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Technical Report

CRDLR 3330

Duration of Passive Immunity to Botulinum Toxin Offered by Antitoxin in Mice and Rabbits

by

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James W. Crook
Fred W. Oberst

November 1965

EDGEWOOD ARSENAL, MARYLAND 21010

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FOREWORD

The work described in this report was authorized under Project 1C622401A097, Medical Defense Aspects of Chemical Agents (U). The experimental data are contained in notebook MN-1721. This work was started in September 1963 and completed in December 1963.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Acknowledgments

The authors are grateful to Frank Vocci, Leo Feinsilver, Willie Mae Lawson, and Ronald K. Biskup for supplying solutions of botulinum toxin with analytical data on their potency.

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Disposition

When this report has served its purpose, DESTROY it.

DIGEST

Mice and rabbits were given varying doses of bivalent botulinum antitoxin, types A and B, by the intraperitoneal and intravenous routes. The persistence of passive immunity in these animals was determined by challenging them with type A toxin at various times. Resulting mortalities from either constant or graded doses served as the basis for determining the extent of passive immunity, which was estimated by utilizing the concept of the median lethal ratio (LR50); i.e., toxin dose:antitoxin dose administered. The decrease in the LR50 with time reflected the lowered activity of the antitoxin in animals and hence a fall in passive immunity.

Passive immunity conferred by botulinum antitoxin, type A, declines steadily in mice and rabbits with time. If the dose of antitoxin is sufficiently large, however, some passive immunity in mice will persist for at least 49 days.

CONTENTS

DURATION OF PASSIVE IMMUNITY TO BOTULINUM TOXIN OFFERED BY ANTITOXIN IN MICE AND RABBITS

I. INTRODUCTION.

Food poisoning in humans from the ingestion of botulinum toxin is caused by the slow absorption of the material into the bloodstream via the gastrointestinal tract. The recommended treatment includes repeated massive doses of specific antitoxin. The required number of doses and dose levels, as well as the duration and extent of passive immunity following neutralization of the toxin in the bloodstream, have not been clearly established. Presumably, in the treatment of the disease, the antitoxin titer dose is sufficiently large to neutralize the absorbed free toxin. The capacity of the antitoxin to inactivate the residual associated or unabsorbed toxin is in part the basis for repetitive dosing with antitoxin in the management of the disease. This capacity of the antitoxin to sustain its neutralization effectiveness after a single and excessive dose is the bas s of the study reported herein. The experiments were designed to determine the duration and extent of passive immunity to Clostridium botulinum toxin, type A, in animals following administration of a single dose of antitoxin. Knowledge of this effectiveness should be useful in evaluating the requirements for repeated doses in the time course and treatment of the disease.

II. MATERIALS.

A. Animals.

Mice, weighing 25 gm, and rabbits, weighing approximately 2 kg, were used as the test animals.

B. Agent.

The botulinum toxin, type A, was similar to that described by Lamanna, McElroy, and Eklund* for a partially purified toxin having a potency of approximately 3×10^{-4} µg/MU. (MU = mouse intraperitoneal LD50 for mice weighing 25 gm.) The stock solution was prepared by personnel of the Basic Toxicology Branch from the powdered material dissolved in a sterile gelatin-phosphate buffer solution (10 gm Na_2HPO_4 and 2 gm Difco gelatin in 1 ℓ distilled water); pH was adjusted to 6.8 by the addition of concentrated

* Lamanna, C., McElroy, O. E., and Eklund, H. W. The Purification and Crystallization of Clostridium Botulinum Type A Toxin. Science 103, 613-614 (1946).

HCl. All stock solutions were bio-assayed in mice for potency before they were used in these studies. The stock solutions were diluted with saline immediately prior to use, so that the injected volumes were usually 0.5 ml in the mice and 1 ml in the rabbits.

C. Antitoxin.

Bivalent botulinum antitoxin (equine origin), globulin-modified, 500 units/ml each of types A and B (Lederle), was used for the antitoxin.

III. PROCEDURES.

A. Test A. Mice Given 500 MU of Toxin at Various Times Following Administration of Graded Doses of Antitoxin.

Doses of antitoxin in saline, ranging from 0.063 to 200 units of antitoxin (u), were administered to groups of 9 or 10 mice by the intraperitoneal route. At subsequent times (6 min, 24 hr, 7 days, and 14 days), the animals were challenged with a constant dose of 500 MU of toxin by the same route. The group size for each time of challenge varied from 40 to 128 mice. Based upon the resulting mortalities, the LR50 was calculated. In all tests performed, the SD50 was also estimated and is defined as the median antitoxin dose that results in 50% survival after challenge with the toxin. These parameters were calculated for both species tested.

To establish an unequivocally effective antitoxin dose, premixtures of the toxin and antitoxin were prepared in solutions with ratios ranging from 10,000 to 80,000 MU:1 u. (The dose of toxin was fixed at 250 or 500 MU, and the dose of antitoxin ranged from 0.00313 to 0.025 u as necessary to yield the desired ratio.) After standing for 10 min, the solutions were injected intraperitoneally into mice, and the LR50 was estimated from mortality.

B. Test B. Rabbits Given 500 MU of Toxin at Various Times Following Administration of Graded Doses of Antitoxin.

Similar tests were run on groups of two to four rabbits at 10 min, 24 hr, and 14 days after intravenous administration of antitoxin. For each time of challenge, the total number of animals employed was smaller than in the mouse experiments. The antitoxin dose ranged from 0.063 to 16.7 u; the toxin dose was constant at 500 MU/rabbit, administered intravenously.

Similarly, the premixture test was run on this species, with solutions containing the toxin and antitoxin in ratios of 10,000:1 to 40,000:1, to establish an efficacious antitoxin dose.

C. Tests C to L. Mice and Rabbits Given Varying Doses of Toxin Following Administration of Graded Doses of Antitoxin.

These tests differed from the preceding two (A and B) in that the toxin dose was varied following the administration of graded doses of antitoxin by either the intraperitoneal or intravenous route. . Mice and rabbits were challenged from 7 to 49 days, also by either route. In effect, these tests, along with the premixture tests, served the useful purpose of range-finding for an effective antitoxin dose for the time-course study.

IV. RESULTS.

A. Test A (Mice) and Test B (Rabbits) at a Constant Dose Level of Toxin.

Table A1 (all tables, A1 through A7, are in appendix A) shows the doses of toxin and antitoxin, their corresponding ratios, and the resultant mortalities in groups of mice challenged with 500 MU of toxin at various times after antitoxin administration. Table A2 presents similar data for rabbits. The LR50's are listed in tables A3 and A4 for the mice and rabbits, respectively, and are shown graphically in figure B1 (all figures, B1 through B4, are in appendix B). Premixtures of toxin and antitoxin injected intraperitoneally into mice yielded 50% mortality when the ratio was 35,700 MU:1 u. The LR50's for the 6-min and 14-day challenge times were 6,670:1 and 147:1, respectively. The SD50 was calculated directly from the LR50. These figures are included in tables A3 and A4. and are plotted in figure B2.

An examination of figure B1 reveals that, by the routes of administration used, the degree of passive immunity at any particular time is very similar in mice and rabbits. A mathematical model of the frequency distribution of mortality in mice and rabbits given antitoxin and challenged at various times with 500 MU of toxin is given in figure B3.

B. Tests C to J (Mice) and Tests K and L (Rabbits) at Various Doses of Toxin.

Tables A5 and A6 show the graded doses of toxin and antitoxin, their corresponding ratios, and subsequent mortalities for mice and rabbits challenged at various times. The LR50's are listed in table A7 and are shown graphically in figure B4. The SD50 is also included in table A7.

The results of mouse tests C and D, plotted in figure B4, indicate that a lower protection level is afforded by a small dose of antitoxin against a correspondingly small dose of toxin than is afforded by much larger doses of antitoxin against large doses of toxin. In other words, the 14-day challenge in mice receiving 500 MU of toxin after 3.40 u of antitoxin (table A3) would result in an LR50 of 147:1, whereas mice that receive 12.5 MU of toxin after 0.69 u of antitoxin (table A7) would have an LR50 of 18:1. For mouse tests E, G, and H, the challenge time was 46 to 49 days after administration of the antitoxin, which was given at median levels of 25, 88, and 75 u for the three tests. The survival of some mice at LR50's of 7.8:1, 2.4:1, and 2.4:1, respectively, indicated a very significant protection against toxin at these times.

V. DISCUSSION.

Two alternative experimental methods were considered for determining the change with time in passive immunity conferred by antitoxin. In the first method, a constant dose of antitoxin could have been followed at selected intervals of time with serial doses of toxin to determine which dose would kill 50% of the animals. The amount of toxin required would be a measure of the amount of available antitoxin remaining in the blood at the challenge time, or the amount of immunity.

The second method would be to follow a wide range of antitoxin doses with a constant dose of toxin at various times to determine which antitoxin dose was still present in sufficient amount to protect 50% of the animals. Most of the tests reported here were done by the second method, with the challenge dose of toxin a constant 500 MU in both mice and rabbits.

The following table is a mathematical model of the change in passive immunity in mice and rabbits challenged with a constant dose of 500 MU of toxin.

Passive immunity in mice and rabbits	Time (t) of toxin challenge after antitoxin	Dose of antitoxin	Ratio of toxin dose to antitoxin dose that yields 50% mortality
% of initial value*	days	u	MU:u
100	8 (min)	0.079	6,335:1
50	1.2	0.158	3,168:1
25	2.4	0.316	1,584:1
10	4.5	0.79	634:1
5	6.6	1.58	317:1
2.5	14	3.16	158:1

The present authors assume that the binding capacity of toxin to antitoxin is constant and that the fall in passive immunity with time reflects the fall in the amount of antitoxin in the bloodstream. Relatively high doses of antitoxin (SD50 = 3.4 u) yield greater protection at 14 days as measured by the LR50 (LR50 = 147:1 for a challenge of 500 MU) than do lower doses of toxin (SD50 = 0.69 u; LR50 = 15.8 for a challenge of 12.5 MU). This indicates that the degree of passive immunity remaining is dependent not only on the elapsed time but also on the absolute amount of antitoxin initially injected. In mixtures (table A1), half the dose of antitoxin does not protect against half the dose of toxin as well as twice the dose of antitoxin protects against twice the dose of toxin. Apparently, larger percentages of large doses of antitoxin persist longer in the blood. The elapsed time, however, is by far the larger factor.

The results of this work indicate that passive immunity to botulinum toxin can persist in mice and rabbits for a prolonged period of time. In test D, for example, a dose of 3.3 u of antitoxin offered some protection to mice (7/10 survived) against 5 MU of toxin given 21 days later. When the challenge was administered as much as 49 days later (tests G and H), there were still some survivors. This is a much longer time than passive immunity against other diseases usually persists. In general, the effectiveness of the antitoxin in protecting against the toxin steadily declines with time.

VI. CONCLUSIONS.

Passive immunity conferred by botulinum antitoxin, type A, declines steadily in mice and rabbits with time. If the dose of antitoxin is sufficiently large, however, some passive immunity in mice will persist for at least 49 days.

* Percent of initial value = 100 ($LR50_t / LR50_{8\ min}$).

APPENDIXES

APPENDIX A

TABLES

TABLE A1

TEST A: MORTALITY IN MICE AFTER CHALLENGE WITH 500 MU* OF TOXIN AT VARIOUS TIMES AFTER ANTITOXIN ADMINISTRATION

(Both antitoxin and toxin given intraperitoneally)

Dose of antitoxin	Dose of toxin	Ratio of toxin dose to antitoxin dose	Occurrence of toxic signs (fraction)	Mortality fraction	Number of deaths	
					1st day	2nd day
u	MU	MU:u				
A. Antitoxin and Toxin Administered Simultaneously as a Mixture						
0.025	250*	10,000:1	0/10	0/10	0	0
0.025	500	20,000:1	0/9	0/9	0	0
0.0125	250*	20,000:1	3/10	0/10	0	0
0.020	500	25,000:1	0/8	0/8	0	0
0.0083	250*	30,000:1	10/10	6/10	6	0
0.0166	500	30,000:1	0/9	0/9	0	0
0.0143	500	35,000:1	0/9	0/9	0	0
0.00625	250*	40,000:1	10/10	10/10	10	0
0.00313	250*	80,000:1	10/10	10/10	10	0
B. Toxin Challenge 6 Min After Antitoxin						
0.167	500	3,000:1	0/10	0/10	0	0
0.125	500	4,000:1	10/10	2/10	2	0
0.100	500	5,000:1	10/10	2/10	1	1
0.083	500	6,000:1	10/10	4/10	3	1
C. Toxin Challenge 24 Hr After Antitoxin						
0.56	500	900:1	10/10	1/10	1	0
0.38	500	1,333:1	10/10	0/10	0	0
0.25	500	2,000:1	10/10	1/10	1	0
0.17	500	3,000:1	10/10	3/10	2	1
0.125	500	4,000:1	10/10	6/10	6	0
0.100	500	5,000:1	10/10	7/10	5	2
0.063	500	8,000:1	10/10	7/10	7	0
D. Toxin Challenge 7 Days After Antitoxin						
4.17	500	120:1	10/10	0/10	0	0
2.50	500	200:1	10/10	4/10	4	0
1.43	500	350:1	10/10	6/10	6	0
1.00	500	500:1	10/10	9/10	9	0
0.25	250*	1,000:1	10/10	10/10	9	1
0.25	500	2,000:1	10/10	10/10	10	0
0.17	500	3,000:1	10/10	10/10	10	0
E. Toxin Challenge 14 Days After Antitoxin						
200	500	2.5:1	0/10	0/10	0	0
100	500	5:1	1/9	1/9	1	0
50	500	10:1	0/10	0/10	0	0
50	500	10:1	9/9	2/9	2	0
25	500	20:1	0/10	0/10	0	0
25	500	20:1	10/10	1/10	1	0
12.5	500	40:1	1/10	0/10	0	0
12.5	500	40:1	10/10	4/10	4	0
6.25	500	80:1	10/10	2/10	2	0
6.25	500	80:1	3/10	3/10	3	0
3.85	500	130:1	10/10	3/10	2	1
2.50	500	200:1	10/10	8/10	7	1
1.56	500	320:1	10/10	8/10	8	0

* In several range-finding tests included in this table, the challenge dose of toxin was 250 MU.

TABLE A2

TEST B: MORTALITY IN RABBITS AFTER CHALLENGE WITH 500 MU OF TOXIN AT VARIOUS TIMES AFTER ANTITOXIN ADMINISTRATION

(Both antitoxin and toxin given intravenously)

Dose of antitoxin	Dose of toxin	Ratio of toxin dose to antitoxin dose	Occurrence of toxic signs (fraction)	Mortality fraction	Number of deaths					
					1st day	2nd day	3rd day	4th day	5th day	6th day
u	MU	MU:u								
A. Antitoxin and Toxin Administered Simultaneously as a Mixture										
0.05	500	10,000:1	0/4	0/4	0	0	0	0	0	0
0.025	500	20,000:1	3/4	3/4	0	0	0	0	0	3
0.0125	500	40,000:1	4/4	4/4	0	1	3	0	0	0
B. Toxin Challenge 10 Min After Antitoxin										
0.125	500	4,000:1	0/2	0/2	0	0	0	0	0	0
0.083	500	6,000:1	1/2	1/2 a/	0	0	0	0	0	0
0.125	1,000 b/	8,000:1	2/2	2/2	0	0	0	0	1	1
C. Toxin Challenge 24 Hr After Antitoxin										
0.250	500	2,000:1	0/4	0/4	0	0	0	0	0	0
0.125	500	4,000:1	3/3	3/3 c/	0	1	0	0	0	1
0.063	500	8,000:1	4/4	4/4	0	2	2	0	0	0
D. Toxin Challenge 14 Days After Antitoxin										
16.7	500	30:1	1/2	1/2	0	0	0	0	1	0
8.33	500	60:1	0/4	0/4	0	0	0	0	0	0
4.35	500	115:1	2/4	2/4	1	1	0	0	0	0
4.17	500	120:1	0/4	0/4	0	0	0	0	0	0
2.17	500	230:1	3/4	3/4	2	1	0	0	0	0
1.09	500	460:1	4/4	4/4	0	4	0	0	0	0

a/ One death on 14th day.

b/ In a range-finding test included in this table, the challenge dose was 1,000 MU of toxin.

c/ One death on 9th day.

TABLE A3

TEST A: RATIOS OF TOXIN DOSE TO ANTITOXIN DOSE THAT ARE LETHAL TO 50% OF THE MICE (LR50 VALUES) AT VARIOUS CHALLENGE TIMES

(Both antitoxin and toxin given intraperitoneally)

LR50 (19/20 confidence limits)	Bliss slope (b)	SD50* (calculated from LR50)	Number of mice	Median dose of antitoxin (range)	Dose of toxin
MU:u		u		u	MU
A. Antitoxin and Toxin Administered Simultaneously as a Mixture					
35,700:1 (31,800 - 40,200)	9.81745	0.014	85	0.0143 (0.00313 - 0.025)	200 - 500
B. Toxin Challenge 6 Min After Antitoxin					
6,670:1 (568 - 78,200)	5.39369	0.075	40	0.113 (0.083 - 0.167)	500
C. Toxin Challenge 24 Hr After Antitoxin					
4,173:1 (3,045 - 5,718)	2.81093	0.120	70	0.17 (0.063 - 0.56)	500
D. Toxin Challenge 7 Days After Antitoxin					
273:1 (213 - 350)	4.69336	1.83	70	1.00 (0.17 - 4.17)	250 - 500
E. Toxin Challenge 14 Days After Antitoxin					
147:1 (83 - 259)	1.48477	3.40	128	12.5 (1.56 - 200)	500

* SD50 = the prophylactic antitoxin dose that will result in 50% survival after challenge with toxin.

TABLE A4

TEST B: RATIOS OF TOXIN DOSE TO ANTITOXIN DOSE THAT ARE LETHAL TO 50% OF THE RABBITS (LR50 VALUES) AT VARIOUS CHALLENGE TIMES

(Both antitoxin and toxin given intravenously)

LR50 (19/20 confidence limits)	Litchfield slope function (S)	SD50* (calculated from LR50)	Number of rabbits	Median dose of antitoxin (range)	Dose of toxin
MU:u		u		u	MU
A. Antitoxin and Toxin Administered Simultaneously as a Mixture					
17,000:1 (8,850 - 32,600)	1.60	0.029	12	0.025 (0.0125 - 0.05)	500
B. Toxin Challenge 10 Min After Antitoxin					
6,000:1 (3,370 - 10,700)	1.66	0.083	6	0.125 (0.083 - 0.125)	500 - 1,000
C. Toxin Challenge 24 Hr After Antitoxin					
2,960:1 (no limits)	1.39	0.169	11	0.125 (0.063 - 0.250)	500
D. Toxin Challenge 14 Days After Antitoxin					
165:1 (110 - 248)	1.68	3.03	22	4.26 (1.09 - 16.7)	500

* SD50 = the prophylactic antitoxin dose that will result in 50% survival after challenge with toxin.

TABLE A5

RANGE-FINDING TESTS IN MICE: MORTALITY AFTER CHALLENGE WITH TOXIN AT VARIOUS TIMES AFTER ANTITOXIN ADMINISTRATION

Test	Time of toxin challenge after antitoxin	Dose of antitoxin	Antitoxin route	Dose of toxin	Toxin route	Ratio of toxin dose to anti-toxin dose	Occurrence of toxic signs (fraction)	Mortality fraction	Number of deaths	
									1st day	2nd day
	days	u		MU		MU:u				
C	14	1.25	Ip*	6.25	Ip	5:1	10/10	1/10	0	1
		2.50	Ip	12.5	Ip	5:1	8/10	7/10	6	1
		1.25	Ip	12.5	Ip	10:1	10/10	2/10	2	0
		1.25	Ip	12.5	Ip	10:1	6/10	5/10	4	1
		0.625	Ip	12.5	Ip	20:1	3/10	0/10	0	0
		1.25	Ip	25	Ip	20:1	9/9	3/9	3	0
		1.25	Ip	50	Ip	40:1	10/10	8/10	6	2
D	21	3.33	Ip	5.0	Ip	1.5:1	6/10	3/10	2	1
		1.67	Ip	7.5	Ip	4.5:1	10/10	6/10	4	2
		0.83	Ip	11.2	Ip	13.5:1	10/10	8/10	7	1
E	46	25	Ip	86	Ip	3.4:1	—	2/6	2	0
F	14	50	Iv**	500	Ip	10:1	0/10	0/10	0	0
		25	Iv	500	Ip	20:1	0/10	0/10	0	0
		12.5	Iv	500	Ip	40:1	0/10	0/10	0	0
		6.25	Iv	500	Ip	80:1	3/10	0/10	0	0
G	46 - 49	125	Iv	86	Ip	0.7:1	—	0/2	0	0
		50	Iv	86	Ip	1.7:1	—	4/10	4	0
H	46 - 49	250	Iv	346	Ip	1.4:1	—	3/5	3	0
		125	Iv	346	Ip	2.8:1	—	1/3	1	0
		25	Iv	346	Ip	14:1	—	5/6	3	2
		12.5	Iv	346	Ip	28:1	6/6	6/6	5	1
J	47	250	Iv	80	Iv	0.32:1	0/1	0/1	0	0
		125	Iv	80	Iv	0.64:1	0/1	0/1	0	0

* Ip = intraperitoneal.

** Iv = intravenous.

TABLE A6

RANGE-FINDING TESTS IN RABBITS: MORTALITY AFTER CHALLENGE WITH TOXIN AT VARIOUS TIMES AFTER ANTITOXIN ADMINISTRATION

Test	Time of toxin challenge after antitoxin	Dose of antitoxin	Antitoxin route	Dose of toxin	Toxin route	Ratio of toxin dose to antitoxin dose	Occurrence of toxic signs (fraction)	Mortality fraction	Number of deaths					
									1st day	2nd day	3rd day	4th day	5th day	6th day
	days	u		MU		MU:u								
K	14	4.35	Ip*	500	Iv**	115:1	4/4	4/4	4	0	0	0	0	0
		2.17	Ip	500	Iv	230:1	4/4	4/4	0	4	0	0	0	0
		1.09	Ip	500	Iv	460:1	4/4	4/4	1	2	1	0	0	0
L	7	0.50	Iv	150	Iv	300:1	1/2	1/2	0	0	0	0	0	1
		0.17	Iv	100	Iv	600:1	2/2	0/2	0	0	0	0	0	0
		0.10	Iv	120	Iv	1,200:1	2/2	2/2	0	1	0	1	0	0

* Ip = intraperitoneal.

** Iv = intravenous.

TABLE A7

RANGE-FINDING TESTS IN MICE AND RABBITS: RATIOS OF TOXIN DOSE TO ANTITOXIN DOSE THAT ARE LETHAL TO 50% OF THE ANIMALS (LR50 VALUES) AT VARIOUS CHALLENGE TIMES

Test	Time of toxin challenge after antitoxin	LR50 (19/20 confidence limits)	Bliss slope (b)	SD50 a/ (calculated from LR50)	Number of animals	Antitoxin		Toxin	
						Dose median (range)	Route	Dose median (range)	Route
	days	MU:u		u		u		MU	
				A. Mice					
C	14	18:1 (11 - 31)	1.89111	0.69	69	1.25 (0.625 - 2.50)	Ip b/	12.5 (6.25 - 50)	Ip
D	21	3.3:1 (1.1 - 9.8)	1.43784	2.27	30	1.67 (0.83 - 3.33)	Ip	7.5 (5.0 - 11.2)	Ip
E	46	Est 7.8:1 (no limits)	–	11.0	6	25	Ip	86	Ip
F	14	>80:1 (no limits)	–	<6.25	40	18.8 (6.25 - 50)	Iv c/	500	Ip
G	46 - 49	2.4:1 (1.1 - 5.3)	1.58691	35.8	12	88 (50 - 125)	Iv	86	Ip
H	46 - 49	2.4:1 (1.1 - 5.3)	1.58691	144.2	20	75 (12.5 - 250)	Iv	346	Ip
J	47	>0.64:1 (no limits)	–	<125	2	188 (125 - 250)	Iv	80	Iv
				B. Rabbits					
K	14	<115:1 (no limits)	–	>4.3	12	2.17 (1.09 - 4.35)	Ip	500	Iv
L	7	600:1 (no limits)	–	0.200	6	0.17 (0.10 - 0.50)	Iv	120 (100 - 150)	Iv

a/ SD50 = the prophylactic antitoxin dose that will result in 50% survival after challenge with toxin.

b/ Ip = intraperitoneal.

c/ Iv = intravenous.

APPENDIX B

FIGURES

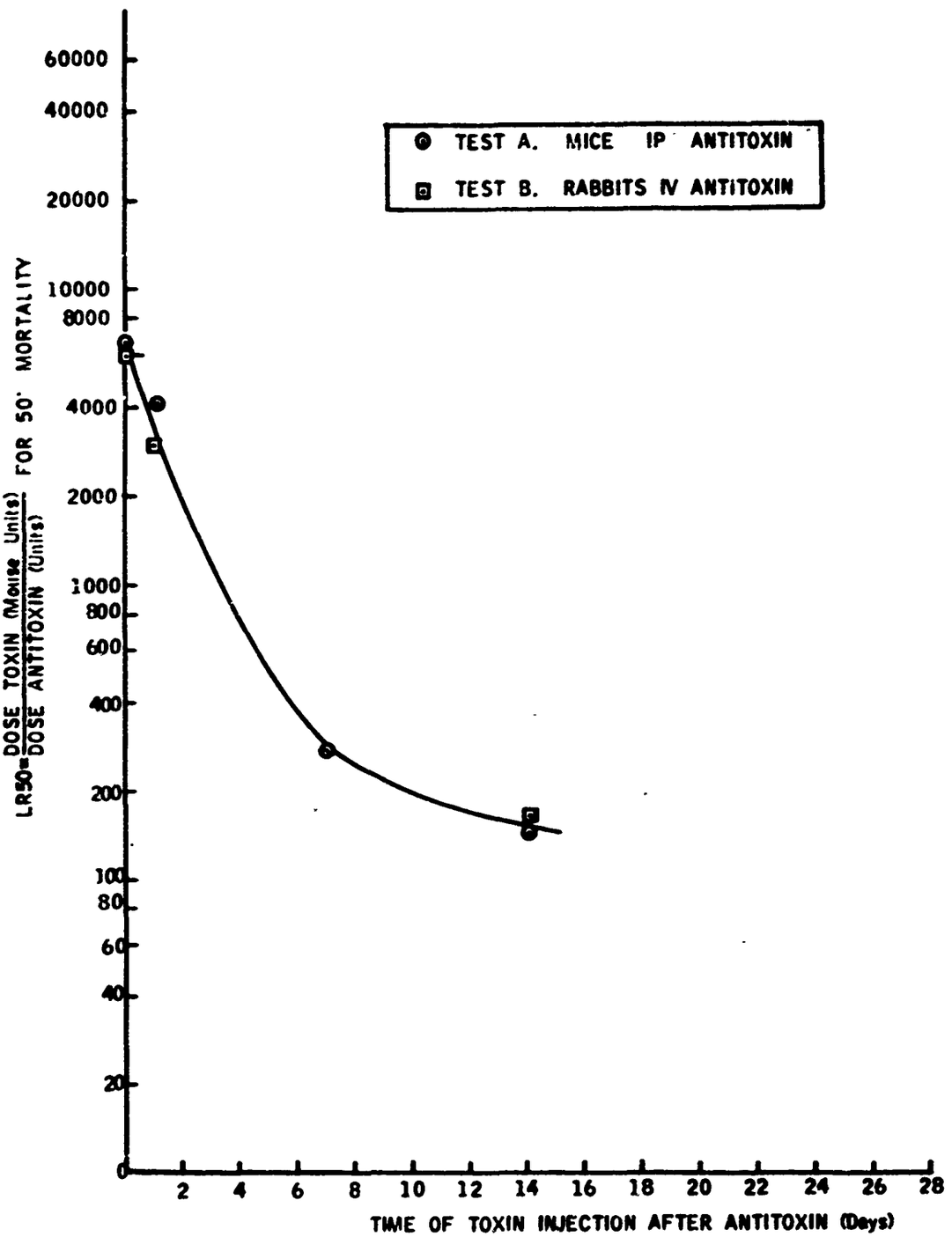


FIGURE B1

MEDIAN LETHAL RATIOS (LR50'S) OF TOXIN TO ANTITOXIN AT VARIOUS TIMES AFTER ANTITOXIN ADMINISTRATION

(Challenge dose of toxin is equal to 500 MU per animal)

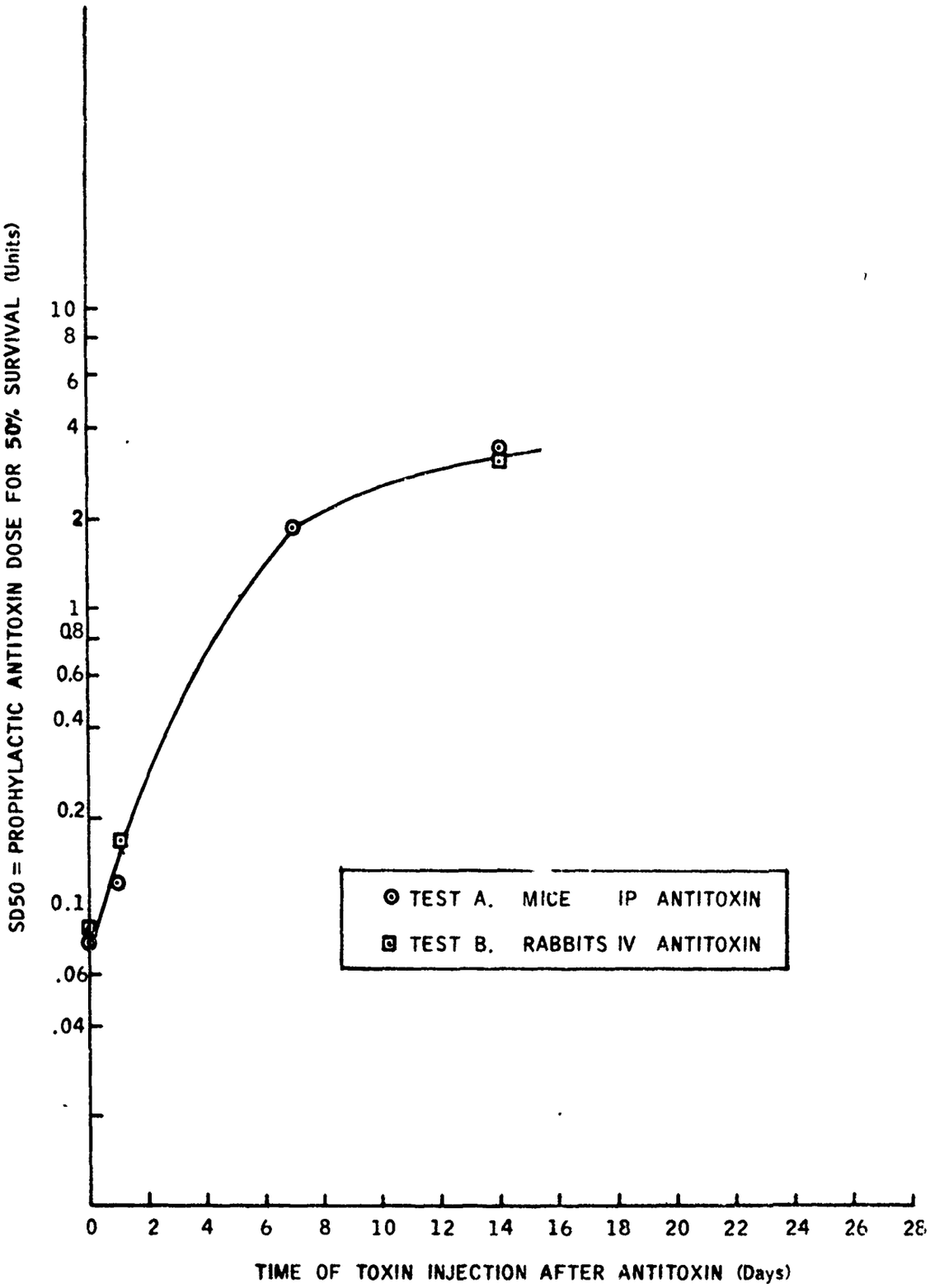


FIGURE B2

DOSES OF ANTITOXIN REQUIRED FOR 50% SURVIVAL (SD50'S) IN ANIMALS CHALLENGED WITH 500 MU OF TOXIN AT VARIOUS TIMES AFTER ANTITOXIN ADMINISTRATION

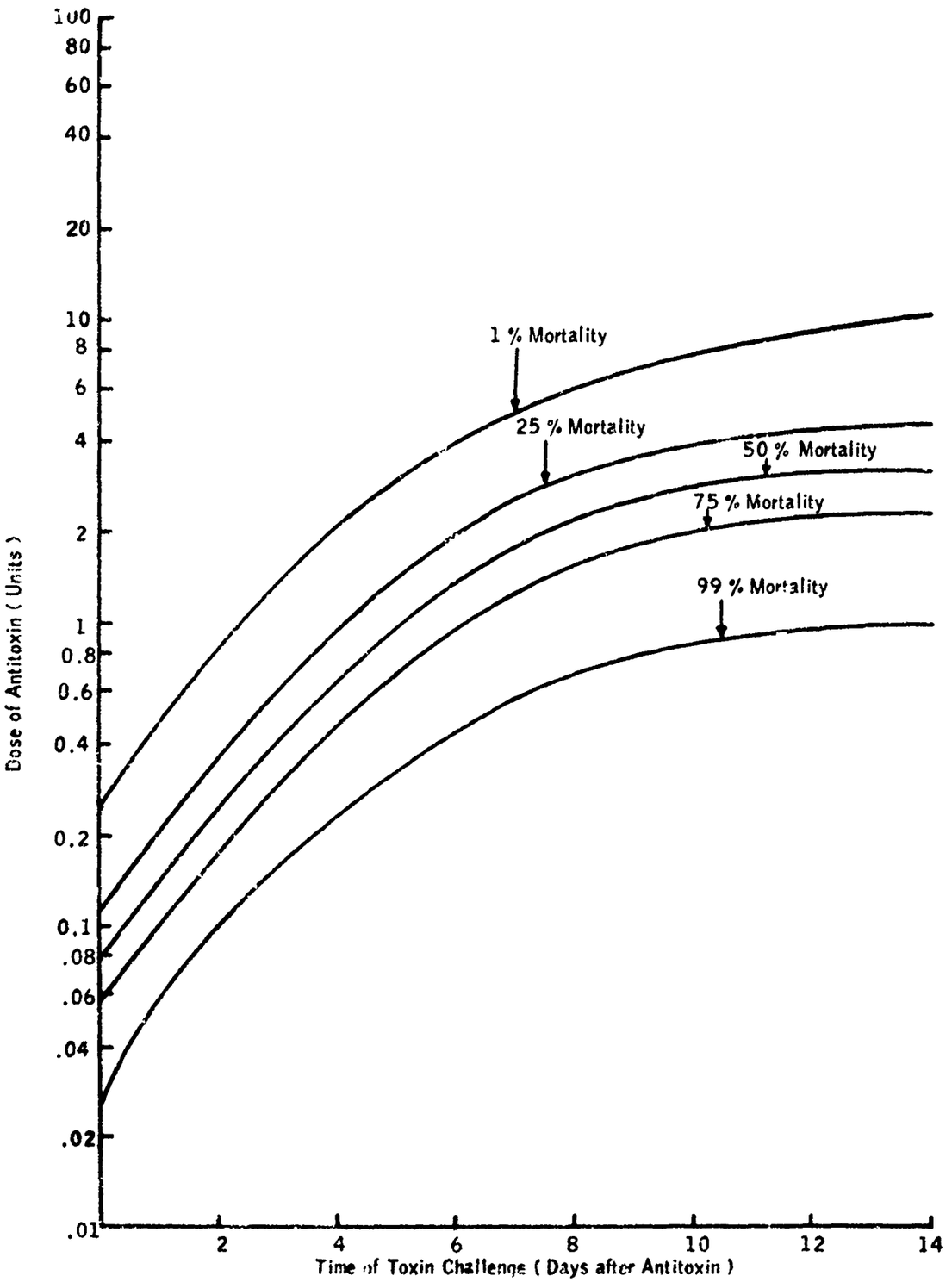
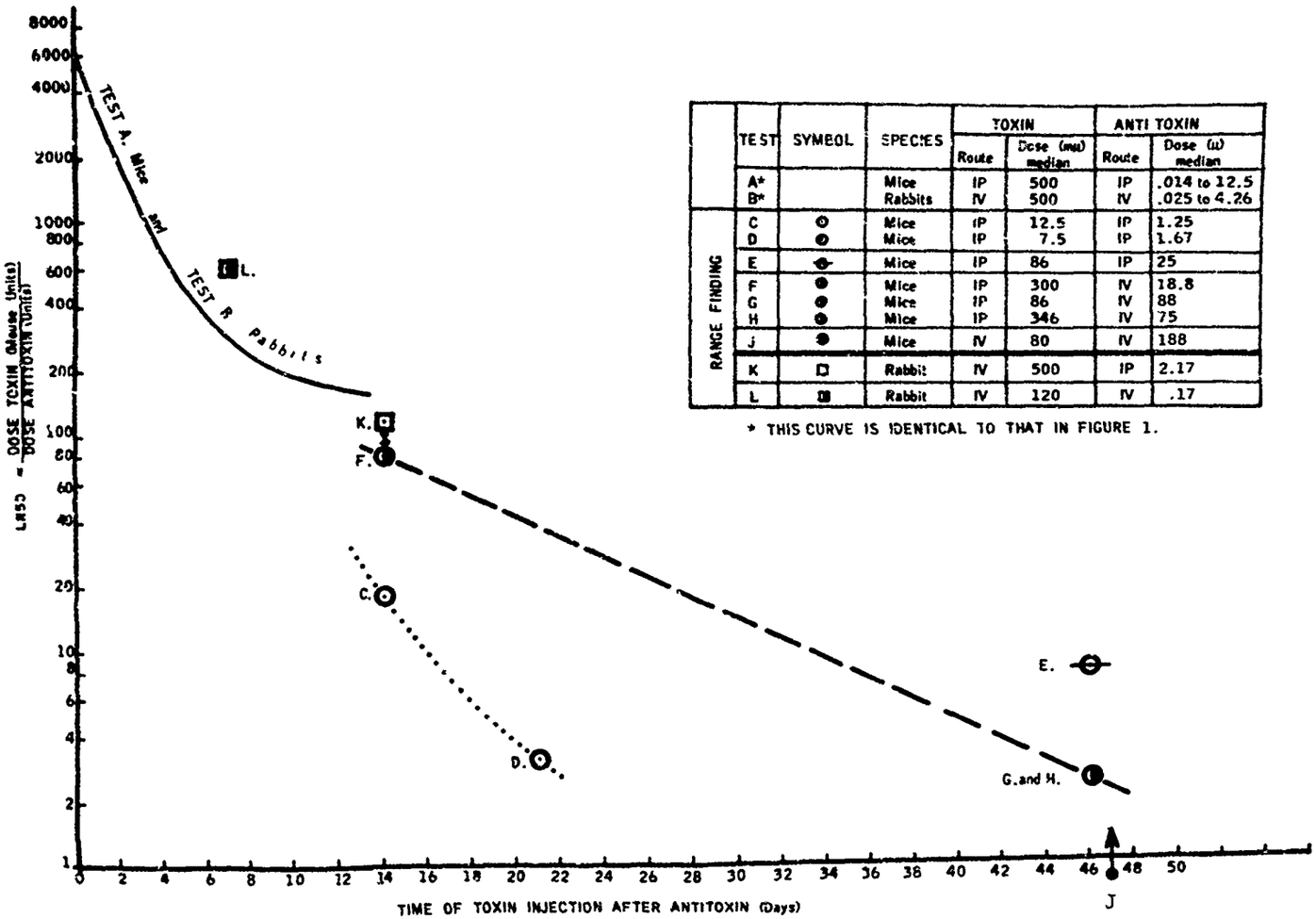


FIGURE B3

FREQUENCY DISTRIBUTION OF MORTALITY IN MICE AND RABBITS GIVEN ANTITOXIN AND CHALLENGED AT VARIOUS TIMES WITH 500 MU OF TOXIN

(Average of intraperitoneal mouse and intravenous rabbit data, using a median slope function of 1.66 for dose-mortality curves)



	TEST	SYMBOL	SPECIES	TOXIN		ANTI TOXIN	
				Route	Dose (mu) median	Route	Dose (u) median
	A*		Mice	IP	500	IP	.014 to 12.5
	B*		Rabbits	IV	500	IV	.025 to 4.26
RANGE FINDING	C	⊙	Mice	IP	12.5	IP	1.25
	D	◉	Mice	IP	7.5	IP	1.67
	E	⊖	Mice	IP	86	IP	25
	F	●	Mice	IP	300	IV	18.8
	G	●	Mice	IP	86	IV	88
	H	●	Mice	IP	346	IV	75
	J	●	Mice	IV	80	IV	188
	K	□	Rabbit	IV	500	IP	2.17
	L	■	Rabbit	IV	120	IV	.17

* THIS CURVE IS IDENTICAL TO THAT IN FIGURE 1.

FIGURE B4

RANGE-FINDING TESTS

[Median lethal ratios (LR50's) of toxin to antitoxin at various exposure times after antitoxin administration]

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DURATION OF PASSIVE IMMUNITY TO BOTULINUM TOXIN OFFERED BY ANTITOXIN IN MICE AND RABBITS

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This work was started in September 1963 and completed in December 1963.

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13. ABSTRACT (U) Various doses of botulinum antitoxin, types A and B, were administered to mice and rabbits by the intraperitoneal and intravenous routes. The persistence of a passive immunity to the type A toxin was determined by (1) administering challenge doses of the toxin at various times after the antitoxin and (2) determining the median lethal ratios (LR50's) of toxin:antitoxin (MU:u) that produced a 50% mortality rate at each challenge time. Decreases in LR50 with time reflect the fall in amount of antitoxin in the blood of the animals and decreased passive immunity to the toxin. A graph prepared from the data represents a mathematical model of mortality frequency distribution in mice and rabbits after 500 MU of toxin. Passive immunity declines steadily with time; however, a sufficiently large dose of antitoxin in mice can produce passive immunity lasting for at least 49 days.

14. KEYWORDS

Botulinum toxin
Botulinum antitoxin, type A
Botulinum antitoxin, type B
Passive immunity
Mice
Rabbits
Intraperitoneal administration
Intravenous administration
Challenge time
Serum level

CONTINUED

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14. KEYWORDS (CONTINUED)

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Mouse units
Mortality
Frequency distribution
Time
Mathematical model