

TEN TITRATIONS OF COBRA ANTI-SERUM IN WHICH
PARAMECIA WERE USED AS TEST AGENTS

Amount of cobra venom per cc of mixture	Amount of serum per cc of mixture	Number of paramecia added to 1 cc of mixture	Number of animals remaining alive after 1 hour. Tem- perature 20° C.									
	Anti- serum		Titration number									
gram	cc		1	2	3	4	5	6	7	8	9	10
0.000005	0.0005	4	0	0	0	0	0	0	0	0	0	0
"	0.0010	4	0	0	0	0	0	0	0	0	0	0
"	0.0014	4	0	0	0	0	0	0	0	0	0	0
"	0.0019	4	2	0	3	0	1	0	0	3*	0	1*
"	0.0024	4	4	0	3	0	2	2	0	3	1	1
"	0.0029	4	4	4	4	3	4	4	0	4	4	4
"	0.0033	4	4	4	4	4	4	4	4	4	4	4
"	0.0038	4	4	4	4	4	4	4	4	4	4	4
	Normal horse serum, cc											
"	0.0005	4	0	0	0	0	0	0	0	0	0	0
"	0.0010	4	0	0	0	0	0	0	0	0	0	0
"	0.0014	4	0	0	0	0	0	0	0	0	0	0
"	0.0019	4	0	0	0	0	0	0	0	0	0	0
"	0.0024	4	0	0	0	0	0	0	0	0	0	0
"	0.0029	4	0	0	0	0	0	0	0	0	0	0
"	0.0033	4	0	0	0	0	0	0	0	0	0	0
"	0.0038	4	0	0	0	0	0	0	0	0	0	0

* Animals in pathological condition.

The medium used in making all dilutions from the venom and anti-serum stock solutions consisted of a 0.025 per cent. solution of beef extract in non-toxic distilled water. This medium was brought to a pH of 7.3 by the addition of Na_2HPO_4 and was kept in sterile condition until time for use.

The mixtures of venom and anti-serum which are described in the accompanying table were allowed to stand at 5° C. for from 10 to 60 minutes before testing their effects on paramecia. It was found that under these conditions neutralization of the venom was as complete in 10 as in 60 minutes. It was also found that the venom in these mixtures deteriorated to some extent when the solutions stood at room temperature and in the presence of sunlight.

The animals used in these titrations came from a culture of *Paramecium multinucleatum* obtained from Dr. L. L. Woodruff, of Yale University. All animals used were the descendants of one animal isolated at the beginning of the investigation.

It is evident from an inspection of the data in the accompanying table that the least amount of the

anti-serum required to sufficiently neutralize 0.000005 gram of venom so that paramecia are able to live in the mixture is from 0.0019 to 0.0029 cc of the anti-serum per cc of mixture. Considering the fact that these animals are normally able to tolerate 0.0000016 gram of cobra venom per cc of mixture it follows that 0.0000034 gram of the venom is neutralized by from 0.0019 to 0.0029 cc of the anti-serum. From this it may be calculated that 1 cc of the anti-serum neutralizes from 0.0011 to 0.0017 gram of cobra venom. The Pasteur Institute, using warm-blooded animals as test agents, reported this anti-serum to have a neutralizing value of from 0.0009 to 0.00125 gram of cobra venom per cc. The titre obtained with the use of paramecia and that obtained by the Pasteur Institute, where warm-blooded animals were used in the test, are essentially in agreement.

It is the belief of the writer that the above described method of determining the strength of cobra anti-serum is reliable and that it could be used routinely.

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