CONCENTRATION OF JAPANESE ENCEPHALITIS VIRUS WITH AMMONIUM SULFATE

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[Following is the translation of an article by V. N. Uvarov, Institute of Virology imeni D.I. Ivanovskogo, USSR Academy of Medical Sciences, Moscow, published in the Russian-language periodical Voprosy virusologii (Problems of Virology), No 4, 1965, pages 486--488. It was submitted on 24 May 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Together with the physical methods for concentrating viruses, a chemical method of purifying and concentrating them with ammonium sulfate is being used successfully [2, 3, 4, 5, 8, 9]. This method was also used for the concentration of the Japanese encephalitis virus /7/. The aim of our investigations was obtaining a concentrated and purified Japanese encephalitis virus, by precipitating it with ammonium sulfate, from an infected cultural liquid, and also determining the optimum conditions under which the highest level of concentration of hemagglutinins is achieved.

Materials and Methods

The work was carried out on a primary culture of trypsinized chick fibroblasts. Suspensions of cells in a quantity of 8×10^5/ml were sown in liter flasks and incubated for 2 days. The growth medium of the tissue culture was made up of 45% Hanks solution, 45% amniotic fluid and 10% bovine serum. The cells of chick fibroblasts were infected with a brain suspension of virus (strain P-1) in a dilution of 10^{-1} on medium No 199; 0.4 ml of virus in 0.6 ml of medium No 199 was placed on a cell monolayer. After a 45 minute period of contact of the virus with the cells at room temperature the nonadsorbed virus was removed by means of washing the cells of the tissue culture with Hanks solution and after addition of the cultural medium the cells of the culture were incubated in 50 ml of medium No 199 for 48--72 hours at 37°.

During all the phases of concentrating the Japanese encephalitis virus with ammonium sulfate we carried out a quantitative determination of the concentration of virus with the help of the RGA, RTGA and infection of white mice. [RGA - hemagglutination reaction; RTGA - hemagglutination inhibition reaction.]

We used an 0.25% suspension of goose erythrocytes in the RGA and the RTGA. The reactions were set up on plastic plates according to the generally accepted method at pH 6.2 and temperature of 4° /1/.

Precipitation of the Japanese encephalitis virus with $(NH_4)_2SO_4$. In 48--72 hours after the infection of chick fibroblast cells with the Japanese encephalitis virus the infected cultural fluid was collected in 100 ml flasks and centrifuged at 2000 rpm for 4 minutes.

We added ammonium sulfate to the supernatant fluid and placed it in a refrigerator, where during the course of 18--24 hours the salting out of the virus in the form of an amorphous precipitate took place.

For establishing the optimum pH zones 50--100 ml of virus was poured into flasks. Ammonium sulfate in a concentration of 50 g% was added and with the help of a 1 n. solution of H_2SO_4 and a saturated solution of NH_4OH we established a pH of 3.0, 5.2, 6.2, 4.2 and 8.5 in each flask. The pH of these solutions was determined with the help of a potentiometer.

For obtaining the maximum precipitation of adsorbed virus we carried out centrifuging at 3000 rpm for one hour. The precipitate was diluted in 3 ml of borate buffer with pH 7.0--9.0 and the dissolved precipitate became a brick-red color. Dissolved salts of ammonium sulfate, which were found in concentrated solutions of virus containing suspensions, were removed by dialysis against a borate buffer solution with pH 7.0--9.0 at 4° for 2--3 days. For elimination of high molecular cellular proteins the concentrated and dialyzed virus was centrifuged at 20,000 rpm for 45 minutes.

The results of the tests showed that the optimum amount of ammonium sulfate, guaranteeing the most complete salting out of the virus in 18--24 hours at 4°, equaled 35--70 g per 100 ml (table 1).

As it was already pointed out, the salting out was carried out in various pH zones. At a pH of 5.2--8.5 the same concentration of hemagglutinins was reached, but the infectious ability of the virus was considerably lowered. It was noted that at 0° the concentration of virus takes place somewhat better than at 4° (table 2).

With the help of the stated method we were able to concentrate hemagglutinins by 32--64 times, however, the concentrated preparations of virus possessed a lowered infectious ability. In all probability at the moment of salting out the virus is decomposed and the disintegration of the infectious viral RNA takes place as a result of the influence of hydrogen ions and the dissociated salts of the solution on the virus particle /6/. Our data on the infectious stability of the virus during the process of its salting out with ammonium sulfate do not correspond with available data from other authors, who carried out investigations with plant viruses and poliomyelitis virus [4, 5]. These differences are apparently explained by the chemical structure of viruses and their resistance to the influence of chemical substances.

Conclusions

1. Japanese encephalitis virus hemagglutinins in an infected cultural fluid ~~may be~~ concentrated with ammonium sulfate.

2. Precipitation of hemagglutinins ~~takes~~ place with ammonium sulfate saturation of 35--70 g in 100 ml at 4--0° in different pH zones (3.2--8.5) in the course of 24 hours.

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Table 1

Concentration of Japanese encephalitis virus hemagglutinins depending on the amount of ammonium sulfate

No. of test	Amount of sulfate (in g%)	Initial titer of hemagglutination	End titer of hemagglutination
1	17.5	1:1024	1:4096
2	35.0	1:1024	1:32000
3	70.0	1:1024	1:64000

Table 2

Concentration of Japaneses encephalitis virus hemagglutinins by ammonium sulfate at various pH of the medium and temperature

No. of test	pH	Initial titer of hemagglutinins	End titer of hemagglutinins at 0°	Time (in hours) of salting out and temperature (in degrees)
4	3.2	1:128	1:512	
5	5.2	1:128	1:2048	
6	6.2	1:128	1:2048	24,4
7	7.5	1:128	1:2048	
8	6.2	1:32	1:1024	
9	7.0	1:32	1:1024	24,0
10	8.5	1:32	1:1024	

Note. The amount of ammonium sulfate in all the tests equaled 50 g%.