

PROTECTIVE DOSE OF ANTITOXIN IN EXPERIMENTAL TETANUS

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The protective dose of antitoxin guaranteeing prevention of tetanus is above the level of 1.0 i.u./g body weight. A dose of 0.1 i.u./g body weight of antitoxin protected about 50% of guinea pigs against experimental infection.

Many investigators who have set out to determine the protective dose of antitoxin in experimental tetanus have indicated that a dose of 0.05 i.u./g body weight is inadequate [3-7].

The object of the present investigation was to study the prevention of experimental tetanus by means of antitoxin.

EXPERIMENTAL METHOD AND RESULTS

The protective dose of antitoxin was determined in guinea pigs into which a "splinter" (a match 2.5 cm long), preliminarily sterilized and then soaked in a suspension of spores of Clostridium tetani for 1 h at 20°, was introduced, subcutaneously in the dorsal region.

The experiments to study prevention of tetanus were regarded as demonstrative only if all animals in the control group died from the infecting dose.

To protect the guinea pigs against experimental tetanus a mixture of tetanus antisera prepared two years previously by the "Diaferm-2" method, was used. The antitoxin contained 900 i.u./ml.

Noninbred guinea pigs weighing 250-300 g were used in the experiments. The skin was painted with alcohol, and a small incision made into which the "splinter" was introduced with forceps. Various doses of antitoxin were injected into the right thigh of the infected guinea pigs 1 and 6 h later.

The animals were inspected daily for one month.

TABLE 1. Infecting Dose of Spores Adsorbed on "Splinters"

	Undiluted suspension of spores	Concentration of suspension of spores			
		1:10	1:100	1:1000	1:10 000
n	28	6	6	6	6
Survival	0	1	3	5	6
Died	28	5	3	1	0
Mortality	100%	83.3%	50%	16.66%	0
\bar{x}	4.43	5	5.33	5	0
V	2.48	2.3	4.0	—	—
S	0.47	0.585	2.31	—	—
CL	3.51—5.35	3.87—6—13	0.8—9.86	—	—

Legend. n denotes number of animals in experiment; \bar{x} arithmetic mean incubation period (in days); V standard deviation of incubation period; S standard error; CL Confidence limits.

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TABLE 2. Incubation Period of Tetanus in Guinea Pigs Developing Disease

Dose of antitoxin (in i.u./g)	No. of guinea pigs in expt.	n	\bar{x}	v	s	CL
Antitoxin injected 1 h after infection						
1	10	1	20	—	—	—
0.1	20	10	15.7	1.67	0.53	14.66—16.74
0.01	29	22	8.59	3.08	0.66	7.31—9.87
0.001	20	16	6.25	3.2	0.8	4.68—7.82
Antitoxin injected 6 h after infection						
1	10	1	21	—	—	—
0.1	20	9	9.55	7.4	0.84	4.71—14.39
0.01	20	14	6.86	4.1	1.10	4.71—9.01
0.001	10	10	4.7	1.25	0.395	3.93—5.47
Control	28	28	4.43	2.48	0.47	3.52—5.35

Legend. n denotes number of animals developing disease; remainder of legend as in Table 1.

TABLE 3. Protection of Guinea Pigs Against Tetanus by Antitoxin

Dose of antitoxin (in i.u./g)	No. of g. pigs in expt.	Developed disease	P rel. to morbidity	Survived	P rel. to surv. rate	PD ₅₀ of antitoxin*	\bar{x}	CL
Antitoxin injected 1 h after infection								
1	10	1	0.10	9	0.90	0.022 ME/e	(20)	—
0.1	20	10	0.50	10	0.50	0.022 »	16.7	14.64—18.76
0.01	29	17	0.59	12	0.41	0.022 »	13.5	11.89—15.11
0.001	20	13	0.65	7	0.35	0.022 »	10.7	7.29—14.11
Antitoxin injected 6 h after infection								
1	10	1	0.10	9	0.90	0.032 ME/e	(21)	—
0.1	20	9	0.45	11	0.55	0.032 »	17.1	13.69—20.51
0.01	20	13	0.65	7	0.35	0.032 »	11.5	9.94—13.06
0.001	10	8	0.80	2	0.20	0.032 »	9.5	8.13—10.87
Control of infecting dose of spores								
	28	28	1.0	0	0.00	—	5.36	3.65—7.07

Legend. \bar{x} means time of death of animals in days; P probability; CL Confidence limits.

*By Ashmarin's method [6-7].

The time of onset of the disease, the severity of its course, and the time of death of the animals were recorded. Results pertaining to the infecting dose of spores are given in Table 1.

By Ashmarin's method [1, 2], $\log LD_{50} = -2.0$ (i.e., 1:100), with confidence limits of -1.5 and -2.5. The infecting dose (undiluted suspension) contained 100 LD_{50} and killed the guinea pigs on the average within 5.36 days (confidence limits 4.29 and 6.42).

In the present experiments, 1 ml of undiluted suspension contained from $8 \cdot 10^8$ to $5 \cdot 10^9$ spores capable of development.

Data showing the incubation period of tetanus in guinea pigs protected by different doses of antitoxin are given in Table 2.

The larger the dose of antitoxin, and the sooner it was injected after infection, the greater the difference between the incubation period of tetanus in the control animals and in animals protected by antitoxin.

The most important task was to determine the dose of antitoxin protecting the guinea pigs from death. These results are given in Table 3.

They show that a dose of antitoxin of 0.1 i.u./g body weight is evidently inadequate to give protection against tetanus. The protective dose of antitoxin must exceed 1 i.u./g body weight.

Bearing in mind the fact that the guinea pig is close to man in its sensitivity to tetanospasmin, it is evident that the protective dose of antitoxin for man must be revised.

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