Onderstepoort J. vet. Res. 38 (4), 237–238 (1971)

TRYPTIC ACTIVATION OF *CLOSTRIDIUM BOTULINUM* TYPE Cβ TOXIN

B. C. JANSEN and P. C. KNOETZE, Veterinary Research Institute, Onderstepoort

Abstract

JANSEN, B. C. & KNOETZE, P. C., 1971. Tryptic activation of *Clostridium botulinum* type Cβ toxin. *Onderstepoort J. vet. Res.* 38 (4), 237–238 (1971).

The toxicity of factor C_2 produced by *C. botulinum* type Cβ is increased by exposure to 0,1% trypsin at pH 7,5 for 30 minutes. If the tryptic action is allowed to continue at pH 7,5 at room temperature, destruction of the factor results.

Introduction

During studies on the antigenicity of *C. botulinum* type Cβ toxin, difficulty was experienced in obtaining a culture filtrate sufficiently toxic for toxin-antitoxin neutralization tests. According to Jansen (1971a) only the toxic factor C_2 is produced by this organism. The inadequate toxicity applied to four strains of unknown origin available in the culture collection of the Onderstepoort Veterinary Research Institute and to strain ATCC 11772, which was ultimately selected for further experimental work.

In considering the reason for the inability to prepare cultures of sufficient toxicity, it became apparent that the antigen might be produced in the form of a prototoxin which requires activation by a proteolytic enzyme analogous to the situation in *C. botulinum* type E (Duff, Wright & Yarinsky, 1956). This possibility was, therefore, investigated experimentally.

Materials and Methods

Culture filtrates were prepared by growing the organism according to the method of Sterne & Wentzel (1950) at 37°C for eight days, harvesting the liquid portion of the culture and filtering it through Seitz clarifying pads. The Lf value of the filtrate was determined by the method of Jansen (1971b) and expressed in terms of the volume of laboratory standard antitoxin indicating the neutral point when mixed with 1,0 ml of filtrate.

Toxicity titrations were performed by diluting the filtrate with a 0,4% dibasic sodium phosphate solution and adjusting the pH to the desired level with dilute hydrochloric acid. White mice of 18 to 20 g mass were injected intravenously with 0,2 ml aliquots of the dilutions of the filtrate and observed for three days. Four mice were used per dilution.

Treatment with trypsin was carried out by adjusting the pH of 50,0 ml of filtrate to 7,5, heating it to 37°C in a waterbath and adding 0,1% trypsin (Merck). After a further 30 minutes in the waterbath the pH of the filtrate was adjusted to 5,8, the level at which filtrates were found to retain their toxicity. It was then cooled to 4°C and stored.

Results

The Lf value and toxicity of a series of culture filtrates before and after trypsin treatment were compared. The results are recorded in Table 1.

From Table 1 it is evident that the toxicity of all culture filtrates was increased by the trypsin treatment while the total antigen content as reflected by the Lf value remained unchanged. In three filtrates the toxicity was increased tenfold.

In a further experiment, the action of the trypsin on the filtrate was allowed to continue at pH 7,5 at 4°C and at room temperature and the Lf value was compared to that of untreated filtrate kept at the same temperatures. The results are given in Table 2.

Table 1 The Lf value and toxicity of culture filtrates before and after trypsin treatment

Before		After	
Lf (ml)	MLD/ml	Lf (ml)	MLD/ml
0,01	10	0,01	100
0,01	0	0,01	50
0,008	10	0,008	100
0,02	10	0,02	100

Table 2 The Lf value (in ml serum) of culture fluid following the action of trypsin for varying periods at both room temperature and 4°C

Exposure period (weeks)	Treated filtrate		Control	
	4°C	Room temp.	4°C	Room temp.
1	0,02	0,008	0,02	0,02
2	0,02	0,006	0,02	0,02
3	0,02	0,006	0,02	0,02
4	0,02	0,004	0,02	0,01
8	0,02	—	0,02	0,01
12	0,02	—	0,02	0,01

The results in Table 2 show that at pH 7,5 the antigen remained stable at 4°C with or without trypsin treatment. At room temperature, however, the control sample deteriorated to half its original value in four weeks while the trypsinized sample showed a significant loss of value in one week. By the eighth week it was completely devoid of detectable antigen.

Discussion

From the results of these experiments it is evident that trypsin activates the toxic factor produced by *C. botulinum* type Cβ. If, however, treatment with 0,1% trypsin is allowed to continue at room temperature, destruction of the antigen takes place. It might be inferred that the increase in toxicity is the result of release of toxin from intact organisms present in the culture. This is, however, not the case because filtered culture material was used and in the process of activation no increase in Lf value occurred. The results of these experiments confirmed that the antigen is produced in the form of a prototoxin and provided an explanation for the inability to obtain sufficiently toxic cultures during the previous studies.

Summary

The toxicity of the main antigen produced by *C. botulinum* type Cβ is increased by exposure to 0,1%

Received 7 October 1971—Editor

trypsin at pH 7,5 for 30 minutes. If the tryptic action is allowed to continue at room temperature, destruction of the antigen takes place.

REFERENCES

DUFF, J. T., WRIGHT, G. G. & YARINSKY, A., 1956. Activation of *Clostridium botulinum* type E toxin by trypsin. *J. Bact.* 72, 455-460.

JANSEN, B. C., 1971a. The toxic antigenic factors produced by *Clostridium botulinum* types C and D. *Onderstepoort J. vet. Res.* 38 (2), 93–98.

JANSEN, B. C., 1971b. The quantitative determination of the toxic factors produced by *Clostridium botulinum* types C and D. *Onderstepoort J. vet. Res.*, 38 (3), 177–180.

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

Printed in the Republic of South Africa for the Government Printer, Pretoria, by Heer Printing Co. (Pty) Ltd., Pretoria.