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Susceptibility of Mice to Intracerebral Inoculation of *C. Diphtheriae* and Diphtheria Toxin*

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Hitherto, mice have been regarded as not highly susceptible to virulent diphtheria bacilli or to diphtheria toxin. The experiments of Kolle and Schlossberger,[1] Hippke,[2] Wolff,[3] Lewy,[4] and Zinnemann[5] showed that relatively large subcutaneous, intraperitoneal, and intravenous doses of virulent *C. diphtheriæ* or diphtheria toxin are required to produce illness or death of the mice, and frequently fail to do even this. However, a rather distinctive reaction indicating involvement of the central nervous system, has been observed frequently to follow such infections. The striking features of this reaction have been variously described as tic-like, or choreiform movements with hyperirritability and clonic convulsions. None of the workers referred to made use of the intracerebral method of inoculating mice; neither did any of them investigate the effect of cultures of *C. diphtheriæ* believed to be avirulent for guinea pigs, or of any of the diphtheroids.

The present series of experiments was prompted by the results of a preliminary test to determine the effect of intracerebral injections of broth cultures of rabbit-virulent *C. diphtheriæ* in mice as compared with subcutaneous and intraperitoneal injections of the same material.

Each of 5 strains, including the gravis, mitis and indeterminate types, was inoculated intracerebrally into 4 mice of different breeds, making 20 mice in all. For purposes of comparison the same strains, plus one other, were inoculated intraperitoneally and subcutaneously. Included were mice belonging to the Swiss, Sherman, C-57, and B. H. breeds. Of the 20 mice inoculated intracerebrally (0.03 cc) all died within 48 hours, while of 24 mice inoculated intraperitoneally (0.5 cc) 10 died and 14 survived, and of 24 inoculated subcutaneously

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1 Kolle, W., and Schlossberger, H., *Z. f. Hyg.*, 1920, **90**, 193.

2 Hippke, E., *Z. f. Hyg.*, 1922, **98**, 432.

3 Wolff, E. K., *Virch. Arch.*, 1922, **238**, 237.

4 Lewy, F. H., *Virch. Arch.*, 1922, **238**, 252.

5 Zinnemann, K., *J. Path. and Bact.*, 1940, **50**, 243.

(0.5 cc), all but one survived. No evidence was seen indicating marked differences between the effectiveness of different types of diphtheria bacilli in killing the mice, nor did any one of the 4 breeds of mice seem to be much more susceptible than the others. After intracerebral inoculation, nearly all the mice showed the peculiar tic-like movements, frenzied activity, and prolonged clonic convulsions referred to above, but no such reactions were noted following subcutaneous or intraperitoneal inoculations.

In more extensive experiments, attempts were made to infect normal and antitoxin-injected mice by the intracerebral route, not only with strains of *C. diphtheriæ* of known virulence for rabbits and chicks[6] but with others of twice-proven avirulence for rabbits and chicks as well as with several strains of diphtheroids. As controls, 2 heat-killed cultures of virulent strains were included. The results of one group of such inoculations are summarized in Table I.

As additional controls, various organisms of entirely different genera (*E. coli, E. typhosa, N. intracellularis, S. aureus, D. pneumoniæ,* and others), and sterile broth were inoculated in a similar manner into a total of 63 mice. A number of the mice receiving these latter materials died, but none of them showed a reaction in any way comparable with that seen in mice receiving living *C. diphtheriæ.* There was no evidence of any nerve involvement.

TABLE I.
Effect of Various Organisms When Injected Intracerebrally into Mice.

Material injected	Result of rabbit and chick virulence test	Strains	Mice	Dead*	Ill and recovered	No visible reaction
C. diphtheriæ (no antitoxin)	Positive	7	48	46	1	1
C. diphtheriæ (antitoxin)‡	"	7	60	26†	17	17
C. diphtheriæ (heat-killed)	"	2	12	0	0	12
C. diphtheriæ‡ (no antitoxin)	Negative	13	18	8	9	1
C. xerosis	"	9	39	0	0	39
C. pseudodiphthericum	"	6	36	0	0	36

*Only deaths within 4 days, or after longer period only following typical spastic and frenzied seizures, are included here.

†8 of these died in a convulsive seizure within about an hour after the infective dose.

‡Antitoxin was given in doses of from 200 to 400 units intraperitoneally from 3 to 18 hours prior to the infective dose. In one instance phenol-free antitoxin was also mixed with the culture for 1 hour before injection intracerebrally. There seemed to be no special advantage in this.

[6] Frobisher, M., Jr., *Science,* 1940, **92**, 88.

It was observed that nearly all of the mice receiving virulent culture plus antitoxin were definitely ill and were affected, in most respects, much as were the unprotected mice. However, as noted in Table I, about half recovered from their illness and survived without further evidence of damage. Two weeks later these survivors were inoculated intracerebrally with a virulent strain of *C. diphtheriæ,* whereupon all died or showed the typical signs described above.

It is particularly important to note that the mice inoculated intracerebrally with *rabbit- and chick-avirulent* strains showed the same distinctive signs, although of a somewhat lesser intensity, as those seen in the mice inoculated with rabbit- and chick-virulent strains.

In order to verify these results, the virulence of 12 of the "avirulent" strains was retested in rabbits. When again found wholly avirulent, these strains were again inoculated intracerebrally into mice with essentially the same results as before. Of 68 mice inoculated, 12 died in 18 hours and 13 more within one week. All of the latter, as well as most of the mice that survived more than a week showed typical choreiform movements and occasionally clonic spasms and convulsions.

Thirty-six mice inoculated intraperitoneally with 0.5 cc of 48-hour broth cultures of 6 of the same strains showed no sign of illness of any sort.

Effect of Toxin. An experiment was carried out to determine the susceptibility of mice to intracerebral inoculations of sterile, toxic, culture-filtrate of *C. diphtheriæ.* A standardized and well seasoned lot of toxin was selected. The M.L.D. for guinea pigs was .0036 cc. Dilutions were so prepared that the dose finally received by the mice (.03 cc) would contain about 5, 1, and 0.2 M.L.D., respectively, after the diluted material was mixed with an equal part of sterile broth or antitoxin containing 50 units per cc. The mixtures stood at room temperature for about one hour before inoculation into the mice. The details of the experiment are shown in Table II.

TABLE II.

Effect of Intracerebral Inoculation of White Mice with Different Amounts of Diphtheria Toxin.

Guinea pig M.L.D.'s of toxin received by the mice	Mice	Dead	Ill and recovered	No visible reaction
5 (with antitoxin)	6	0	0	6
5 (without ")	6	6	0	0
1 (with ")	6	0	0	6
1 (without ")	6	4	2	0
0.2 (with ")	6	0	0	6
0.2 (without ")	6	0	0	6

The effect of the toxin was, in general, quite comparable with that of cultures of *C. diphtheriæ,* especially that of the largest dose. There were, however, no early deaths; indeed, no definite symptoms were noted before 48 hours had elapsed. The mice which received 5 M.L.D. then began to show the characteristic hyperirritability and hyperactivity observed in those infected with live cultures. The period of illness was, in general, greatly prolonged, but all of the unprotected mice which became ill eventually died. The mice receiving 1.0 M.L.D. became ill a day or two later than those receiving 5 M.L.D. Those receiving the smallest dose showed no ill effect. The antitoxin controls showed no reaction. It is probable that the toxin, in the toxin-antitoxin mixture, was completely neutralized before injection.

These results indicate that mice are much more susceptible to toxin introduced by the intracerebral route than to toxin introduced subcutaneously or intraperitoneally, since it has been found by earlier workers that doses of from 80 to 100,000 guinea-pig-M.L.D.'s were required to kill mice by the latter routes.[5] These doses are from 16 to 2000 times the largest dose (5 g.p. M.L.D.) used in the present experiments.

It would appear that, by using the intracerebral route of inoculation in mice, degrees of susceptibility to *C. diphtheriæ* and its toxin not hitherto suspected may be demonstrated.

From the above observations, it is evident that diphtheria toxin, when introduced intracerebrally into mice, produces a reaction which is similar in all respects to that resulting from the intracerebral inoculation of live, rabbit-virulent cultures such as are generally known to be toxigenic. Moreover, *the same type of reaction is produced by rabbit-avirulent strains which have never been shown to be toxigenic.* The implication is clear that the effect of the latter is due to the production of minute amounts of toxin which become manifest when elaborated in intimate contact with nervous tissue of the mouse.

It seems, therefore, that the term "avirulent" when applied to this group of organisms can be used only in a relative sense, and that there may be a closer biologic relationship between virulent and avirulent diphtheria bacilli, than has been believed to be the case. This method of inoculation may provide a new tool with which to study the rôle of rabbit-avirulent diphtheria organisms in the epidemiology of the disease.

Summary. These experiments have revealed four main facts: (a) White mice are highly susceptible to intracerebrally-injected 48-hour broth cultures of rabbit-virulent strains of *C. diphtheriæ;* 99% of the mice so inoculated dying within 10 days. (b) Mice are like-

wise susceptible, but in somewhat lesser degree, to similar injections of rabbit-*avirulent,* and hitherto supposedly harmless, strains of *C. diphtheriæ;* about 85% of the mice showing highly characteristic reactions within 10 days. (c) Mice receiving minute doses of toxin intracerebrally exhibit the same definitive and fatal results observed in the mice receiving the rabbit-virulent and rabbit-avirulent strains. (d) A number of cultures of diphtheroids *(C. xerosis* and *C. pseudodiphthericum)* were harmless for mice when injected as indicated above. Several other organisms have failed to produce any reactions in any way resembling those produced by *C. diphtheriæ* and diphtheria toxin.

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Comparative *in vitro* Study of Various Sulfanilamide Derivatives on Typhoid-Dysentery Organisms.

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Following the introduction of sulfathiazole by Fosbinder and Walter[1] it soon became evident that the thiazole derivatives exhibited bacteriostatic action against a number of organisms *in vitro.*[2-5] Previous experience in investigating infections of the urinary tract[6] due to *B. coli, B. lactis aerogenes* and *B. proteus* suggested that sulfathiazole might be an effective bacteriostatic agent against other Gram-negative organisms. The present study was undertaken to determine the comparative action *in vitro* of sulfanilamide, sulfapyridine, sulfamethylthiazole and sulfathiazole on representative organisms of the typhoid-dysentery group. The organisms used in this study were *Eberthella typhosa, Salmonella paratyphi, Salmonella schottmuelleri, Shigella dysenteriæ, Shigella paradysenteriæ*

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