THE ACTION OF DIPHTHERIA TOXIN ON MICE.

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It has been generally accepted that mice are almost insusceptible to diphtheria toxin. Roux and Yersin (1888) found that mice were susceptible only to concentrated toxin and were killed by 80 times the minimal lethal dose for a guinea-pig.

Behring and Kitashiwa (1901) found that the lethal dose per grm. of mouse was 6000 to 10,000 times the lethal dose per grm. of guinea-pig. The lethal dose for a mouse would therefore be from 300 to 500 times the lethal dose for a guinea-pig.

Kolle and Schlossberger reinvestigated the question in 1919 and concluded that white mice were practically insusceptible to large doses (these authors however used only about 100 guinea-pig m.l.d.) of diphtheria toxin, but were susceptible to fairly small doses of living bacilli.

By using potent toxin, prepared by concentration (Glenny and Walpole, 1915), we have been able recently to compare not only the minimal lethal dose for mice with that for guinea-pigs, but also to compare the neutralising effect of antitoxin upon toxin in the two animals.

Table I gives the experiments upon guinea-pigs to determine the m.l.d., L_0 and L+ values for the toxin used.

Table I. *Showing titration for m.l.d., L_0 and L+ values in the guinea-pig.*

Toxin c.c.	Antitoxin	Result of subcutaneous injection into guinea-pigs	Conclusion
0·0005	—	Large swelling. Living 6th day	
0·0006	—	,, ,,	
0·0006	—	,, ,,	
0·0007	—	,, ,,	
0·0008	—	,, Died 4½ days	m.l.d. = 0·0008 c.c.
0·0008	—	,, ,, 5 ,,	
0·0010	—	,, ,, 3 ,,	
0·016	1 unit	No swelling. Living 6th day	
0·018	,,	Medium swelling. Living 6th day	L_0 = between 0·016 and 0·018 c.c.
0·020	,,	Large swelling. Died 5 days	L+ = 0·020 c.c.
0·025	,,	,, ,, 3 ,,	

The m.l.d. for mice was determined by both intravenous and intramuscular injection. Table II gives the data relating to all mice injected. Our general experience and that probably of most observers is that mice are far more prone to infection and to deaths under adverse conditions than are guinea-pigs. A few discrepant deaths appear in the table, but otherwise the results are fairly uniform. For the purpose of tabulation, the sixth day was taken as the

limiting day for significant deaths, but a number of deaths occurred in order on the seventh and eighth day. Thus, of the four mice injected intravenously with 0·05 c.c. toxin and recorded as living, one died on the seventh day, and two on the eighth day, while two of those injected intramuscularly with the same dose died on the seventh day. In a few of the early experiments, the mice were not observed after the sixth or seventh day.

From Table II it would appear that 0·05 c.c. can be taken as the intravenous m.l.d. for this toxin in mice, *i.e.* 60 times the subcutaneous m.l.d. for guinea-pigs, while the intramuscular m.l.d. was 0·08 c.c., *i.e.* 100 times the subcutaneous m.l.d. for guinea-pigs.

To determine the neutralisation value of antitoxin for toxin when injected intravenously into mice, 0·2 c.c. of toxin was taken as a test dose. This amount was chosen in order to keep the volume of material injected as small as possible, while using as a test dose more than two fatal doses of toxin (in this case, 4 m.l.d.), and also to use a dose, that, unneutralised, killed in considerably less time than the maximum significant death time. Thus, from Table II, we

Table II. *Showing the results of intravenous and of intramuscular injection of diphtheria toxin into mice.*

	Results of intravenous injection into mice			Results of intramuscular injection into mice		
Dose c.c.	Death time in days	Total number dying within 5 days	Total number living after the 5th day	Death time in days	Total number dying within 5 days	Total number living after the 5th day
0·01	—	0	2	—	0	1
0·02	3·6	2	4	—	0	3
0·03	5	1	2	—	0	1
0·04	6	1	2	6	1	2
0·05	3, 4, 4, 5, 5, 5, 6, 6	8	4*	4, 5	2	7†
0·06	—	—	—	5	1	1
0·08	—	—	—	5, 5	2	0
0·10	2, 3, 4, 4, 5, 5, 5, 5, 5, 6, 6, 6	13	1	2½, 3, 3, 4, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6	15	3
0·20	2, 2, 2, 2, 2, 3, 3, 3, 4, 4	10	0	2, 3, 4, 4, 4, 4, 5, 5, 6	9	0

* 1 died 7. 2 died 8.

† 2 died 7.

see that the average time of death of mice injected with 0·2 c.c. toxin was three days, while six days has been taken as the maximum significant death time.

Table III. *Showing the neutralising power of antitoxin for toxin by intravenous injection into mice.*

Toxin c.c.	Antitoxin units	Death time in days	Total number dying	Total number living
0·2	5	3, 5	2	0
0·2	6	3, 5, 6	3	0
0·2	8	2, 4, 4, 5, 5	5	0
0·2	10	2, 3, 3, 6, 6, 6	6	1
0·2	12	5, 5, 5, 6	4	3
0·2	13	3	1	1
0·2	14	—	0	2
0·2	15	—	0	6

Table III shows that 10 units of antitoxin failed to neutralise 0·2 c.c. of toxin, 12 or 13 units gave an equal number of deaths and survivals, while 14 units was more than sufficient to neutralise 0·2 c.c. toxin injected intravenously into mice. These figures correspond to those that would be deduced from the L_0 and L+ values determined in guinea-pigs, recorded in Table I. In order to make a direct comparison between the neutralising value of antitoxin for toxin, in mice and in guinea-pigs, similar mixtures were injected into guinea-pigs, *i.e.* 0·2 c.c. of toxin (10 L+ doses) with varying quantities of antitoxin; the results are recorded in Table IV.

Table IV. *Titration of antitoxin against* 10 *L+ doses of toxin in guinea-pigs.*

Toxin c.c.	Antitoxin units	Result of subcutaneous injection into guinea-pigs
0·2	6	Died in 24 hours
0·2	10	„ 2 days
0·2	12	„ 2½ „
0·2	13	Large swelling—surviving 6th day
0·2	14	No „ „ „
0·2	15	„ „ „ „

It will be seen by comparing Tables III and IV, that a mixture containing 0·2 c.c. of toxin and 10 units of antitoxin is toxic both to mice and guinea-pigs, while a mixture containing 14 units with the same amount of toxin is non-toxic to both species. In guinea-pigs, the test dose used, 0·2 c.c. of toxin, contained 250 m.l.d.; the end point is reached more abruptly than in mice, the same test dose for which contained only 4 m.l.d. Allowing for the marked difference in the number of m.l.d. for the two species, it can be said that antitoxin has approximately the same neutralising power for toxin in mice as in guinea-pigs.

A few experiments on the neutral point determined by intramuscular injection into mice gave irregular results. This was to be expected, because the test dose used (0·2 c.c.) contained only 2½ m.l.d., and, unneutralised, killed, on the average, after the fourth day, a time limit too near to the maximum significant death time. The general trend of the experiments showed that between 8–10 units of antitoxin would neutralise 0·2 c.c. of toxin. It is obvious that as the test dose of toxin approximates more closely to the minimal lethal dose, proportionately smaller doses of antitoxin are necessary to prevent death. The results obtained by intramuscular injection, although not very conclusive, tend to confirm the results obtained by intravenous injection, that the neutralising power of toxin for antitoxin in mice is of the same order as that in guinea-pigs.

Since this work was concluded, a paper has appeared by Coca, Russell and Baughman (1921), on "The Reaction of the Rat to Diphtheria Toxin." They show that "the rat is not absolutely immune to diphtheria toxin. Although it usually survives the injection of 1000 m.l.d. (for the guinea-pig), it regularly succumbs to 4000 such units." They suggest that toxin is less rapidly absorbed by the cells of the rat as compared with the guinea-pig, and have demonstrated

the presence of more free toxin in the blood of rats six hours after an intraperitoneal injection of toxin, than was found in the blood of guinea-pigs at the same interval of time, after a similar injection. They further show that "the rat is capable of the production of antitoxin upon the repeated injection of diphtheria toxin."

CONCLUSIONS.

1. For the particular toxin tested, the intravenous m.l.d. for mice was 60 times and the intramuscular m.l.d. for mice 100 times, the guinea-pig subcutaneous m.l.d.

2. Antitoxin has the same neutralising power for toxin in mice as in guinea-pigs.

REFERENCES.

BEHRING and KITASHIWA (1907). *Berlin. klin. Woch.* p. 157.
COCA, RUSSELL and BAUGHMAN (1921). *Journ. of Immunology*, VI. 387.
GLENNY and WALPOLE (1915). *Biochem. Journ.* IX. 298.
KOLLE and SCHLOSSBERGER (1919). *Arb. a. d. Inst. f. Exp. Therap. z. Frankfort. A. M.* H. 8.
ROUX and YERSIN (1888). *Ann. Inst. Pasteur*, II. 629.