ENTEROTROPIC PROPERTIES OF Bacillus cereus TOXIN

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The toxin was separated from a component possessing lecithinase activity by fractionation on a column with Biogel P-150. Toxin of Bacillus cereus, deprived of its lecithinase activity, caused death of noninbred albino mice and rabbits. By contrast with lecithinase, the lethal toxin of B. cereus gave rise to symptoms of food poisoning in experiments on cats.

An extensive literature has now accumulated on the pathogenicity of Bacillus cereus to man and animals. Particular attention must be paid to results indicating the role of B. cereus in gastro-intestinal ailments in man.

Many authorities have attempted to account for the toxic effects of B. cereus by the organism's ability to produce lecithinase C [3, 5, 6, 10, 15], although indirect evidence has been obtained that B. cereus also possesses a toxin [7, 9, 14, 16]. The absence of unanimity on this problem is largely due to the fact that for a long time it proved impossible to obtain lecithinase or toxin as homogeneous preparations and to demonstrate their individual or collective activity. The present writers first obtained an exotoxin of B. cereus and showed that it was not identical with lecithinase [2].

In the investigations described below the biological action of the toxin and of the lecithinase C of B. cereus obtained by fractionation on a Biogel column was studied.

EXPERIMENTAL METHOD

Lethal toxin of B. cereus was prepared by the method developed previously by the writers [4]. Protein was determined by Lowry's method [11]. Fractionation took place on a column with Biogel P-150 (Bio Rad) in 0.015 M NaCl solution, pH 7.0. Fractions were collected in volumes of 3.0-3.5 ml, using the KhKOV-1 chromatographic collector. The distribution curve was plotted from the absorptions at 280 nm. Absorption was determined on a model 137 U. V. Perkin-Elmer spectrophotometer. Lecithinase activity was determined by the lecitho-vitellin reaction [12] and expressed in L. V. units. Hemolytic activity was determined by hemolysis of sheep's erythrocytes [13]. Toxicity was studied by intravenous injection of the preparations into rabbits (weighing 2.5-3.5 kg), or noninbred albino mice (weighing 14-16 g) by the method of Haines et al. [8], and also by intracardiac injection of the preparations into guinea pigs (weighing 300-330 g). The enterotropic properties of the toxin and lecithinase were tested on cats (weighing 2.7-2.9 kg) by injecting the preparations into the femoral vein.

EXPERIMENTAL RESULTS

To isolate the toxin and lecithinase from the culture filtrates, the method of salting out with ammonium sulfate (75% saturation) was used. The toxin and lecithinase were separated by fractionation on a column with Biogel P-150. The material precipitated with ammonium sulfate was applied to the column in

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TABLE 1. Results of Tests of Toxin and Lecithinase from B. cereus on Animals

Animals	Toxin fraction			Lecithinase fraction			
	dose (in mg protein)	total No. of animals	number dying	dose (in mg protein)	dose (in L. V. units)	total No. of animals	number dying
Noninbred albino mice	0.35-0.5 (MLD)	20	20	5-10	256-512	20	0
Rabbits	3-4.8 per kg body weight (MLD)	2	2	25.8 per kg body weight	512 per kg body weight	1	0
Guinea pigs	1.3 per kg body weight	4	0	77.4 per kg body weight	1536 per kg body weight	3	0

TABLE 2. Results of tests of Lecithinase C and Toxin of B. Cereus on Cats

Substances tested	Dose (in mg protein/ kg body weight	Dose (in L.V. units/ kg body weight)	Number of animals	Time of beginning of reaction after injection of preparation (in min)	Type of reaction
Toxin preparation, batch 1	7.3	0	2	50-60	Violent vomiting, weakness
" 2	13.0	0	2	50-60	Vomiting, weakness
Lecithinase preparation, batch 1	7.43	1517	2	0	Slight apathy
" 2	15.0	2625	2	0	" "

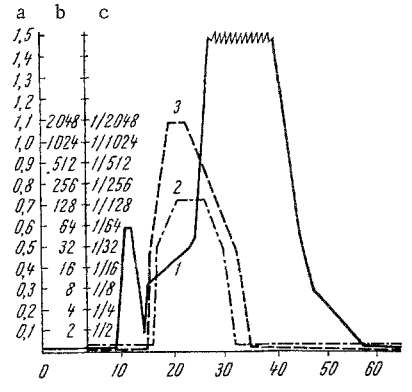

Fig. 1. Fractionation of B. cereus toxin on column with Biogel P-150: 1) optical density; 2) lecithinase activity; 3) hemolytic activity. Ordinate: a) optical density, b) lecithinase activity (in L.V. units/ ml), c) hemolytic activity (dilution of sample); abscissa, sample No.

a dose of 300 mg. By using 0.015 M NaCl solution, pH 7.0, as eluate, the toxin was separated from a component with lecithinase activity. Under these conditions of fractionation the distribution curve showed two peaks (Fig. 1). The fraction corresponding to the first peak had neither lecithinase nor hemolytic properties but always possessed marked toxic activity. The second fraction, which constituted part of the second peak, had no toxic activity, but exhibited marked lecithinase and hemolytic properties.

The homogeneity of the isolated B. cereus toxin was confirmed by the results of electrophoretic and serological analysis and also by ultracentrifugation [1].

The lethal toxin of B. cereus and the lecithinase C, separated on the biogel column, were tested for biological activity (Table 1).

As Table 1 shows, a lethal effect on mice and rabbits could be obtained only by the use of the toxin. All the animals survived after receiving very large doses of lecithinase. Guinea pigs were resistant to the dose of toxin tested, and also to the lecithinase. Mice died 5-20 min after injection of the toxin, and rabbits 4-20 h after its injection.

To study the enterotropic action of the lecithinase C and toxin of B. cereus they were tested on cats, which are a convenient model for the investigation of enterotoxins, such as those produced by staphylococci (Table 2).

The results in Table 2 show that after injection of the toxin into cats clearly defined symptoms of poisoning were observed. As a rule the reaction to injection of the preparation began 50-60 min after the injection and was accompanied by severe vomiting with enterotoxic manifestations, which continued for 2 h after the onset of the reactions. Although usually the experiment ended with recovery of the cats, in one experiment after injection of toxin of batch 1, one of the cats died 21 h after the injection. Death was preceded by all the symptoms described above.

After injection of lecithinase into cats in doses much greater than the dose of toxin when expressed as protein content, the animals showed weakness but no other symptoms of poisoning (Table 2).

The results of the tests on cats thus showed that the toxic features observed in food poisoning due to B. cereus are evidently due to the action of the toxin and not to the action of the lecithinase C.

After heating preparations of the toxin of B. cereus for 20 min at 60°C they lost both their lethal and their enterotropic properties. It can thus be concluded from these results that the enterotoxic effect is due to the action of a thermolabile toxin of B. cereus.

Despite the fact that many workers have expressed the view that lecithinase plays an important role in food poisoning of bacterial etiology, there is no general agreement on this matter [15, 17].

So far as B. cereus is concerned, the results of the present experiments on cats indicate that lecithinase does not cause symptoms of food poisoning. It must be noted that the dose of the lecithinase preparation injected was considerable (1500-2500 L. V. units/kg body weight of the cat).

Although no symptoms of food poisoning occurred after injection of lecithinase, the lethal toxin of B. cereus exhibited well-marked enterotropic properties. The reaction of the cat to the toxin was very violent and it occurred 50-60 min after injection of the preparation. On the basis of the results of this investigation an important role can be ascribed to the lethal thermolabile toxin in cases of food poisoning caused by B. cereus.

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