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RESISTANCE OF GUINEA PIGS
IMMUNIZED WITH BOTULINUM TOXOIDS
TO AEROGENIC CHALLENGE WITH TOXIN

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ABSTRACT

Data are presented showing that active immunization of guinea pigs with botulinum toxoids afforded a high level of protection to challenge with botulinum toxins by the respiratory route. Resistance to challenge by this route was similar to resistance afforded to challenge by the parenteral and oral routes. The high survival percentages obtained in these studies made it impossible to establish any relationship between serum antitoxin titer and resistance to challenge.

Efficacy of botulinum antigens in the immunization of man and laboratory animals is based on development of serum antitoxin titers and the increase in resistance afforded laboratory animals to parenteral challenge. Minimum satisfactory levels of antitoxin have somewhat arbitrarily been selected as twice the standardization level or twice the lowest measurable titer. Guinea pigs immunized with univalent toxoids and whose sera contained approximately the satisfactory levels of antitoxin withstood intraperitoneal challenge with 10^6 to 10^8 LD₅₀ of homologous toxin.*

It has been established in our laboratory that inhalation of bacterial toxins in moderate doses can be lethal to a variety of laboratory animals. It was considered that respiratory challenge might provide a more severe test of the efficacy of toxoids than challenge by other routes. Support for this view was provided by Jakolev's finding that higher levels of antitoxin were required for prophylaxis against inhalation of botulinum toxin than against challenge by other routes. This report presents results of some of our studies to determine resistance afforded actively immunized guinea pigs to challenge with botulinum toxins by the respiratory route.

The toxins used in initial studies were produced from cultures grown in cellophane tubing immersed in nutrient medium. Cultures were clarified by centrifugation and used without further concentration or purification, since unaltered cell-free preparations were considered to be most suitable. Type E toxin was activated with trypsin prior to clarification of culture and subsequently stored in the frozen state. The type A toxins used in later studies were partially purified preparations stored in either the liquid or dried state.

* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Intraperitoneal and oral LD₅₀ estimates of toxins were determined in mice and guinea pigs. Respiratory LD₅₀ estimates were determined in guinea pigs exposed bodily to static aerosols of the toxins. More than 60 per cent of the particles in the aerosols were five microns or less in diameter. The guinea pig inhaled dose, in terms of MIPLD₅₀ units, was estimated by titration of the collecting fluid from impinger samplers. Serum antitoxin titers of the immunized animals were determined on sera obtained by cardiac bleeding of the animals the day before toxin challenge.

Before immunized guinea pigs were challenged the LD₅₀'s for normal guinea pigs were obtained. These estimates for the five toxins for normal guinea pigs challenged by the various routes, expressed in mouse IPLD₅₀, are shown in Table I. The animals were most susceptible by the IP route, as expected, and were more susceptible to toxin administered by the respiratory route than by the oral route.

Expression of LD₅₀ estimates in guinea pig intraperitoneal LD₅₀ suggests that normal guinea pigs show essentially similar susceptibility to the five toxins administered by the respiratory route. The respiratory susceptibility is more closely related to their oral susceptibility than to their intraperitoneal susceptibility with at least four of the five types.

In our first series of experiments, groups of guinea pigs were immunized by the subcutaneous route, each with a single injection of univalent toxoid. Each of the univalently immunized groups of guinea pigs was divided into three subgroups, which were challenged 36 to 40 days after immunization with homologous toxin by the oral, respiratory, and intraperitoneal routes respectively. Normal control groups were challenged in the same manner.

TABLE I. TOXICITY OF BOTULINUM TOXINS FOR THE GUINEA PIG BY VARIOUS ROUTES

Type	Mouse IPLD ₅₀		
	IP	Respiratory	Oral
A	5.2	141	717
B	4.2	350	306
C	1.6	87	177
D	4.1	186	436
E	34.3	778	178,000

The respiratory challenge results are summarized in Table II; the respiratory challenge doses are expressed in guinea pig respiratory LD₅₀. A high percentage of the actively immunized guinea pigs survived the respiratory challenge doses. Although not shown in this table, 70 to 100 per cent of the animals in the subgroups challenged by the intra-peritoneal and oral routes survived challenge of 10⁴ to 10⁵ LD₅₀ respectively.

Average antitoxin titers ranged from 10 to 50 times measurable levels, and although a considerable variation in antitoxin titers was obtained, the high percentage of survivors made it impossible to establish any relationship between serum antitoxin titers of individual animals and resistance to challenge.

Higher levels of challenge would be necessary to determine the magnitude of resistance to respiratory challenge. A second test in this series of experiments was designed to accomplish this.

Groups of guinea pigs were immunized with either univalent type D or pentavalent botulinum toxoids (combined typed A, B, C, D, and E) prior to challenge with varying doses of type D toxin by the respiratory route. The type D toxin was selected, of the toxin types available, because of its high potency in culture, enabling higher challenge doses. Immunization with pentavalent toxoid was included so that the effectiveness of an antigen in a multivalent preparation and a univalent antigen could be compared.

TABLE II. RESPONSE OF GUINEA PIGS TO CHALLENGE WITH BOTULINUM TOXINS

Toxin Type	Antitoxin Titer	Challenge Respiratory LD ₅₀	No. Survivors Total Exposed	Per Cent Survival
A	0.3 <0.04 - 0.96	5	40/44	91
B	0.059 <0.005 - 0.8	7	30/38	79
C	0.4 <0.02 - 1.9	8	38/44	86
D	6.1 <0.32 - 21	9	35/39	90
E	0.029 <0.0025 - 0.2	3	26/32	81

Two subgroups were employed for the pentavalent immunizations. The first pentavalent group received 1.0 milliliter of toxoid as an immunizing dose and was challenged 50 days after immunization. One-half milliliter immunizing dose was employed for the second pentavalent group and for the univalent group, and these groups were challenged 40 days after immunization. Antitoxin titers of pools of eight sera obtained the day before challenge were determined.

Subgroups were challenged with varying doses of type D toxin by the respiratory route. Smaller groups of guinea pigs immunized in the same manner served as controls for intraperitoneal and oral challenges. Normal guinea pigs were challenged in a similar fashion.

Table III shows that essentially all of the actively immunized guinea pigs survived 20 to 2000 respiratory LD₅₀. Although not shown in this table, 60 to 100 per cent of the animals in comparable immunization groups survived 10⁶ guinea pig LD₅₀ when challenged either by the intraperitoneal or by the oral route.

TABLE III. CHALLENGE OF IMMUNIZED GUINEA PIGS WITH TYPE D TOXIN BY THE RESPIRATORY ROUTE

Type Immunization and Volume Injected	Antitoxin Titer	Challenge Respiratory LD ₅₀	No. Survivors Total Exposed	Per Cent Survival
	3.8	22	40/40	100
Univalent 0.5 ml	3.2 - 5.1	162	40/40	100
		2100	40/40	100
	0.63	26	20/20	100
Pentavalent 0.5 ml	<0.16 - 1.9	277	20/20	100
		2090	16/20	80
	1.5	26	20/20	100
Pentavalent 1.0 ml	0.32 - 5.8	230	20/20	100
		1980	20/20	100
None	-	1	6/20	30

A third test in this series of experiments was designed to determine the resistance afforded pentavalently immunized guinea pigs to challenge with purified type A toxin by the respiratory route. Groups of guinea pigs were subcutaneously immunized with a 0.5-milliliter dose of pentavalent toxoid and challenged after 40 days by the respiratory route with purified type A toxin.

The results of the type A respiratory challenge are shown in Table IV. A high percentage of the actively immunized guinea pigs survived. A small percentage of the nonimmunized guinea pigs survived the same challenge doses. Fifty per cent of the animals in comparable immunized groups survived intraperitoneal challenge doses of 10^5 LD₅₀ and 100 per cent survived 10^6 oral LD₅₀.

A fourth test in this series of experiments was designed to determine the resistance afforded pentavalently immunized guinea pigs to challenge with type A toxin at various times after immunization. Guinea pigs were immunized with a 0.5-milliliter dose of pentavalent toxoid and challenged after 15, 30, and 45 days by the respiratory route with dried type A toxin. Table V shows that at the three immunization periods tested, essentially all the immunized animals survived the challenge doses obtained.

To achieve even higher challenge doses, additional experiments were conducted with dynamic aerosols generated in the Henderson apparatus. Approximately 90 per cent of pentavalently immunized guinea pigs survived 10^3 to 10^4 LD₅₀ of type A toxin administered by the respiratory route 40 days after immunization.

With type D toxin, approximately 75 per cent of similarly immunized guinea pigs survived 10^3 LD₅₀, and 50 per cent survived 10^4 LD₅₀. Similar results were obtained with types B, C, and E.

TABLE IV. CHALLENGE OF GUINEA PIGS WITH TYPE A TOXIN

Challenge Respiratory LD ₅₀	Immunized ^{a/}		Nonimmunized
	No. Survivors Total Exposed	Per Cent Survival	No. Survivors Total Exposed
50 - 150	71/72	99	0/24
10 - 30	69/72	96	2/24
1 - 3	72/72	100	3/24

a. Antitoxin Titer
Average 0.16
Range <0.02 to 0.96

TABLE V. CHALLENGE OF IMMUNIZED GUINEA PIGS WITH TYPE A TOXIN BY THE RESPIRATORY ROUTE

Challenge Respiratory LD ₅₀	No. Survivors Total Exposed		
	15 Days ^{a/}	30 Days ^{a/}	45 Days ^{a/}
1	30/30	30/30	30/30
12	30/30	30/30	30/30
60	29/30	30/30	30/30

a. Days after immunization.