Allantoic Fluid as a Source of Arbovirus Hemagglutinin

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Noninfectious hemagglutinins were prepared from the allantoic fluids of embryonated chicken eggs infected with Sindbis virus or with Murray Valley encephalitis virus.

Since hemagglutinating activities were first described (9), hemagglutinins have been prepared from many arboviruses, usually from the brain or other organs of suckling mice. Infected organs have been treated with acetone-ether (1), sucrose-acetone (3), or Tween-ether (7) to extract hemagglutinin. Methods for the production of hemagglutinin from infected cell cultures have also been developed (4). Both complement-fixing antigens (6, 8) and hemagglutinin (8) have been extracted from chick embryos infected with Eastern and Western equine encephalitis viruses. Some other arboviruses will multiply in developing chick embryos, and high titers of Sindbis virus and Murray Valley encephalitis (MVE) virus have been demonstrated in the allantoic fluid of infected embryonated eggs (2). The production of hemagglutinin from allantoic fluid is described below.

MVE virus (strain MRM 66) and Sindbis virus (strain MRM 39) were kindly supplied by R. L. Doherty of the Queensland Institute of Medical Research, Brisbane. Virus stocks were maintained by intracerebral passage in suckling mice, and hemagglutinins of mouse brain origin were prepared by sucrose-acetone extraction (3). Chick embryos, after 10 days of incubation, were inoculated into the allantoic cavity with 0.2-ml volumes of 10^{-2} suspensions of infected mouse brain in tris(hydroxymethyl)amino-methane-buffered Hanks balanced salt solution containing 0.2% bovine albumin. The eggs were then incubated at 37 C, and when the embryos died allantoic fluid was harvested and stored at −50 C. The following materials were tested for hemagglutinating activity: untreated allantoic fluid, allantoic fluid treated with Tween-ether (7), allantoic fluid treated with arcton-heptane (5), and allantoic fluid that had been subjected to both treatments. Allantoic fluid from uninoculated eggs was similarly treated. Hemagglutination and hemagglutination-inhibition tests, with goose erythrocytes as indicator cells, were performed as described by Clarke and Casals (3), except that a microtiter adaption of the test was used. Antigens prepared from mouse brain and allantoic fluid were tested for infectivity by intracerebral inoculation of suckling mice. Hemagglutination-inhibition tests were performed with all antigens, by using chicken antiserum to Sindbis virus and MVE virus, respectively.

Hemagglutinins were present at titers as high as 1:128 for Sindbis virus and 1:256 for MVE virus in allantoic fluid that had been treated with both Tween-ether and arcton-heptane. The order of treatments did not affect the results. Fluids that had received only one of the treatments contained very small amounts of hemagglutinin, and untreated allantoic fluid had no activity. Uninfected allantoic fluid did not yield hemagglutinin. Hemagglutinin produced from allantoic fluid had no detectable infectivity, in contrast to mouse brain antigens which were infective for suckling mice. Homologous antisera inhibited both types of antigen to the same titer. Optimum pH values for hemagglutination were similar, except for a shift to the acid side when Sindbis virus allantoic fluid was treated with arcton-heptane before Tween-ether treatment.

Allantoic fluid proved to be a useful source of hemagglutinin for a group A virus (Sindbis) and a group B virus (MVE). Allantoic fluid is more convenient to harvest than suckling mouse tissues, and treatment with Tween-ether and arcton-heptane is more rapid than sucrose-acetone extraction. The advantage of a noninfectious antigen is obvious. The Sindbis virus antigen from allantoic fluid was of higher titer.
than the mouse brain antigen usually prepared in this laboratory. The MVE allantoic fluid antigen was much lower in titer than that obtained from mouse brain, but it was still a useful antigen. Allantoic fluid may have general use as a source of noninfectious hemagglutinin for arboviruses that multiply in chick embryos.

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LITERATURE CITED