EARLY DETECTION OF ARBOVIRUSES IN TISSUE CULTURES BY HEMAGGLUTINATION

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[Following is the translation of an article by S. Ya. Gaydamovich and V. A. Vagzhanova, Institute of Virology imeni D.I. Ivanovskogo, USSR Academy of Medical Sciences, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) 9(6) 1964, pages 712--714. It was submitted on 2 Aug 1963. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Investigations, conducted by us with the viruses of Japanese and tick-borne encephalitises, showed that in the absence of a cytopathic effect in infected cultures virus indication could be accomplished with the help of the reactions of complement fixation and hemagglutination [1, 2]. Since hemagglutinins were detected in early periods following infection, we decided to use the hemagglutination test also in working with arboviruses, which possess a clearly expressed cytopathic activity. In the work we used the viruses of the Venezuelan and Western variants of American equine encephalomyelitis.

Materials and Methods

The virus of Venezuelan encephalomyelitis and the virus of the Western variant of American equine encephalomyelitis were obtained from the virus collection of the Institute of Virology imeni D. I. Ivanovskogo, AMN USSR, in the form of lyophilized brain of infected mice. The investigations were carried out with 1--7 passages in a tissue culture.

We used initially trypsinized cultures of chick fibroblasts. After 24--48 hours of growth we poured off the medium and deposited the virus in various dilutions on the cellular layer. After 20--30 minutes of contact at room temperature the virus was removed, the culture was washed one time with Hanks solution, and medium No 199 without serum was added. The volume of the medium for Karrel dishes was 15 ml, and for test tubes and penicillin flasks -- 1.5 ml. The virus for infection comprised 1/10 of the corresponding volume of the medium.

The hemagglutination reaction was set up with an 0.25% solution of goose erythrocytes at a pH of from 5.4 to 7.0. Dilution of the virus was performed in a borate buffer, pH 9.0, and the suspension of erythrocytes - in a phosphate buffer with varrying pH's.

Results

During hemagglutination with arboviruses there is great importance in the conditions for the reaction, especially the pH. We did not find any literary data on the optimum pH for the Venezuelan encephalomyelitis virus incubated in a tissue culture, therefore, we initially carried out a series of tests in order to study these conditions. Five experiments were set up with the Venezuelan encephalomyelitis virus and 4 with the virus of the

Western variant of American equine encephalomyelitis. These experiments showed that both variants have the same reaction range -- from pH 5.4 to pH 6.6. The optimum pH for appearance of hemagglutinins was 5.8--6.0.

We studied the time for the appearance of hemagglutinins in the cultures in various periods after infection with an interval of several hours. For eachtest we selected material either from one dish or we combined the cultural fluid from several test tubes or flasks. In the first tests we selected samples in 3, 6, 9, 12, 18 and 24 hours after infection, then the time for the appearance of hemagglutinins between the 3rd and 6th hours was studied with an interval of one hour. The reaction was set up at the optimum pH indicated.

During infection with mass doses of virus in the form of the whole cultural fluid with an approximate titer up to 10^7 -- 10^9 based on the CPD, the hemagglutinin of the Venezuelan encephalomyelitis virus could be detected in 5--6 hours.

Similar results were obtained with the virus of the Western variant of American equine encephalomyelitis.

In subsequent tests we compared the time for the detection of hemagglutinins and the cytopathic effect depending on the dose of the virus used for infection. These tests were set up only with the virus of Venezuelan encephalomyelitis. The results of the 2 tests are presented in the table.

As the results showed, even during infection of the cultures with small doses of virus the hemagglutinins were detected earlier than the cytopathic effect was displayed. The titers of hemagglutinins in the early periods were 1:4 --1:7. By 15 hours they reached 1:1024 in individual tests. Based on the hemagglutination phenomenon it is possible to titrate the virus in a tissue culture and obtain an answer in 12--15 hours. The method of hemagglutination may be somewhat simplified -- instead of setting up the reaction on polystyrene boards with pockets, it is possible to add the suspension of erythrocytes directly into the test tube or flask in which the cultures were incubated. After addition of the erythrocytes the test tubes are set up vertically in a rack, and the flasks are turned in the tray with the cellular layer upwards, so that the erythrocytes slide easier along the glass.

Conclusions

1. The hemagglutination reaction with the virus of Venezuelan encephalomyelitis and the Western variant of American equine encephalomyelitis, incubated in tissue cultures of chick fibroblasts, takes place at 4° in a pH zone of 5.4--6.6; optimum pH 5.8--6.0.

2. During infection of the cultures with large doses of virus within the limits of 10^7--10^9 CPD_{50} the hemagglutinins appear in the cultural fluid in 5--6 hours following infection, and the cytopathic effect is detected after 24 hours.

2. The hemagglutination phenomenon may be used for the early detection of a virus in cultures.

Literature

1. Gaydamovich, S. Ya., Obukhova, V. R., Vopr. virusol. (Problems of Virology), 1962, No 4, p 42.

2. Gaydamovich, S. Ya., Duan, Suan-myou, Titova, N. G., Ibid., No 1, p 43.