**PURIFICATION AND CONCENTRATION OF GDVII STRAIN
OF MOUSE ENCEPHALOMYELITIS VIRUS II. REPORT.
PURIFICATION AND CONCENTRATION OF GDVII
VIRUS BY MEANS OF RED CELL STROMA**

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Since a procedure of purification and concentration of virus by means of adsorption to and elution from erythrocytes is a simple and effective method, some experiments of purification of pneumotropic and neurotropic viruses capable of hemagglutination have been made by this method. The studies on purification and concentration of the GDVII strain of mouse encephalomyelitis virus were described by Lahelle and Ward, (1) and Nakagawa and Nakamura (2) (3) in recent years. However, that the concentration of the virus by this procedure did not produce satisfactory yields of virus because of hemolysis appearing in the process of concentration of the virus by elution from erythrocytes, and that the combination of the methods by means of methanol and acetone precipitation at low temperature with the method of hemagglutination was attempted for obtaining more satisfactory results, were reported in previous papers (2) (3).

The purpose of the present paper is to report the research on further more effective method by means of adsorption to and elution from the red cells stroma (4) which are capable of adsorption of viruses.

MATERIALS AND METHODS

Virus The GDVII virus used was maintained by intracerebral passages in adult mice in this laboratory. Brains were removed from exsanguinated mice shortly after the appearance of signs indicating infection of the central nervous system, and a ten per cent suspension was made up in saline solution (pH 7.0), and were removed the coarse particles by centrifugation at 3000 RPM for 5 minutes. These crude suspensions were employed promptly after preparation as the starting materials.

Stroma (5) A part of five per cent saponin (Merck) solution added to nine part of ten per cent human group O erythrocytes suspension with the saline solution, and after hemolysis occurred, the stroma suspension were made by three times washing and resuspending with saline solution.

Purification procedure of virus. The crude suspension was mixed with the equal

volume of human Group O erythrocytes suspension in saline solution. After the agitation every five minutes for one hour in the cold, the mixed solution was allowed to stand at 4 C overnight, and was separated the supernatant fluid from erythrocytes by centrifugation at 3000 RPM for 15 minutes. One third of the original volume of saline solution as eluant was added to erythrocytes sedimented, and was kept 37 C with occassional agitation for one hour. The supernatant fluid separated by centrifugation at 3000 RPM for 15 minutes was mixed with the equal volume of stroma suspension, and after occassional agitation for one hour in the cold, the mixture was allowed to stand at 4 C over night. After centrifugation at 3000 RPM for 15 minutes, the Sörensen buffer solution (pH 7.0) as eluant was added to the stroma sedimented which might adsorb the virus particles, and elution of virus was carried out by keeping at 37 C for one hour. The stroma was centrifuged off at 9000 RPM for 20 minutes in the cold, and the supernatant fluid was obtained as the final product.

Hemagglutination procedure (6). Briefly, serial 2-fold dilution of the suspension prepared tested in saline solution (pH 7.0) and 0.3% human Group O erythrocytes suspension were mixed. The agglutination were performed at 4 C. Readings were made after 2 hours by observing the agglutination pattern with the naked eye and taking the endpoint as the highest dilution.

Infectivity. Titration of the content of the virus in the materials was expressed as 50% infectivity endpoint (LD_{50}) by the inoculation of the materials into mice by the intracerebral route.

Nitrogen content. The nitrogen content of all materials was measured by the micro-Kjeldahl method.

EXPERIMENTAL

Determination of dosage of stroma and time of adsorption in the adsorption and elution of virus procedure with stroma. One, five and ten per cent stroma suspension were prepared, and each suspension was added to the equal volume of the crude virus suspension, and then was kept in the cold for 2, 4 or 18 hours. The data of determination of the effect for virus adsorption to the stroma by measurement of hemagglutination titer of supernate after centrifugation are shown in Table 1. The results indicated that the greater amounts of virus particles in the starting materials were adsorbed to the stroma during the adsorption procedure for 18 hours in the cold, but that the adsorption of virus to the stroma suspension was less effective than that of virus to the erythrocytes. Therefore, a twenty per cent of the stroma suspension was employed for the following experiments.

TABLE 1
Relationship between dosage of stroma and time of adsorption in
the GDVII virus-hemagglutination procedure

% Stroma	Time of adsorption (hour)	Hemagglutination titer	
		Crude	Stroma supernate
1	2	2,560	128
1	4	2,560	80
1	18	2,560	80
5	2	2,560	640
5	4	2,560	80
5	18	2,560	80
10	2	2,560	640
10	4	2,560	80
10	18	2,560	80

Purification and concentration of GDVII virus by means of adsorption to and elution from erythrocytes and stroma. In the first step, the virus was adsorbed to the human O Group erythrocytes, and was eluted from erythrocytes by saline solution in the volume which did not occur hemolysis, and finally the virus eluted into the saline solution was adsorbed to and eluted from the twenty per cent stroma suspension.

The results obtained are shown in Table 2. The studies on the concentration of virus in the one two-hundredth of the original volume were attempted in this experiment. It is obvious that the concentration procedure of virus by stroma is more

TABLE 2
*Purification of GDVII-virus by means of adsorption and elution
with erythrocytes and stroma*

Exp. No.	Expected conc.	H A T LD ₅₀		Observed conc.	r N per ml		% removal N	H A T units LD ₅₀ units/mg N	
		Crude	Purified		Crude	Purified		Crude	Purified
1	1	256	256	1	1484.0	117.6	92.1	$1 \times 10^{2.55}$	$1 \times 10^{3.64}$
2	20	4096	4096	16	1078.0	8.4	99.2	$1 \times 10^{2.96}$	$1 \times 10^{5.99}$
3	100	256	524288	2048	981.4	1.8	99.8	$1 \times 10^{2.72}$	$1 \times 10^{8.76}$
4	200	256	2096152	8192	909.1	1.6	99.8	$1 \times 10^{2.75}$	$1 \times 10^{9.42}$
5	100	256	1048576	4096	991.2	5.6	99.5	$1 \times 10^{2.71}$	$1 \times 10^{8.57}$
5	100	$10^{-5.24}$	$10^{-5.62}$		991.2	5.6	99.5	$10^{-6.944}$	$10^{-9.571}$
Normal brain	100	0	0	0	918.4	28.0	97.0	0	0

effective than that of erythrocytes for the removal of total nitrogen in the final product, that is, the former is able to concentrate the virus in more approximately 1×10^6 hemagglutinating units per mg N, and 1000-fold LD_{50} per mg N than that of the original materials. These results are unable to obtain by adsorption to and elution from erythrocytes procedure because of hemolysis.

DISCUSSION

In previous reports (2) (3), the studies on purification and concentration of GDVII virus by means of hemagglutination were described. The results of the experiments indicated that the purification of the virus by means of adsorption and elution produce with erythrocytes was very effective method, however, that the concentration of the virus by this procedure did not produce satisfactory yields of virus because of hemolysis in the concentrating elution of the virus from erythrocytes. To avoid this hemolysis, various kinds of eluants of the virus from erythrocytes were examined, but unfortunately it was confirmed that all solutions employed could not avoid the hemolysis in the concentration procedure of virus, for instance, in the case of 10-fold concentration of virus by elution into one tenth volume of saline, hemolysis occurred without exception, and then the nitrogen content in the final products increased. Therefore, the concentration method by means of methanol and acetone precipitation at low temperature was employed for obtaining the good yield of virus in the purified material.

In the present paper, a very simple method of purification and concentration of virus which utilized the virus hemagglutinative capacity only, without the precipitation procedure, was reported. As there was an obstacle of hemolysis for getting good yield of virus in the final product in previous experiments, the stroma which has not consideration with hemolysis was employed in the present experiments.

The data of these experiments demonstrated the hemagglutination titer of the original crude suspension, in the experiment No. 4, was 256 and the titer of the final concentrated preparation was 2,096,152 with reduction of 99.8 per cent of the nitrogen, as well as in the experiment No. 5, the LD_{50} of the crude material was $10^{-5.24}$ and that of the final product was $10^{-5.62}$ with reduction of 99.5 per cent of the nitrogen. In other words, the final product contained 9.571 ($-\log LD_{50}$) units per mg N while the crude material contained 6.944 ($-\log LD_{50}$) units per mg N, i.e. it is indicated that virus is concentrated in approximately 1000-fold per ml with the removal of non-virus protein from the starting material. Lahelle and Ward (1) attempted the purification of the GDVII virus by means of adsorption to and elution from stroma corresponding to 10 per cent red cells, and got more effective yields of virus than that of

hemagglutination procedure. However, they did not make an experiment of concentration of virus. Homma et al (7) demonstrated that the chicken red cell stroma is suitable to adsorb and elute the influenza virus for production of influenza viral vaccine, and that the capacity of the stroma to adsorb and elute the influenza virus was heat resistant (100 C for 30 minutes) and was unchanged when stored at 4 C as long as 4 weeks.

It may be emphasized, therefore, that a procedure by means of adsorption to and elution from stroma is a simple, effective, and valuable method for the purification and concentration of virus capable of hemagglutination. However, as the removal of normal components (8) contained in the normal brains which have the dispute in the case of purification and identification of virus by observing the electron micrographs has not been made by this method, the following experiments, in future, should develop to solve the problems concerning the normal components.

SUMMARY

Purification and concentration of the GDVII strain of mouse encephalomyelitis virus by means of adsorption and elution with red cell stroma was attempted. This method is a simple, effective and useful procedure in high concentration of virus without consideration of hemolysis.

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