

ments on the building. The currents during most of the time since have been large enough to measure directly with the type R galvanometer, or of the order of  $5 \times 10^{-9}$  ampere. These are about the same as those of June, 1930, and in the same direction, *i.e.*, upward currents.

It will be noted in this connection that our results, except during the very driest part of the last summer, are in direct contradiction to the fair weather results of the investigators mentioned above. Our results indicate that in this vicinity the earth is nearly always gaining negative charge. Our results check the others for the prevailing direction of the current during a rain, *i.e.*, that the earth is gaining negative charge. It was also noted that, during a thunderstorm, the direction of change of field during a lightning stroke, was predominately in one direction.

Most of the changes noted occurred when the storm was overhead so that the distance to the points of discharge were comparatively short. All the changes in field—with two exceptions—were positive, *i.e.*, the fields set up by the discharges were downward. One of these two exceptions occurred as the storm was approaching and was quite small. The other occurred while the storm cloud was overhead and was comparatively strong. These results check those of Schonland and Craig for near discharges. (*Proc. Roy. Soc.*, A vol. 114, p. 229, 1927). As there was only one observer and he was continually occupied with the measuring instruments and as the storm was approaching from the opposite side of the building, there was no opportunity to note the character of the discharges or to make any actual estimates of the distances to the points of discharge.

Thinking that there might possibly be some error in our method of determining the direction of the current, an electrometer was set up and the direction again determined. Both galvanometers and the electrometer gave the same results. Frequent tests were also made to determine whether the direction of the currents noted might not be due to small e.m.f.'s developed in the switches but no evidence to this effect was found.

While the apparatus was set up in the field, it was noted that whenever an auto passed stirring up a cloud of dust which drifted across the apparatus, there was an added current set up. This was always in one direction and was interpreted as being due to tribo-electricity generated between the rubber tires of the car and the cinders on the road. Without exception, however, this was followed at an interval of about thirty seconds by a reverse current. This reverse current has not been explained.

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## USE OF PROTOZOA IN MEASURING THE NEUTRALIZING VALUE OF COBRA ANTI-SERUM<sup>1</sup>

IN previous studies of the effect of toxins and venoms on protozoa<sup>2</sup> it has been shown that paramecia may be used in measuring the potency of venoms. Attempts were made to use paramecia in measuring the neutralizing value of the anti-serum of *Crotalus* (rattlesnake) venom, but results were not wholly satisfactory. More recently the study has been extended to the use of paramecia in measuring the strength of cobra anti-serum. In this the results have been more satisfactory. It is the purpose of this paper to describe the methods used and the results obtained in ten different titrations where paramecia were used in measuring the neutralizing value of a sample of cobra anti-serum.

The method which was used in measuring the strength of the cobra anti-serum consisted in placing paramecia (in each case four animals in 1 cc) in various mixtures of cobra venom and anti-serum and thus determining the least amount of anti-serum required to protect the animals from a given amount (0.000005 gram) of venom. The method and results of ten different titrations are shown in the accompanying table.

The venom which was used in these titrations was supplied in desiccated form by the Pasteur Institute. A stock solution of this venom was made by dissolving 0.05 gram of the dry venom in 9 cc of distilled water to which 1 cc of glycerine had been added. This solution was brought to a pH of 7.0 by the addition of  $\text{Na}_2\text{HPO}_4$ . This stock solution did not deteriorate in strength during the period of the investigation and was used in making all dilutions of venom indicated in the accompanying table. The solution was kept at from 5 to 10° C. Repeated titrations showed that the least concentration (minimal lethal concentration) of this venom required to kill paramecia (*P. multimucleatum*) was 0.000002 gram per cc and that the maximum tolerance of this species of paramecia for the venom was 0.0000016 gram per cc of medium.

The anti-serum used in these titrations was a sample supplied in desiccated form by the Pasteur Institute. A fresh stock solution of the desiccated anti-serum was made up for each titration shown in the accompanying table. This, in each case, was made by weighing 0.05 gram of the desiccated anti-serum (the equivalent in this case of 0.5 cc of liquid anti-serum) and dissolving in 10 cc of neutral distilled water. From such stock solution all dilutions of the anti-serum shown in the table were made.

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<sup>2</sup> C. H. Philpott, *Jour. Exp. Zool.*, vol. 56, No. 2; *Jour. Morph. and Physiol.*, vol. 46, No. 1; *Proc. Soc. Exp. Biol. and Med.*, vol. 26; *Biol. Bull.*, vol. 60, No. 1.

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  $\text{Na}_2\text{HPO}_4$  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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