NEUTRALIZATION WITH ANTITOXIN OF TETANUS TOXIN BOUND WITH BRAIN SUBSTANCE

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Experiments using purified tetanus toxin labeled with I^{131}, horse antitoxin of the "Diaferm-3 IEM" brand, and the substance protagon isolated from bovine brain tissue, have shown that the protagon–toxin complex, when treated with antitoxin in vitro, loses its toxicity, although the radioactivity of the complex remains unaffected after washing. It is postulated that protagon-bound toxin can undergo specific neutralization by antitoxin.

The possibility of specific neutralization by antitoxin of tetanus toxin when bound by nerve tissue has not yet been settled. It is generally considered that no such neutralization takes place, although facts have been obtained to suggest that toxin, when adsorbed by brain substance, can be neutralized by antitoxin in vitro [3].

In the investigation described below, the possibility of neutralization by antitoxin of tetanus toxin bound on protagon was studied. Protagon is a substance isolated from brain tissue, containing gangliosides, cerebrosides, and other components.

Since the role of receptor of tetanus toxin in nerve tissue is played by gangliosides combined in a certain manner with cerebrosides or other substances forming a water-insoluble complex [4, 7, 8], protagon is able to bind tetanus toxin [6], and it is regarded as the unpurified receptor of the toxin.

EXPERIMENTAL METHOD

Protagon was obtained by a slightly modified method of Thierfeld and Klenk from freshly isolated and cooled bovine brain. Dry toxin was added to a suspension of protagon in physiological saline in a dose of 1 mg toxin to 50 mg protagon in 5 ml saline, and the mixture was incubated at 37° for 45 min. To remove free and loosely bound toxin, the suspension was centrifuged and the residue washed in physiological saline (6000-10,000 rpm for 10 min). After the 3rd or 4th washing, no toxin was found in the supernatant. The residue, possessing toxicity (200-400 MLD for mice /10 mg protagon), was mixed with different doses of "Diaferm" antitoxin. The mixture was incubated for 45 min at 37°, centrifuged, and the residue was washed 3 times with physiological saline to remove free antitoxin. The washed residue was titrated on mice and its toxicity estimated from the scale prepared by the writers. After addition of different doses of serum, the pH was stabilized. Three batches of dry, unpurified toxin from the N. F. Gamaleya Institute of Epidemiology and Microbiology and 3 samples of protagon were tested (in experiment No. 1 activity of the toxin was 8000 MLD for mice/mg, in experiment No. 2 it was 11,000 MLD/mg, and in experiment No. 3, 15,000 MLD for mice/mg).

In the experiments with radioactive toxin, dry tetanus toxin of strain No. 228 from the Leningrad Institute of Vaccines and Sera was used. I^{131}-labeled toxin was prepared from toxin purified by Pillemer's method [5] by the method described previously [1, 2]. To obtain the protagon–toxin complex, 40 μg of toxin-I^{131} was added to 50 mg protagon. The mixture was incubated under the same conditions, after which

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TABLE 1. Results of Treatment of Protagon–Toxin I^{131} Complex with Antitoxin

Experiment No.	Dose of antitoxin (in i.u.)	Radioactivity of fractions, pulses/min				
		residue before treatment	Supernatant washings			Residue after treatment with antitoxin*
			I	II	III	
1	0,05	2 869	160	135	126	2 448 (85%)
	5	2 115	117	72	144	1 782 (84%)
	50	1 863	126	108	54	1 575 (84%)
	Control	2 756	72	72	72	2 547 (92%)
2	0,05	855	27	9	9	810 (94%)
	5	965	108	56	18	783 (81%)
	50	1 026	180	27	18	801 (79%)
	Control	840	81	0	39	720 (85%)
3	0,05	3 429	450	54	—	2 925 (85%)
	5	4 374	684	153	—	3 537 (81%)
	50	3 771	729	99	—	2 943 (79%)
	Control	3 762	450	423	—	2 889 (76%)

* Total radioactivity of residue after treatment with antitoxin plus radioactivity of supernatant washings taken as a 100%.

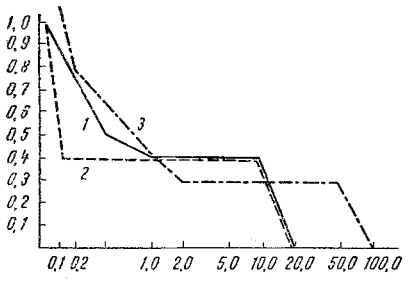

Fig. 1. Toxicity of protagon–toxin complex after treatment with antitoxin. 1, 2, 3) Experiment No. Abscissa, dose of antitoxin (in i. u.); ordinate, toxicity of residue (in MLD).

the residue was washed repeatedly by centrifugation (10,000 g at 2°), and the radioactivity of the supernatant was tested. After the 4th washing, it was very low (15%). To the washed residue containing protagon–tetanus toxin complex, from 0.05 to 50 i. u. "Diaferm" antitoxin was added and the volume was made up to 5 ml with 0.85% NaCl solution. The mixtures were incubated at 37° for 45 min and the residue washed 3 times by centrifugation. After the last washing, the radioactivity of the residue was tested. The same procedure was carried out with the control sample in which, instead of serum, physiological saline was added. Control tests were carried out with binding of free I^{131} by protagon. They showed that such binding is negligible (under 2%). The radioactivity of the samples specified above was determined under the SI-26 end-window tube of a B-1 apparatus (in 0.5 ml of alkalified solution).

EXPERIMENTAL RESULTS

Following addition of antitoxin to the protagon–toxin complex, the toxicity of the complex fell (Fig. 1). A large part of the toxin was neutralized after addition of small doses of antitoxin (0.02-0.01 i.u.) to the complex, and part of it could be neutralized by the addition of a further, comparatively large dose of antitoxin. The same type of neutralization curve was obtained for all three experiments performed. Differences observed were purely quantitative and evidently dependent on the quality of the protagon and toxin. In experiments Nos. 1 and 3, for instance, freshly prepared protagon was used, but in experiment No. 2 the protagon was older.

The experiments with purified radioactive toxin showed that I^{131}-labeled toxin is bound by protagon in the same way as ordinary toxin. Following treatment of the residue containing protagon–toxin complex with antitoxin in doses of 0.01-0.05 i. u., partial neutralization of the toxin took place, while in doses of 25-50 i. u., neutralization of the toxin was complete. At the same time, as Table 1 shows, no decrease took place in the radioactivity of the treated and repeatedly washed residue by comparison with the control. Since free iodine is not bound with protagon to any significant extent, it can be concluded that the radioactivity of the residue was due to its content of labeled toxin.

These results suggest that toxin bound with protagon may be neutralized by antitoxin.

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