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SUSCEPTIBILITY OF MINK TO CLOSTRIDIUM BOTULINUM TYPE E TOXIN

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It is well known that there is great variation in the susceptibility of different species to Clostridium botulinum toxins. Dogs, cats and foxes are very resistant (*Quortrup & Gorham* 1949, *Johannsen* 1965, *Skulberg* unpublished), while mink are known to be very susceptible (*Hall & Stiles* 1938, *Dinter & Kull* 1950). The difference in susceptibility seems mainly to be noticeable upon oral administration. Highly resistant animals like cats succumb rapidly after parenteral injection of small amounts of Clostridium botulinum toxin (*Skulberg* unpublished).

In addition, a certain variation exists between the species as to the susceptibility to different types of Clostridium botulinum toxins. The susceptibility of mink to botulinal toxins given orally has previously been investigated by *Quortrup & Gorham, Wagenaar et al.* (1953) and *Dinter & Kull* (1955). All authors agree that mink are highly susceptible to type C toxin, but there is some discrepancy as to susceptibility to types A, B and E toxins. *Wagenaar et al.* found that mink were resistant to types A, B and E, while *Quortrup & Gorham* found mink moderately susceptible to types A and B, but "immune" to type E. *Dinter & Kull* (1955) found that mink were about 400 times less susceptible to type A than to type C by oral administration.

Outbreaks of botulism in mink caused by type E have been mentioned in a communication from the State Veterinary Serum Laboraty in Copenhagen (*Meddelelse fra Serumlaboratoriet* 1956) and also described by *Nordstoga* (1961) and *Skulberg* (1961).

The present study was done in order to try to elucidate the susceptibility of mink to Clostridium botulinum type E toxin given by mouth.

MATERIALS AND METHODS

Clostridium botulinum type E strain Frederiksberg was used for toxin production. The culture was obtained from Dr. H. O. Pedersen, Copenhagen, who isolated the strain from an outbreak of botulism in man (*Pedersen* 1953). Toxin was produced by cultivation in corn steep medium according to the technique described by *Sterne & Wentzel* (1950). The culture was incubated until reaching maximal toxicity, centrifuged, dialyzed for 24 hrs. and activated by addition of 0.1 % trypsin (Difco 1:250) (*Duff et al.* 1956). The toxic supernatants were afterwards diluted 1:3 with glycerol and stored at — 20°C (*Cardella et al.* 1958). The activity of the toxin was determined by intraperitoneal injection into Swiss mice weighing 18—20 g (MLD-determination). The toxic activity of the preparation was determined to be 2×10^4 MLD per ml, henceforth referred to as MLD (i. p. mice).

The toxin was both fed and injected subcutaneously. The feeding experiments were performed by introducing 15 ml of toxin dilutions directly into the stomach by means of a stomach tube and a syringe. In order to ensure that all the toxic solution was applied in the stomach 5 ml of sterile physiological saline was afterwards passed through the tube by means of the same syringe.

The animals were restrained for about 30 min. after the removal of the stomach tube to prevent them from vomiting. Subcutaneous injections of toxic solutions were given behind the left shoulder. The animals were observed at certain intervals for 96 hrs.

Solutions of toxins were also given orally to Swiss mice weighing about 30 g. 0.5 ml of the toxin solutions was introduced directly into the stomach by means of a glass tube connected to a syringe.

Neutralization tests were performed by injecting mice of the same size with Clostridium botulinum type E antitoxin (Institut Pasteur) in quantities sufficient to neutralize 1×10^4 MLD toxin. One hour after the antitoxin injection toxin solutions were given orally in quantities corresponding to 1×10^2, 5×10^2 and 1×10^3 MLD (i. p. mice).

Table 1. Results of feeding experiments in mink with Clostridium botulinum type E toxin.

Mink no.	Weight g	Dose MLD (i. p. mice)	Survived /dead	Symptoms
321	1800	2×10^4	Survived	Reduced appetite, but no other symptoms
691	1200	2×10^4	„	
641	800	2×10^4	„	
232	1500	1×10^5	„	
692	1500	1×10^5	„	
538	1500	1×10^5	„	
664	1500	2×10^5	„	
463	1400	2×10^5	„	
674	1000	2×10^5	Died after 45 min.	Spasms started 20 min. after feeding
631	800	4×10^5	Died after 65 min.	Typical botulism symptoms with paralysis
630	1100	4×10^5	Died after 35 min.	Spasms initiated after 25 min. Intermittent paralytic stages

Table 2. Results of subcutaneous injections in mink with Clostridium botulinum type E toxin.

Mink no.	Weight g	Dose injected MLD (i. p. mice)	Survived /dead	Symptoms
633	1000	2×10^2	Survived	No symptoms
643	800	2×10^2	„	„
665	1200	2×10^3	„	Paralysis after 24 hrs., but recovered
516	1200	2×10^3	Died after 48 hrs.	No symptoms observed
509	1200	1×10^4	Died after 48 hrs.	Paralysed after 24 hrs.
285	800	1×10^4	Died after 48 hrs.	„
284	900	2×10^4	Died after 48 hrs.	„
864	900	2×10^4	Died after 24 hrs.	No symptoms observed
670	800	4×10^4	Died after 24 hrs.	„
634	800	4×10^4	Died after 48 hrs.	Paralysed after 24 hrs.

Table 3. Results of feeding experiments in mice with Clostridium botulinum type E toxin.

Dose MLD (i. p. mice)	Average survival time*)
1×10^3	40 min.
5×10^2	40 min.
1×10^2	4 hrs. 10 min.**)
1×10^1	> 72 hrs.

*) Time interval between feeding and death.
**) Variation: 50 min. — 10 hrs. 35 min.

RESULTS

The results of the feeding and injection experiments in mink are given in Tables 1 and 2, and the results of the feeding experiments in mice are given in Table 3.

Spasms were the most characteristic symptoms in the mice dying less than 1 hr. after feeding. More typical symptoms dominated by respiration paralysis occurred at the lower doses where some of the mice succumbed several hours after feeding.

The animals which were protected by injections of antitoxin solutions prior to feeding did not show any symptoms of botulism and survived.

DISCUSSION

Two striking observations were made in the feeding experiments with mink. First, quite large amounts of Clostridium botulinum type E toxin were required to kill mink, 2×10^5 MLD (i. p. mice) or approximately 200 MLD (i. p. mice) per g. For comparison it might be mentioned that the oral lethal dose of Clostridium botulinum type C toxin for mink was determined by *Tjaberg & Skulberg* (1968) to approximately 5×10^3 MLD (i. p. mice) or approximately 8 MLD (i. p. mice) per g.

These results might indicate that mink are approximately 25 times more susceptible to type C botulinal toxin than to type E by oral administration.

Secondly, and perhaps more surprising was the short time interval between feeding and the onset of symptoms and the death of the animals. It may be seen from Table 1 that all animals died very quickly, most of them even less than 1 hr. after feeding. Generally such a short time interval may be described as atypical with regard to botulism. With the exception of 1

animal (the one which survived longest) the symptoms were not typical, since spasms were predominant. Paralysis which is regarded as a typical symptom was observed only in 1 mink. It is well known that for type E there is a shorter period from administration of toxin (feeding or parenteral injection) to the onset of symptoms and death than is generally the case with other types of Clostridium botulinum. This is particularly the case when large amounts of toxin are given. These results might therefore indicate that mink are rather resistant to type E toxin given per os, but when very large doses are given the animals will succumb with atypical symptoms.

The feeding experiments in mice were performed mainly in order to find out the time interval between feeding and appearance of symptoms and deaths in this species. The results of these experiments indicate that large amounts of type E toxin given by mouth to mice cause a rapid death. The time interval is of the same order of magnitude as that observed in the feeding experiments in mink.

On the basis of the exceptionally short period between feeding of the toxin and the onset of the symptoms one might suspect that a second toxic component apart from Clostridium botulinum type E toxin might have been present in the culture supernatant, for instance toxic peptides or histamins. The fact that mice protected with Clostridium botulinum type E antitoxin survived oral application of corresponding amounts of toxin indicates, however, that the rapid deaths in mice are caused by type E toxin.

Similar experiments aiming at determining the relation between the oral and intraperitoneal lethal doses of Clostridium botulinum type E toxin in mice have been performed by *Licciardello et al.* (1967). They found that the oral lethal dose was 50—100 times greater than the intraperitoneal dose, and this corresponds fairly well with the results obtained in these experiments. In their publication, however, there is no reference to the time interval between administration of the toxin and the onset of symptoms and deaths of the mice.

The results obtained in experiments involving subcutaneous injections of Clostridium botulinum type E toxin into mink were much clearer. Similar to the results of the feeding experiments mink were less susceptible to type E toxin than to type C. In these experiments it was found that 2×10^3 MLD (i. p. mice) or

approximately 2 MLD (i. p. mice) per g of type E toxin were necessary to kill a mink by subcutaneous injection. Results from similar experiments with Clostridium botulinum type C toxin (*Tjaberg & Skulberg*) indicate that toxin quantities slightly above 1×10^2 MLD (i. p. mice) will kill a mink kit weighing 600 g. This corresponds to less than 0.2 MLD (i. p. mice) per g. These results indicate accordingly that type E toxin is approximately 10 times less toxic than type C when injected subcutaneously into mink. Later observations by *Loftsgård & Yndestad* (personal communication) indicate that the critical level of type C toxin by subcutaneous injections in mink is lower, about 0.025 MLD (i. p. mice). The time interval between subcutaneous injection of type E toxin and the onset of symptoms and deaths was as expected.

A comparison between the oral and subcutaneous lethal dose of Clostridium botulinum type E toxin in mink indicates on the basis of these results that the toxin is approximately 100 times more potent when injected subcutaneously than after being fed. A similar comparison for type C is difficult due to the discrepancy in the data on the susceptibility after subcutaneous injection. On the basis of the results mentioned above it might be indicated that type C toxin is 40—350 times more potent when injected subcutaneously into mink as compared to feeding.

A major question in this connection is the validity of the previous reports of type E outbreaks in mink. In the outbreak described by *Skulberg* (1961) only very minute amounts of toxin (5 MLD per g) could be demonstrated in the food. Compared to the large quantities of toxin required to kill a mink shown in these experiments it seems unlikely that type E could have caused the outbreak.

It is important to be aware of the difficulties involved in demonstrating and identifying botulinal toxins in samples of mink food which are suspected to be responsible for outbreaks of botulism. *Dinter & Kull* (1951) state that "the demonstration of toxin is not the rule but the exception". The possibility therefore clearly exists that in the outbreak described by *Skulberg* (1961) where Clostridium botulinum type E was regarded as responsible type C toxin might also have been present, but impossible to demonstrate. In fact this seems likely.

The effect of proteolytic enzymes on Clostridium botulinum toxins may have some relevance in this connection. It is well

known that precursors of types A, B and E toxins are activated by proteolytic enzymes. Later observations concerning the effects of proteolytic enzymes on Clostridium botulinum toxins (*Skulberg* 1964) indicate, however, that an inactivation of types A and B toxins may occur after prolonged treatment with proteolytic enzymes. Similar experiments with the other types of Clostridium botulinum toxins have not yet been performed, and definite conclusions as to the possibility of eventual inactivation of types C and E toxins in the mink food in question cannot be drawn. Substantial production of microbial proteinases is, however, likely to occur in mink food where a broad spectrum of microorganisms is generally present.

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SUMMARY

Experiments aiming at elucidation of the toxicity of Clostridium botulinum type E for mink are described. The observations indicate that amounts in the order of 2×10^5 intraperitoneal MLD (mice) or approximately 200 MLD per g of type E toxin will kill a mink after oral administration. The symptoms observed in the animals were atypical as there was an unusually short period between administration of the toxin and the onset of symptoms and deaths of the animals. Similar results were obtained when Clostridium botulinum type E toxin was fed to Swiss mice. When mice were protected by subcutaneous injections of type E antitoxin prior to feeding the animals survived without showing any symptoms.

Subcutaneous injection of type E toxin in amounts of the order of 2×10^3 intraperitoneal MLD (mice) killed mink, and typical symptoms of botulism were observed. This quantity corresponds to approximately 2 intraperitoneal MLD (mice) per g.

Comparison is made with previous observations obtained in similar experiments made with Clostridium botulinum type C toxin. It is shown that mink are substantially less susceptible to type E than to type C toxin when the toxins are given by mouth. On this basis previous results in reports on outbreaks of botulism in mink caused by Clostridium botulinum type E may be regarded as questionable.

ZUSAMMENFASSUNG

Die Empfänglichkeit von Nerzen gegenüber Botulinumtoxin Typ E.

Versuche, die vorgenommen wurden im Hinblick darauf die Toxizität von Clostridium botulinum Typ E gegenüber Nerzen zu beurteilen, werden beschrieben. Die Ergebnisse zeigen, dass Botulinumtoxin

Typ E in der Grössenordnung 2×10^5 MLD (bei intraperitonealer Injektion an Mäusen bestimmt) oder etwa 200 MLD pro g die Nerze töten wird, wenn das Toxin per os gegeben wird. Die Tiere starben unter Symptomen, die nicht ganz typisch für Botulismus waren, indem sie ungewöhnlich schnell nach Eingabe des Toxins eintraten (35—65 Minuten nach der Dosierung). Entsprechende Ergebnisse wurden erreicht, wenn Typ E Toxin peroral an Mäuse verabreicht wurde. Subkutane Injektion von Typ E Antitoxin beschützte die Tiere bei einer darauffolgenden peroralen Belastung mit Typ E Toxin.

Subkutane Injektion von Typ E Toxin in der Grössenordnung 2×10^3 (Mäuse) tötete die Nerze unter typischen Symptomen. Dieses entspricht einer Toxinmenge von etwa 2 MLD pro g.

Diese Ergebnisse wurden mit früheren Observationen von entsprechenden Versuchen mit Botulinumtoxin Typ C verglichen. Es zeigt sich, dass Nerze bedeutend weniger empfänglich gegenüber dem Typ E Toxin als gegenüber dem Typ C Toxin sind wenn das Toxin per os verabreicht wird. Unter Hinweis auf die grosse Toxinmenge vom Typ E Toxin, die erfordert wird um Nerze durch perorale Eingabe zu töten, werden frühere Rapporte über Fälle von Vergiftungen bei Nerzen vom Botulinumtoxin Typ E verursacht diskutiert.

SAMMENDRAG

Minkens mottagelighet for botulinumtoksin type E.

Det beskrives forsøk med henblikk på å belyse toksisiteten av *Clostridium botulinum* type E for mink. Resultatene viser, at botulinumtoksin type E i størrelsesorden 2×10^5 MLD (bestemt ved intraperitoneal injeksjon på mus) eller ca. 200 MLD pr. g vil drepe mink, når toksinet gis per os. Dyrene døde under symptomer, som ikke var helt typiske ved botulisme, idet symptomene satte inn usedvanlig hurtig etter at toksinet ble gitt (35—65 min. etter doseringen). Tilsvarende resultater fikk man, når type E toksin ble gitt peroralt til mus. Subcutan injeksjon av type E antitoksin beskyttet dyrene ved en følgende peroral belastning med type E toksin.

Subcutan injeksjon av type E toksin i størrelsesorden 2×10^3 MLD (mus) drepte mink under typiske symptomer. Dette tilsvarer en toksinmengde på ca. 2 MLD pr. g.

Disse resultater er sammenlignet med tidligere observasjoner fra tilsvarende forsøk med botulinumtoksin type C. Resultatene viser, at mink er betydelig mindre mottagelig for type E toksin enn type C når toksinet gis per os. På bakgrunn av den store toksinmengde av type E toksin, som skal til for å drepe mink ved peroral dosering, blir tidligere rapporter om forgiftningstilfeller hos mink forårsaket av botulinumtoksin type E diskutert.

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