THE FATE OF EXOTOXIN FROM *Bacillus thuringiensis* IN MICE

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Exotoxin from *Bacillus thuringiensis* administered intraperitoneally to mice is rapidly excreted in urine in an unaltered form. In addition, it is dephosphorylated so that its concentration in the tissue decreases. The actual concentration of exotoxin in different mouse organs is discussed in connection with the inhibition of RNA biosynthesis.

Exotoxin produced by *Bacillus thuringiensis* is an effective inhibitor of DNA-dependent RNA polymerase both in microorganisms and in higher organisms. This is also the cause of its pronounced toxicity for mammals[1-4]. In view of the fact that the period of its toxicity depends on the rate of its excretion from the organism and on its enzymic dephosphorylation to a nontoxic product, the two processes were examined and the distribution of exotoxin in mouse organs was analyzed.

EXPERIMENTAL

Reagents and material. Labelled ^{32}P-exotoxin of specific radioactivity of 200 μCi/mg P (6·2 μCi/μmol exotoxin) was prepared biosynthetically[5]. Before application, the specific activity was adjusted to 4·0 μCi/ μmol. The experiments were done with white female mice, strain H (20 g), kept under standard conditions. The exotoxin was administered intraperitoneally (2·66 μCi/500 μg per animal) to groups of four mice. The excreted urine was collected in every instance in metabolic cages and, when killing the animal, on Whatman paper No 3, and then eluted with water. At suitable time intervals the mice were killed by decapitation, the excised organs were weighed and processed at 4°C.

Extraction. The organs were homogenized in a Potter-Elvehjem homogenizer with 0·2M-$HClO_4$ at 0°C. The suspensions were centrifuged for 15 min at 2000 g. The supernatants were neutralized with 7M-KOH, and, after removing the precipitate, the neutral supernatant was used for determination of the radioactivity and for separation by paper electrophoresis. The sediments after extraction with 0·2M-$HClO_4$ were further extracted twice with ethanol–ether (3 : 1) for 5 min at 70°C. After centrifugation, the supernatants were used for radioactivity assay and the sediments were extracted twice with 0·5M-$HClO_4$ (20 min at 70°C). The supernatants were used for the radioactivity assay. The volume of the extracts varied between 2 and 4 ml, depending on the weight of the extracted organ.

Paper electrophoresis was employed in the modification according to Markham[6] on Whatman No 3MM in 0·05M primary sodium citrate (pH 3·8) for 80 min at a potential gradient of 25 V/cm. Radioactivity was assayed in a gas-flow counter (Frieseke-Hoepfner). Following electrophoresis, radioactive spots were eluted and counted.

RESULTS

The study of exotoxin excretion was facilitated by the finding that the urine of experimental animals contains at all time intervals only exotoxin and a very small amount of inorganic phosphate. Excretion of urine-bound exotoxin sets in immediately after its administration and reaches maximum values after approximately 25 min (Fig. 1). The rapid drop of excretion is shown in Fig. 1, it results also from the analyses of urine collected when killing the animal at later periods after application of exotoxin. After 30 min the urine radioactivity was such that, at the time of killing, the urine of sacrificed animals contained as much radioactivity as had been excreted up to that moment during the whole period of exposure. On the other hand, after 120 min and 240 min exposures, the urine collected at the time of killing contained only 2-3% of total excreted radioactivity.

As to the distribution of exotoxin in the organism, the situation is complicated by its enzymic degradation, and by the reutilization of the inorganic phosphate. The extent of the two processes may be seen from the result of sequential extraction of the organs (Table I). The values obtained at different time intervals from the ethanol–ether extracts and from the extracts with hot 0·5M-$HClO_4$ indicate the extent of reutilization of exotoxin phosphate. They show that a steady state is reached in the organism already after 30 min, *i.e.* at a moment when the greater part of exotoxin has already been degraded. This finding is in agreement with the analysis of extracts with cold 0·2M-$HClO_4$ which, in addition to low-molecular products

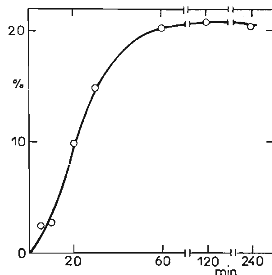

Fig. 1

Excretion of Urinary Exotoxin during Different Time Interval Following its Administration

The amount of exotoxin refers to the amount of exotoxin administered to mice at time zero, and is expressed as per cent. Min, time after the injection of exotoxin.

of reutilized phosphate, contain also undegraded exotoxin. Electrophoresis revealed a separate peak of exotoxin in the kidney only during a 30 min time interval following the administration, and in the liver only during a 5 min period after the injection (5 min after the administration of 500 μg exotoxin per mouse the kidney contained 18·2 μg and the liver 3·0 μg of the substance per g wet weight). At later time intervals the electrophoreograms did not show a separate peak of exotoxin and the values measured permitted to determine only the upper probable limit of exotoxin concentration in the individual organs (Table II).

TABLE I

Distribution of ^{32}P-Exotoxin and of Reutilized ^{32}P in Different Mouse Organs

For details see the Experimental.

Organ	Radioactivity of extracts, c.p.m./mg tissue		
	0·2M-$HClO_4$ (4°C)	ethanol–ether (70°C)	0·5M-$HClO_4$ (70°C)
	30 min after application		
Liver	16·00 ± 0·42	4·50 ± 0·17	3·23 ± 0·15
Spleen	18·25 ± 0·17	0·79 ± 0·05	3·96 ± 0·15
Intestine	11·15 ± 0·23	0·72 ± 0·05	0·88 ± 0·07
Heart	13·00 ± 0·31	0·73 ± 0·03	1·21 ± 0·07
Brain	1·04 ± 0·08	0·50 ± 0·06	0·35 ± 0·02
Kidney	70·10 ± 2·30	9·00 ± 0·40	4·24 ± 0·17
	75 min after application		
Liver	19·05 ± 0·39	7·11 ± 0·29	2·86 ± 0·12
Spleen	14·45 ± 0·27	2·13 ± 0·11	3·83 ± 0·15
Intestine	11·25 ± 0·38	1·69 ± 0·06	0·78 ± 0·05
Heart	8·59 ± 0·36	0·90 ± 0·04	0·92 ± 0·07
Brain	0·74 ± 0·09	0·19 ± 0·03	0·29 ± 0·01
Kidney	97·00 ± 3·20	29·54 ± 0·88	4·19 ± 0·28
	180 min after application		
Liver	21·55 ± 0·51	12·85 ± 0·39	2·34 ± 0·13
Spleen	18·85 ± 0·34	4·45 ± 0·27	5·73 ± 0·10
Intestine	12·85 ± 0·47	5·13 ± 0·22	2·54 ± 0·09
Heart	14·50 ± 0·39	1·87 ± 0·08	1·21 ± 0·06
Brain	0·90 ± 0·08	0·40 ± 0·03	0·56 ± 0·07
Kidney	83·75 ± 2·60	46·25 ± 1·12	4·62 ± 0·31

TABLE II

Upper Limit of Exotoxin Concentration in Mouse Organs in Relation to the Exposure Period

The results are given in μg exotoxin/g fresh weight of organ. Post-injection time is given in min.

Organ	Exotoxin administration	
	30	75
Liver	0·90	0·53
Spleen	0·88	0·35
Heart	0·42	0·13
Intestine	0·46	0·15
Kidney	3·64	1·24

DISCUSSION

The concentration of exotoxin in the organs under investigation drops rapidly due to its excretion and enzymic dephosphorylation to a nontoxic product. The results indicate that 20% of the applied exotoxin is excreted within 25–30 min after the administration. This apparently explains the high concentration of exotoxin and the presence of reutilization products of the radioactive phosphate in the kidneys. Our data indicate that enzymic dephosphorylation in the organism is very intense and that in the tissues it decreases the concentration of exotoxin more than its excretion. Thus 5 min after the administration of 500 μg/mouse the concentration of the inhibitor in the liver reaches the level of 3 μg/g tissue and only of 1 μg/g tissue after 30 min. On the assumption of an even distribution of exotoxin in the tissues these values correspond to those which are inhibitory for animal polymerases[4]. Consequently it is not difficult during a 30 min exposure *in vivo* to explain the decrease of RNA synthesis by the action of exotoxin. However, if one takes into account not only the drop of exotoxin concentration in the tissue following its administration but also the fact that the values represent the upper probable concentration limits, the prolonged inhibition of RNA synthesis in mouse liver[3] (at least 2 h; in rats 8 h; ref.[7]) is rather surprising. This phenomenon is even more complicated in view of the finding that the animal DNA-dependent RNA polymerase is inhibited by exotoxin in a competitive manner[4] as has also been described in bacteria[1,8].

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