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THE ANTIGENICITY OF *CLOSTRIDIUM BOTULINUM* TYPE C TOXIN ADMINISTERED *PER OS*

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ABSTRACT

JANSEN, B. C., KNOETZE, P. C. & VISSER, F. The antigenicity of *Clostridium botulinum* type C toxin administered *per os*. *Onderstepoort J. vet. Res.*, 37 (3), 169–172 (1970).

Large quantities of toxoid given to rabbits *per os* did not elicit a primary immune response. Toxin absorbed through the intestinal wall of guinea-pigs and toxoided produced an anamnestic response on injection into basically-immune rabbits, but had no effect on fully susceptible rabbits. By dosing sufficient toxin or toxoid *per os* to basically-immune rabbits anamnestic responses could be produced.

INTRODUCTION

During the course of experiments to determine the relationship between the serum antitoxin titre and the resistance of cattle to the oral dosing of *Clostridium botulinum* type C toxin, it became necessary to know whether or not toxin exposed to the digestive process and absorbed through the intestinal wall is still antigenic. Also, as the expense of using cattle for experimental purposes forces one to use them repeatedly, it is important to know whether an oral dose of toxin will affect an animal's resistance to subsequent doses. Furthermore it seems essential to establish whether vaccinated animals have their resistance enhanced by the ingestion of toxic material in the field. Since no information on these points could be obtained from the literature, a series of experiments in guinea-pigs and rabbits was undertaken to clarify them.

MATERIALS AND METHODS

Clostridium botulinum type C toxin was prepared by the method of Sterne & Wentzel (1950) using the Onderstepoort Veterinary Research Institute vaccine strain. For conversion of the crude toxin to toxoid, formalin to a final concentration of 0.6 per cent was added and the mixture incubated at 38°C for 8 days. Detoxification was regarded as being complete when 0.2 ml injected intravenously into white mice failed to kill them.

For the vaccination of experimental animals alum-precipitated toxoid was prepared by adding alum to toxoid at pH 6.0 to a final concentration of 2 per cent. Dried toxin was prepared by adding 30 g ammonium sulphate to 100 ml crude toxic culture filtrate and drying the precipitate *in vacuo*. Toxicity and neutralization tests were done in 25 g white mice and deaths recorded for 3 days. The test dose of toxin contained about 50 MLD.

The guinea-pigs used weighed about 0.7 kg and the rabbits about 5 kg.

Per os dosing of rabbits and guinea-pigs was done under light ether anaesthesia using a syringe and lubricated plastic catheter; the animals were starved for 12 hours to prevent regurgitation of the toxic solution, and some physiological saline was injected through the catheter to ensure that all the active material was deposited in the stomach.

To obtain toxic serum guinea-pigs were dosed *per os* with crude toxin and bled at hourly intervals. The most toxic serum was obtained 3 to 4 hours after dosing. The maximum concentration was obtained by dosing 1 ml of crude toxin; bigger doses did not increase the toxicity of the serum. As a standard procedure, therefore, guinea-pigs were dosed with 1 ml of toxin and exsanguinated after 3 hours. The serum contained about 20 mouse MLD/ml.

Toxic serum was detoxified with a final concentration of 0.1 per cent formalin and incubated at 38°C for 8 days. At this stage 0.4 ml injected intravenously into mice was non-toxic. Alum-precipitated serum toxoid was prepared by adding alum to a final concentration of 1 per cent and adjusting the pH to 6.0.

RESULTS

Experiment I

To establish if antibody can be produced by dosing *C. botulinum* antigen *per os* three rabbits were given 10, 25 and 40 ml crude toxoid at monthly intervals and bled 14 days after the last dose. None of them showed a detectable antibody response. There was no doubt about the antigenicity of the toxoid because the same material was used for the preparation of an effective vaccine.

Experiment II

This experiment was planned to investigate whether toxin which has passed from the gut into the blood of a guinea-pig is able to evoke an antibody response. Toxic guinea-pig serum was formalinized and precipitated with alum. Three rabbits were given three subcutaneous injections of 5, 10 and 15 ml respectively of the mixture at fortnightly intervals. Two weeks after the last injection the sera of the rabbits contained no detectable antibody.

Experiment III

As the adequacy of the amount of absorbed toxin used to act as a primary stimulus was in doubt, an experiment was designed to test its ability to elicit an anamnestic response on the premise that it requires a minimal amount of antigen.

Rabbits received one injection of alum-precipitated toxoid to provide them with a basic immunity and were left for 2 months before continuing the experiment. The titres in the different rabbits varied and, in those left as controls, declined to a low level after a few months, e.g. one of the controls had a titre of 110 IU/ml 2 months, 20 IU/ml 3 months and 0.5 IU/ml 6 months after the injection of toxoid.

One group of rabbits received toxoid *per os* and their antitoxin titres were determined on the dates indicated in Table 1.

From Table 1 it can be seen that the oral dosing of toxoid to basically-immune rabbits did have a substantial booster effect in one of the three rabbits. In Rabbit No. 1, which had a high titre initially, the quantity of toxoid was apparently insufficient to maintain the titre.

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TABLE 1 Serum-antitoxin titres of basically-immune rabbits repeatedly dosed *per os* with crude toxoid

Rabbit No.	Day	Initial titre IU/ml	Crude toxoid per os (ml)	Titre IU/ml
1	1	1666.0	5.0	
	8		10.0	1666.0
	15		10.0	1250.0
	22		10.0	1250.0
	29			500.0
2	1	10.0	5.0	
	8		10.0	10.0
	15		10.0	12.5
	22		10.0	10.0
	29			10.0
3	1	166.0	5.0	
	8		10.0	50.0
	15		10.0	50.0
	22		10.0	2500.0
	29			2500.0

A second group of basically-immune rabbits was dosed *per os* with crude toxin and their antitoxin titres determined on the dates indicated in Table 2.

TABLE 2 Serum-antitoxin titres of basically-immune rabbits repeatedly dosed *per os* with crude toxin

Rabbit No.	Day	Initial titre IU/ml	*Per os* MLD crude toxin	Titre IU/ml
1	1	20	20	
	11		20	20.0
	19		20	10.0
	43		20	2.5
	90		20	0.5
	94			Died
2	1	16.7		
	4		30	
	12			16.7
	13		40	
	20		50	250.0
	30			1250.0
3	1	10		
	4		30	
	12			10.0
	13		40	
	20		50	125.0
	30			2500.0
4	1	500		
	4		30	
	12			500.0
	13		40	
	20		50	500.0
	30			500.0

From Table 2 it can be seen that in Rabbit No. 1, which received a constant dose over a longer period than the rest, the antibody titre decreased progressively. Ultimately the antibody content of the blood was too low to protect the rabbit. In Rabbits No. 2 and 3 the higher doses had a decided booster effect. In Rabbit No. 4 the titre should have decreased in about a month if no antigenic stimulus had been given, whereas it remained stationary, possibly indicating that the oral dosing did have an effect.

Experiment IV

In a further experiment rabbits with a basic immunity were given alum-precipitated, formalinized toxic guinea-pig serum subcutaneously. The details are given in Table 3.

TABLE 3 Schedule of subcutaneous injections of alum-precipitated, formalinized toxic guinea-pig serum given to rabbits

Rabbit No.	Day	Initial titre IU/ml	ml/ injected subcut.	Titre IU/ml
1	1	1.0	1.0	
	10			1.0
	11		5.0	
	17			2.5
	18		10.0	
	24			12.5
2	1	1.3	1.0	
	10			12.5
	11		5.0	
	17			16.5
	18		10.0	
	24			16.5

From the results in Table 3 it is clear that a considerable increase in titre was brought about by the injection of the alum-precipitated, formalinized toxic serum.

Experiments done with *C. botulinum* type D toxin yielded similar results.

DISCUSSION AND CONCLUSIONS

The results of this series of experiments show that it is impossible to produce a primary antibody response in rabbits by dosing them *per os* with fairly large volumes of concentrated *C. botulinum* type C toxoid. A likely explanation is that insufficient antigen is absorbed. Even when the toxin contained in guinea-pig serum was converted to alum-precipitated toxoid it was still impossible to elicit a primary response by injecting as much as 15 ml. The results of Experiment IV showed that a fraction of this volume does produce an anamnestic response. In Experiment III the results showed that a booster response can follow when the dose of toxin given *per os* is sufficiently large. A dose as high as 20 *per os* MLD given repeatedly will not stop the decline of the antibody level to a stage where it is no longer protective against that dose. The likelihood of an animal becoming immune following the absorption of a sublethal dose of toxin is, therefore, excluded.

While 30 *per os* MLD given by mouth to a basically-immune rabbit does not elicit a booster effect, 40 *per os* MLD will do so in an animal in which the serum-antibody titre is not too high. It follows that in nature the resistance to botulism in a vaccinated animal will not be enhanced by the ingestion of a small amount of toxic material, but definitely will be by a large amount of toxin provided that the antibody titre of the recipient is sufficiently high to withstand the dose. Seldom, if ever, will the antibody titre of a vaccinated animal be too high to allow ingested toxin to stimulate a booster response.

The fact that toxin absorbed through the intestinal wall is antigenic is confirmed by Experiment IV.

SUMMARY

It was impossible to produce a primary immune response in rabbits by dosing them *per os* with fairly large quantities of *C. botulinum* type C toxoid.

Rabbits injected with alum-precipitated, formalinized serum obtained from a guinea-pig after receiving crude toxin *per os* did not develop serum-antibody titres. Basically-immune rabbits showed booster responses after receiving the same treatment.

By dosing sufficient toxin or toxoid *per os* to basically-immune rabbits anamnestic responses could be produced. It was, however, not possible when the serum-antibody titre was too high.

Essentially similar results were obtained with *C. botulinum* type D toxin.

REFERENCE

STERNE, M. & WENTZEL, L. M., 1950. A new method for the large-scale production of high-titre botulinum formol-toxoid types C and D. *J. Immun.*, 65, 175-183.

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