibid. 1925—XXXIX, 1.—Compt. Rend. Soc. Biol., 1927, XCVI, 30.

Ramon, Berthelot, Grasset et Amoreux, Compt. Rend. Soc. Biol., 1927, XCVI, 30.

3. Weinberg, W. and Goy, P.—Vaccination Anti-botulinique Par Voie sous-cutanee et peos—Compt. Rend. des Sec. de Biol., 93, 1925, 430-432. Ibid 92, 1925, 564-5, Ibid 91, 1924, 1140-41, Ibid 91, 1924, 148-149, Ibid 90, 1924, 269-271.

varied from one to eight weeks, depending somewhat on the amount of formalin and the degree of heat.

The protective or immunizing value of the toxoid was determined in guinea pigs and other animals. Ten days after receiving 1 cc to 3 cc of toxoid guinea pigs were given 5 to 10 lethal doses of unaltered toxin subcutaneously. Death in the control or untreated pigs receiving the unaltered toxin clearly showed the protective character of botulism toxoids. (See Tables 1 and 2). A single injection of B and C toxoids provided protection in guinea pigs, while larger doses of C toxoid (5 cc) provided protection in quarter and half grown chickens.

In attempting to protect horses and mules it was found that a single subcutaneous injection of potent B or C toxoids (20 cc to 30 cc) followed by exposure in ten days failed to protect against lethal injections of the unaltered toxins. (See Table 3). Both B and C toxoids that proved nontoxic in guinea pigs were nontoxic in horses (700 to 1000 pounds) in 20 cc to 30 cc doses subcutaneously, but the immunizing value in single doses of 30 cc was negligible. Two injections of B and C toxoids in 20 to 30 cc doses given a week apart to horses provided protection seven days after the last injection to the unaltered toxin. (See Tables 4 and 5).

A combination of B and C toxoids administered to horses simultaneously in amounts which separately protected failed to protect against a combination of the respective unaltered toxins, while the immunizing value of botulism toxoid A gave irregular and uncertain results in the preliminary trials.

Summary:

1. Formalin (.3 to .6 per cent) added to filtered or unfiltered liquid cultures of *Cl. botulism* B and C upon incubating 1 or more weeks at 37°C. to 42°C. becomes relatively atoxic.

2. A single subcutaneous injection of 1 to 3 cc atoxic botulism toxins B and C possesses immunizing value in guinea pigs. Horses and mules were not protected by single injections of botulism toxoids B and C (20 cc and 30 cc) but two injections a week apart protected against the unaltered toxins.

TABLE I.
PROTECTIVE CHARACTER OF BOTULISM TOXOID B.

Guinea Pig	Weight in Grams	Treatment 10-29-28	Treatment 11-8-27	Results
509	350	1 cc Botulism Toxoid 2699B subcutaneously.	1/500 B. Toxin subcutaneously	11-17-27 Released Healthy
993	310	2 cc Botulism Toxoid 2699B subcutaneously.	1/500 B. Toxin subcutaneously	11-17-27 Released Healthy
390	420	1 cc Botulism Toxoid 1413B subcutaneously.	1/500 B. Toxin subcutaneously	11-17-27 Released Healthy
379	350	2 cc Botulism Toxoid 1413B subcutaneously.	1/500 B. Toxin subcutaneously	11-17-27 Released Healthy
381	320	1 cc Botulism Toxoid 1267B subcutaneously.	1/500 B. Toxin subcutaneously	11-17-27 Released Healthy
395	300	2 cc Botulism Toxoid 1267B subcutaneously.	1/500 B. Toxin subcutaneously	11-17-27 Released Healthy
946	250	Control	1/500 B. Toxin subcutaneously	11-10-27 Died

TABLE 2.
PROTECTIVE CHARACTER OF BOTULISM TOXOID B.

Guinea Pig	Weight in Grams	Treatment 1-5-28	Treatment 1-21-28	Results
883	420	2 cc Botulism Toxoid 6757C subcutaneously.	1/1000 C. Toxin sub-cutaneously.	1-28-28 Released Healthy
899	330	3 cc Botulism Toxoid 6757C subcutaneously.	1/1000 C. Toxin sub-cutaneously	1-28-28 Released Healthy
884	320	Control	1/1000 C. Toxin sub-cutaneously	1-23-28 Died

TABLE III.
NONPROTECTIVE CHARACTER SINGLE INJECTION OF BOTULISM TOXOID B

Horse	Weight	Treatment 9-2-27	Treatment 9-12-27	Results
I	1050 Lbs.	20 cc Botulism Toxoid 1413B subcutaneously	1 cc Botulism Toxin subcutaneously.	9-17-27 Died.
K	950 Lbs.	30 cc Botulism Toxoid 1413B subcutaneously	1 cc Botulism Toxin subcutaneously.	9-15-27 Died.
M	750 Lbs.	Control	1 cc Botulism Toxin subcutaneously.	9-17-27 Died.

TABLE IV.
PROTECTIVE CHARACTER TWO INJECTIONS OF BOTULISM TOXOID B

Horse	Weight	Treatment 9-18-27	Treatment 9-24-27	Toxin 10-1-27	Results
L	850 Lbs.	20 cc Botulism Toxoid 1413B subcutaneously	20 cc Botulism Toxoid 1413B subcutaneously	1 cc B. Toxin subcutaneously	10-26-27 Released Healthy
N	850 Lbs.	20 cc Botulism Toxoid 1267B subcutaneously	20 cc Botulism Toxoid 1267B subcutaneously		10-26-27 Released Healthy
S. M.	1300 Lbs.	Control			10-3-27 Died

TABLE V.
PROTECTIVE CHARACTER TWO INJECTIONS OF BOTULISM TOXOID C

Horse	Weight	Treatment 9-11-27	Treatment 9-21-27	Toxin 9-28-27	Results
C	1100 Lbs.	30 cc Botulism Toxoid 1787 C subcutaneously	30 cc Botulism Toxoid 1787C subcutaneously	1 cc C Toxin subcutaneously	10-7-27 Released Healthy
D	900 Lbs.	30 cc Botulism Toxoid 1674C subcutaneously	30 cc Botulism Toxoid 1674C subcutaneously		10-7-27 Released Healthy
B	1000 Lbs.	30 cc Botulism Toxoid 1774C subcutaneously	30 cc Botulism Toxoid 1674C subcutaneously		10-7-27 Released Healthy
E	950 Lbs.	Control			10-1-27 Died