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The production of *Clostridium botulinum* toxin in mammalian, avian and piscine carrion

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SUMMARY

Mice, birds (chicks, quail) and fish (rudd, goldfish) killed shortly after receiving 1300–2000 spores of *Clostridium botulinum per os* were incubated, usually at 23 °C for 7 days. A 10% (w/v) homogenate of each rotting carcass was then prepared, sterilized by membrane filtration, and assayed for toxin.

In mouse carcasses a type C strain of *C. botulinum* usually produced $> 2 \times 10^5$ mouse intraperitoneal LD/g; in fish carcasses it usually produced less – often much less – than 2×10^4 LD/g. Avian carcasses appeared to be intermediate between those of mice and fish in their ability to support toxigenesis. A type E strain of *C. botulinum*, unlike type C, produced toxin equally well in fish and mouse carrion, usually at a concentration of between 2×10^4 and 2×10^5 LD/g.

INTRODUCTION

Botulism, a disease of vertebrates, is well known in cattle, sheep and horses; on poultry, pheasant, mink and fish farms; in zoo animals and free-living waterfowl; and in man.

Clostridium botulinum types C and D rarely affect man but are the commonest causes of botulism in farm animals. In waterfowl the disease is usually due to type C, but type E has caused large outbreaks in Common Loons (*Gavia immer*) and gulls (*Larus* spp.) on the shores of Lake Michigan (1–4), probably associated with the consumption of toxic fish or fish carcasses; it is also responsible for outbreaks on fish farms (5–7). Most human cases are due to types A, B and E, and those caused by the latter are well known to result from the consumption of toxic fish carcasses and other marine products.

Because carrion is one of the main sources of botulinal toxin for animals, studies of type C and E toxigenesis in the carcasses of mice incubated at different temperatures and for different periods have been made (8, 9). The present report describes experiments in which mouse, bird and fish carrion were compared in respect of their ability to support type C toxigenesis; and type E toxigenesis was studied in mouse and fish carrion.

MATERIALS AND METHODS

Most of the methods have already been described (8, 9). They are briefly outlined below, together with additional information.

C. botulinum *spore suspensions*

Spore suspensions prepared from *C. botulinum* type C (strain FH6513) and type E (strain NCTC 8266) were stored at −20 °C.

Animals

In addition to mice, two avian species and two species of fish were used. The mean weights were: mice 29·9 g; goldfish 8·6 g; rudd 14·8 g; quail (aged *c* 3 weeks) 51 g; chicks (aged 3 days) 42·6 g. Other chicks of the same age had been dosed *per os*, 2 days before use, with 1 ml of a 10% (w/v) suspension of faeces from an adult bird. This changed not only the gut microflora but also the appearance of the caeca and their contents; it also led within 2 days to a weight loss averaging 9·2 g/bird.

Preparation of toxic carcass homogenates

Except where stated otherwise (experiment 3, Table 1) animals were dosed *per os* with 1300–2000 spores (dose volume 0·25 ml) of *C. botulinum* type C or E, prepared from frozen spore suspension by diluting appropriately in distilled water. Mice were dosed by means of an Animal Feeding Needle (18 gauge, 2 inch; Harvard Apparatus Ltd, Edenbridge, Kent) attached to a 1 ml syringe. Fish and birds were dosed by means of a plastic Cat Catheter (size 4FG; Arnold Veterinary Products Ltd, 14 Tessa Road, Reading), cut to a convenient length and attached to a 1 ml syringe.

All animals were killed within 10 min of dosing – mice and birds by cervical dislocation, and fish by a blow to the head. The carcasses were wrapped individually in plastic bags and incubated. After incubation each putrefying carcass was weighed and homogenized in sufficient gelatin phosphate buffer to make a 10% (w/v) suspension. This was then cleared and sterilized by membrane filtration to form 'filtered homogenate' (FH); or merely passed through muslin to form 'unfiltered homogenate' (UH).

Toxin assay

Each FH was examined by injecting decimal dilutions (0·5 ml) into single mice intraperitoneally. Some titrations were made in duplicate, one of the two dilution series being made at pH 6·0 in the presence of 0·5% trypsin. Mice showing signs of rapidly progressing botulism were killed to prevent suffering.

RESULTS

Type C toxigenesis in the carcasses of fish, birds, and mice

Table 1 shows three preliminary experiments in which carcasses were incubated at either 30 °C or 23 °C for 6–7 days. In the first, type C toxigenesis in the carcasses of fish (rudd) was usually much less than in those of mice. The three experiments taken together gave a sight suggestion that the toxigenic capacity of carcasses of chicks and quail was intermediate between that of fish and mouse carrion.

Table 1. *Post-mortem toxigenesis in rudd, quail and mice, killed and incubated after being inoculated with* C. botulinum *type C*

Experiment	Incubation: Temperature (°C)	Incubation: Duration (days)	Carcass (*n*)	Number of carcasses showing the stated LD (log 10) of toxin per g: 2·3–3·3	3·3–4·3	4·3–5·3	5·3–6·3
1	30	6	Rudd (6)	4	1	1	0
			Quail (3)	0	1	1	1
			Mouse (6)	0	0	2	4*
2	23	7	Chick (3)	0	0	3	0
3	23	7	Chick (3)†	0	0	2	1
			Chick (3)‡†	0	0	1	2

* Smith & Turner (8) found that mice usually gave this result.

† These chicks were killed (when moribund) or found dead 18 h after receiving 1 ml of UH prepared from mouse carcasses incubated at 23 °C for 14 days.

‡ Chicks were given adult avian faeces by mouth 3 days before death.

Table 2. *Post-mortem toxigenesis in goldfish, quail and mice, killed and incubated at 23 °C for 7 days after being inoculated with* C. botulinum *type C*

Carcass (*n*)	Number of carcasses showing the stated LD (log 10) of toxin per g: < 2·3	2·3–3·3	3·3–4·3	4·3–5·3	5·3–6·3
Goldfish (6)	3	3	0	0	0
Chick (6)	0	0	1	4	1
Chick (6)*	0	0	2	4	0
Mouse (6)	0	0	0	1	5†

* Chicks were given adult faeces by mouth 2 days before they were killed.

† Smith & Turner (8) found that mice usually gave this result.

These indications were confirmed in a further experiment (Table 2) in which goldfish, chick and mouse carcasses were incubated at 23 °C for 7 days. Modification of the chick gut microflora by feeding faeces from an adult bird appeared not to affect toxigenesis *post mortem*.

Type E toxigenesis in the carcasses of goldfish and mice

Three experiments in which carcasses were incubated at 23 °C for 7 days are described in Table 3. The first showed that *C. botulinum* type E, unlike type C (Tables 1 and 2), produced toxin equally well in goldfish and mouse carcasses. The second and third experiments confirmed that type E toxin was produced much more readily than type C toxin in goldfish carrion.

DISCUSSION

The production of *C. botulinum* toxin in rotting carcasses is probably influenced by numerous factors, including the mixed microflora of carrion and the conditions under which incubation takes place. The effect of the temperature and duration of

Table 3. *Post-mortem toxigenesis in goldfish and mice, killed and incubated at 23 °C for 7 days after being inoculated with* C. botulinum *type C or E*

Experiment	Carcasses contained *C. botulinum* type	Carcass (*n*)	Number of carcasses showing the stated LD (log 10) of toxin per g				
			< 2·3	2·3–3·3	3·3–4·3	4·3–5·3	5·3–6·3
1*	E	Goldfish (5)	0	0	1	4	0
	E	Mouse (6)	0	0	0	6†	0
2*	C	Goldfish (6)	5	1	0	0	0
	E	Goldfish (6)	0	1	0	5	0
3	C	Goldfish (6)	0	1	5	0	0
	E	Goldfish (6)	0	0	0	5	1

* In experiments 1 and 2 toxin was assayed in the presence and absence of trypsin; trypsinization did not increase the titres.

† Smith, Turner & Till (9) also found that mice gave this result.

incubation on type C and E toxigenesis has already been studied (8, 9), but only in mouse carrion. It seemed likely that the microflora and the nature of the tissues of animals belonging to different classes would vary sufficiently to influence toxin production. The experiments described bore out this prediction.

A type C strain of *C. botulinum* produced less toxin in the carcasses of fish than in those of chicks (including those given an adult gut flora), and much less than in those of mice. Type C botulism is a common form of the disease in animals. For example, it occurs in cattle on pastures spread with poultry litter containing avian carrion (10) and on phosphorus-deficient pastures that lead to a craving for mammalian carrion (11). It also occurs on pheasant farms and, on a massive scale, in wild waterfowl; in such outbreaks toxin is often conveyed from avian carrion to potential victims of the disease via the intermediary of sarcophagous maggots.

A type E strain of *C. botulinum*, on the other hand, produced toxin equally well in fish and mouse carrion. This is of interest in relation to the ability of type E (see Introduction) to produce fish-borne botulism in man and in loons and gulls on Lake Michigan, and botulism in farmed fish.

The experiments reported were made with single strains of *C. botulinum* types C and E, and with the carcasses of one mammalian species, two avian species, and two species of fish. Without further similar studies it would be unwise to generalize on type C and E toxigenesis in carrion derived from mammals, birds and fish, but the findings are nonetheless of epidemiological interest.

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