

## A Technique for the Production of Immune Seras for *Paramecium aurelia*

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In connection with studies on the inheritance in certain ciliate protozoa (5) which are being conducted by Professor T. M. Sonneborn at Indiana University, it was desirable to produce immune sera against *Paramecium aurelia*. Some of the phenomena which were being studied suggested that a fractionation of antigens in a manner similar to separation of the somatic and flagellar antigens now widely used with bacteria might be interesting and worthwhile. Such proved to be the case and this paper will outline the technique, which was developed in the fall of 1941, by which we were able to produce satisfactory sera.

The following variations in the preparation of antigen and in the routine of injection were studied in the original and later series of animals:

(#1) Six intraperitoneal injections of 3-4 ml. of a thick suspension of organisms (approximately 75,000) were given at weekly intervals.

(#2) Six intraperitoneal injections of 3-4 ml. of a thick suspension of organisms (approximately 75,000) were given twice weekly.

(#3) Six intravenous injections of a thick suspension of cells which had been steamed for 1 hour in the Arnold sterilizer, were given twice weekly.

(#4) Six intravenous injections of the *supernatant fluid* obtained by centrifuging a culture which had been mechanically agitated in a manner to be described, were given twice or thrice weekly.

(#5) Six intravenous injections of the culture mechanically agitated prior to centrifugation, were given twice weekly.

In (#3), (#4), and (#5) the amounts of the successive injections were approximately as follows: 0.25, 0.5, 0.75, 1.0, 1.0, and 1.0 ml.

In all experiments healthy adult rabbits were used to produce the serum. In no instance did we encounter a normal serum which displayed any reaction when mixed with untreated paramecia. All samples were heated at 55-60° C. for one half hour before using. Beginning on the fifth day or six day after the last injection, all animals were bled for trial titer, and, if found to be satisfactory, additional blood was obtained on succeeding days by marginal ear vein bleeding. The serum was

removed from this blood after overnight refrigeration and stored, without preservation, at 4° C., in rubber capped serum vials or in sterile small screw-capped bottles. Small quantities of serum were withdrawn as needed from these stock bottles, heated in a waterbath at 55°-60° C. for one-half hour, and dilutions prepared for the tests using the solution suggested by Bernheimer and Harrison (1).

The paramecia used for the injections were grown by Professor Sonneborn and collected in the following manner. The races to be used were transferred from stock bottles to 250 ml. flasks containing approximately 100 ml. of lettuce infusion (4) to which pure cultures of *Aerobacter aerogenes* were added to serve as a source of food. The cultures were maintained at a temperature of  $27^\circ \pm 1^\circ$  C. It was unnecessary to handle the flask cultures aseptically. Each culture was used when 3-5 days old. At the time the paramaecia were to be used, they were aggregated in a dense ring immediately below the surface of the fluid and around the contact between fluid and flask. This dense ring of paramecia was withdrawn from each flask with a pipette to which a rubber bulb was attached and the collections from a number of flask cultures of the same race were combined until 20 to 40 ml. were obtained. The combined material was then centrifuged at approximately 1500 r.p.m. for 15 minutes and the sediment resuspended in approximately 5 ml. of liquid.

In techniques #4 and #5 the material was given further treatment before injection. The suspension was drawn into and expelled from a syringe, fitted with a fine bore (#22 gauge) needle, for a minimum of 25 times by which time the paramecia were broken into small fragments. For technique #4 this material was then centrifuged for 1-2 hours at 3500 r.p.m., and the *supernatant fluid* used for injection. This represented an attempt to approximate the conditions previously found to be successful for preparing flagellar suspensions of bacteria (3). microscopic examination of this supernatant material revealed numerous separate cilia. For technique #5, the culture was mechanically agitated but not centrifuged.

### Results

As discussed by Bernheimer and Harrison (1) and others, the criterion for judging the activity of a serum is based upon a phenomenon called an "immobilization reaction". When fresh, living paramecia are mixed with an immune serum there is a disturbance in the rate of locomotion of the animals. In low dilutions of a potent serum, complete immobilization and sometimes death of the paramecia may be observed within a few minutes. In more dilute solutions of the serum, the paramecia become immobilized more slowly and may show spontaneous recovery after a few hours. Higher dilutions of the sera may produce only a retardation of locomotion. We have taken as the titer of a serum that dilution which showed a definite slowing of locomotion of fresh paramecia at the end of a two-hour period. The animals were mixed with the diluted serum in small concavities in thick slides and the mixtures incubated at

27° ± 1° C. All observations on presence or absence of locomotion were made with a wide-field binocular dissecting microscope.

Of the five variations in technique which were tested the best seems to be the injection of the supernatant fluid from the mechanically agitated culture. This material contained a large number of cilia but relatively few, if any, intact cells. By this technique it has been possible to produce with regularity sera giving a titer of approximately 1-1000. In some instances the value was as high as 1-4000. No reaction was obtained when *Aerobacter aerogenes* was mixed with this serum. The titers which we obtained using technique #4 seem to be better than the titers of the sera produced by Bernheimer and Harrison (1, 2) who are the latest workers to publish extensive studies of the serology of *Paramecia*. They used six or seven intraperitoneal injections of whole cells given at weekly intervals.

It is interesting to note that the heated material appears to be non-antigenic if we may judge by the fact that we observed no antibodies following the injections noted above. Reactions were obtained with animals from techniques #1 and #2, but in general the titers of these sera were less satisfactory than that obtained with technique #4. Although the number of animals tested was smaller, reactions obtained with sera produced by technique #5 appear similar to the values obtained by technique #4. In general it would seem that the antigenic material is located on the surface of the paramecia.

### References

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