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THE TAXONOMIC POSITION OF *CLOSTRIDIUM BOTULINUM* TYPE C

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ABSTRACT

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Experimental evidence is produced to justify abandoning the practice of subdividing *Clostridium botulinum* Type C into type C_α and type C_β.

Résumé

LA BIOTAXIE DE CLOSTRIDIUM BOTULINUM *DE TYPE C*

S'appuyant sur des preuves expérimentales, les auteurs croient être justifié d'abandonner la subdivision de Clostridium botulinum *de type C en type* C_α *et type* C_β.

INTRODUCTION

Bengston (1922) isolated a strain of *Clostridium botulinum* from *Lucilia caesar* larvae after observing that chickens which ate the fly larvae sometimes died of a paralytic disease. In the same year Seddon (1922) isolated a similar strain from a beef carcass in Australia. He found the organism highly toxigenic, as is evidenced in his description of results obtained with guinea-pigs: "Oral administration of three drops of the fluid from a culture frequently led to marked symptoms and death within twenty-four hours, though, varying with the age of the culture and the dosage, the onset of symptoms might be delayed for some days and death not occur until one or two days later. Subcutaneous inoculation was likewise attended with fatal results, even with much smaller doses."

In the same publication Seddon refers to Bengston's organism as *Bacillus botulinus* and designates his own isolate *Bacillus parabotulinus*. He also differentiates the one organism from the other by comparing their colonial morphology, the morphology of single organisms, their arrangement in chains, glucose fermentation with respect to acid and gas production, and precipitation of casein in milk. He admits that the differentiation was not taken further by the performance of toxin-antitoxin neutralization tests.

From the work done in many laboratories in subsequent years, it is now known that the different types and strains of *C. botulinum* form a very heterogeneous group and can only be differentiated reliably on the basis of their toxin production. The validity of Seddon's differentiation on the grounds of variable characteristics is, therefore, in doubt. In spite of this, the organisms isolated by Bengston and Seddon have persisted as separated subtypes in the literature (Smith & Holdeman, 1968; Topley & Wilson, 1975). The organism isolated by Bengston has been labelled type C_α; that isolated by Seddon, type C_β.

After more precise methods of identifying the toxins produced by *C. botulinum* type C had been developed (Jansen, 1971), it became possible to investigate the justification for maintaining the difference between types C_α and C_β. To this end a series of *C. botulinum* type C strains was examined for their ability to produce different toxins and the stability of this characteristic.

MATERIALS AND METHODS

Strains

Strains of *C. botulinum* Type C were obtained from various sources as indicated in Table 1.

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TABLE 1 The different strains of *C. botulinum* used and their origin

Strain	Origin
C_β No. 60.....	Pasteur Institute, Paris
C No. 2/5.....	Pasteur Institute, Paris
C Vache foie....	Pasteur Institute, Paris
C_α 8264.....	National Collection of Type Cultures (NCTC) London
C_β 3732.....	National Collection of Type Cultures (NCTC) London
C.....	Nigeria
C_β.....	Virginia, U.S.A.
C.....	Veterinary Research Institute, Onderstepoort

Growth media

The ability of the various strains to produce C_1 and C_2 toxins was determined by growing them in the following medium for 5 days at 37 °C:

Tryptone*.....	3,0 g
Yeast Extract**.....	2,0 g
Glucose.....	1,0 g
Sodium thioglycollate.....	0,1 g
Cysteine hydrochloride.....	0,1 g
Aqua dest.....	ad 100 ml

The pH was adjusted to 7,2 and the medium sterilized at 0,75 atm. for 30 min. Thereafter the medium was distributed into sterile 200×25 mm test tubes containing sterilized meat particles and used within 2 days of preparation.

Surface cultures for the selection of individual colonies were grown on the following medium:

Tryptose**.....	2,0 g
Dextrose.....	0,1 g
NaCl.....	0,5 g
Agar.....	1,5 g
Aqua dest.....	ad 90,0 ml
Defibrinated ox blood.....	10,0 ml

The ingredients without the blood were mixed, the pH adjusted to 7,2 and sterilized in an autoclave at 0,75 atm. for 30 min. Thereafter the mixture was allowed to cool to 50 °C in a water bath, the ox blood added with sterile precautions and the plates poured.

The surface cultures were incubated in a McIntosh and Fildes anaerobic jar at 37 °C for 48 h in an atmosphere of hydrogen.

Production and assay of toxin

In an attempt to produce the highest possible concentration of C_2 toxin (the ordinary cultures in

* Oxoid
** Difco

liquid medium plus meat particles were weakly toxic) the cellophane sac method of Sterne & Wentzel (1950) was used.

For activating C_2 prototoxin, the pH of a sample of culture filtrate was adjusted to pH 7,5, its temperature raised to 37 °C in a water bath and 0,1% trypsin [Merck 2 000 U/g] added. After 30 min at 37 °C the pH was adjusted to 5,8 and the activated filtrate stored at 4 °C.

In testing a culture filtrate for the presence of C_1 or C_2 toxin, both C_2 and C_1 toxins were neutralized with the respective monospecific laboratory standard antiserum. Toxin-antitoxin neutralization tests were done to confirm the presence of a toxin. These tests were done by intravenous injection into white mice having an average mass of 20 g. All deaths occurring within 3 days were recorded.

For testing the toxicity of cultures by oral dosage, guinea pigs, mass about 300 g, were used. They were starved for 12 h before dosing and subsequently observed for 3 weeks.

Tests for detecting the presence of D toxin, which can only be done by indirect means for type C cultures (Jansen, 1971), were not performed because D toxin does not seem to have any decisive value in classifying type C cultures.

Results

The strains tested were found to produce the toxins indicated in Table 2.

TABLE 2 The toxins produced by strains of *C. botulinum* type C

Strain	Toxin	
C_β No. 60	C_1	C_2
C No. 2/5	C_1	
C Vache foie	C_1	C_2
C_α NCTC 8264	C_1	C_2
C_β NCTC 3732		C_2
C Nigeria	C_1	C_2
C_β Virginia	C_1	C_2
C Onderstepoort	C_1	C_2

The results of a random selection of 75 colonies from a surface culture of the Onderstepoort strain, tested for toxin production, are recorded in Table 3.

TABLE 3 Toxin production by individual colonies from a culture of *C. botulinum* type C

No. of colonies	Toxin produced	
17	—	
36	C_2	
22	C_1	C_2

All 10 colonies, harvested from a surface culture of *C. botulinum* type C Onderstepoort strain into separate tubes of meat-particle liquid medium, produced both C_1 and C_2 toxins. On repeated serial subculture in the same medium, 1 of the cultures lost its ability to produce C_1 toxin after 2 passages but continued to produce C_2 toxin.

None of the cultures producing C_2 toxin only became highly toxic. Guinea pigs could only be killed by intraperitoneal injection of as much as 5 ml culture filtrate from a 10-day culture, using Sterne & Wentzel's method. Oral dosing of 80 ml of the same culture fluid over a period of 4 days, i.e. 20 ml per day, had no deleterious effect.

There was no difficulty in obtaining the same degree of toxicity with meat-particle broth cultures producing C_1 toxin only as that described by Seddon for *Bacillus parabotulinus*. The same was achieved with cultures producing both C_1 and C_2 toxins in which the C_2 content of the culture filtrate had been neutralized.

Discussion

From Table 2 it can be seen that, while most of the *C. botulinum* type C strains tested produced both C_1 and C_2 toxins, one strain produced C_1 toxin only and the NCTC strain of *C. botulinum* type C_β produced C_2 toxin only. Table 3, on the other hand, shows that, within a strain producing both C_1 and C_2 toxins, there are individual organisms producing no toxin at all; others produce only C_1 toxin and the rest both C_1 and C_2 toxins.

The fact that the organism originally isolated by Seddon was described as highly toxic when dosed to guinea pigs and when injected into horses, cattle and sheep, suggests that it was unlikely to have produced C_2 toxin only in view of the findings reported above. These results also cast some doubt on whether the NCTC strain *C. botulinum* type C_β represents the same organism as the one described by Seddon. In view of the results shown in Table 3, it seems likely that the NCTC strain C_β was derived from a colony producing C_2 toxin only out of a mixed population in which some organisms produced C_1 as well as C_2 toxin. This would agree with the results reported above.

There is no doubt that the production of C_1 toxin is the most constant feature of the highly toxic *C. botulinum* type C strain, and it is suggested that this be regarded as the distinguishing characteristic of this organism. Furthermore the loss of its ability to produce C_1 toxin as a result of which only the ability to produce C_2 toxin remains, should not be regarded as justification for retaining type C_β as a valid subtype.

From a taxonomic point of view, therefore, there should be only *C. botulinum* type C with the ability to produce C_1 toxin as the distinguishing feature. Organisms that have lost this characteristic should be regarded as aberrant and not included in the type.

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