# A RAPID METHOD FOR THE PURIFICATION OF THE $MEF_1$ STRAIN OF POLIOMYELITIS VIRUS

by

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During attempts at determining the iso-electric point of the $MEF_1$ strain of poliomyelitis virus in extracts of suckling mouse brain it was noted that the extracts, which were free from coarse particles, became turbid when dialysed against dilute buffers at pH values below 7. We therefore examined the effect on the titre of the virus suspensions on dialysis against buffers at different pH values. This led to a simple method of purifying poliomyelitis virus. The fact that SK poliomyelitis virus and encephalomyelitis virus in mice can be precipitated at approximately pH 4.5 and redispersed at pH 8 without loss of activity has been described[1,2].

## EXPERIMENTAL

The virus was the $MEF_1$ strain of poliomyelitis virus adapted to suckling mice in our laboratory[3]. It was prepared as a 10% suspension of the brains in $M/75$ phosphate buffer pH 8.2 containing 0.044 $M$ NaCl and 10% rabbit serum. The crude suspension was spun at 2600 r.p.m. for 1 hour and 10 ml portions of the supernatant fluid were dialysed against 2 litre amounts of phosphate-citrate buffers at different pH values at 4° C for 48 hours. The buffers were those of McIlvaine diluted 1 in 5 with distilled water. The dialysates were spun at 2600 r.p.m. for 1 hour. The deposits were resuspended in $M/15$ phosphate buffer of pH 8.2, which brought a part only of the precipitate into solution. The resuspended deposit and the supernatant fluid were titrated in albino mice 4 weeks old. Serial tenfold dilutions of the material to be tested were made in nutrient broth and each dilution inoculated into groups of 6 mice, each mouse receiving 0.03 ml intracerebrally. The mice were kept under observation for a period of 3 weeks. Titres are expressed as $LD_{50}$ calculated according to the method of REED AND MUENCH[5].

In a second experiment the brain suspension was mixed with an equal volume of McIlvaine's buffer of twice the normal concentration giving a final pH of 4.3. The solution was not dialysed but was kept at 4° C for 48 hours and then spun at 2600 r.p.m. for 1 hour. The deposit was resuspended in $M/15$ phosphate buffer pH 8.2 as in the first experiment and both supernatant fluid and resuspended deposit were titrated in mice as in the first experiment.

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In a third experiment an emulsion of 500 infected suckling mice brains in 1000 ml of a 10% serum phosphate buffer M/75 pH 8.2 was centrifuged at 12,000 r.p.m. for 15 minutes. The clear supernatant fluid was separated and the deposit was resuspended in 400 ml phosphate buffer and again centrifuged for 15 minutes at 12,000 r.p.m. The combined supernatant fluids were dialysed against 10 litres of McIlvaine's buffer (diluted 1 in 5 with distilled water) at pH 4.3 for 48 hours at 4° C. The dialysed fluid was spun at 2600 r.p.m. for 1 hour and the deposit resuspended in 200 ml *M*/15 phosphate buffer of pH 8.2. The suspension was dialysed a second time as above for a period of 24 hours at pH 4.3, and the deposit after centrifugation, resuspended in 200 ml *M*/15 phosphate of pH 8.2. This was kept at room temperature for 2 hours, then cooled in iced water and an equal volume of cold chloroform and amyl alcohol mixture (1 part amyl alcohol to 10 parts of chloroform) added[4]. The mixture was shaken in ice-cold water for 15 minutes. After centrifugation at 2000 r.p.m. for 20 minutes the supernatant was treated as before with fresh chloroform-amyl alcohol and again spun at 2000 r.p.m. for 20 minutes.

The supernatant fluid freed from dissolved chloroform by exposure to a vacuum was centrifuged at 30,000 r.p.m. for 2 hours in the No. 40 rotor of a model L Spinco centrifuge. The pellets were resuspended in 10 ml phosphate buffer of pH 8.2 cooled in ice-water and subjected to chloroform-amyl alcohol treatment for 10 minutes. The chloroform was removed as before and the material centrifuged at 30,000 r.p.m. for 2 hours. To facilitate redispersion of the pellet 1 ml of a concentrated glucose solution was layered at the bottom of the tube before centrifugation. The resulting clear pellet measured 3 to 4 mm in diameter. This was redispersed in 1 ml of 10% rabbit-serum-saline, and the virus content determined by titration in mice.

Because of the small amount of material in the final pellet no direct estimation of its protein content was attempted, but an indirect estimate was obtained from comparison with the size of pellets formed on centrifugation of solutions of haemocyanine which have approximately the same particle size as the poliomyelitis virus. Ten ml amounts of Caminella sincta haemocyanine (sedimentation constant 100 *S*) of different concentrations were centrifuged at 30,000 r.p.m. for 60 minutes in tubes similar to those used for the final concentrations of $MEF_1$ virus. The diameters of the pellets were measured. In Fig. 2 the results are recorded graphically. By interpolating the size of the final pellet of the $MEF_1$ poliomyelitis material into this curve the amount of sedimentable protein present could be estimated.

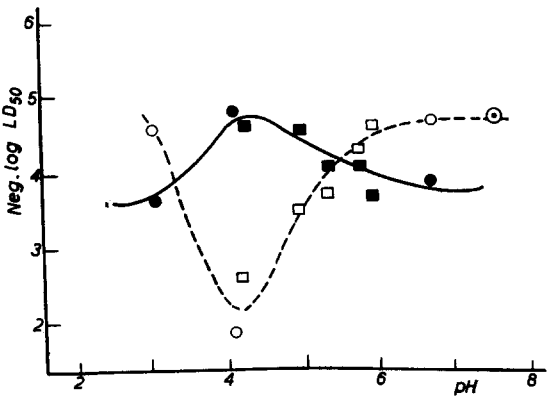


Fig. 1. The open circles and squares represent the titres of the supernatants in two experiments respectively (interrupted line). The solid circles and squares are those of the corresponding deposits (solid line). The value at pH 7.5 is the average titre of the original undialysed emulsion.

## RESULTS

In Fig. 1 are given the results obtained in two of the first experiments. The interrupted line represents the titres (expressed as negative log $LD_{50}$) of the supernatant fluid after dialysis against buffer at pH 4.3,

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whilst the solid line is that of the deposits resuspended in an amount equivalent to the original volume.

It will be noted that with lowering of the pH the titres of the supernatant fluids become progressively reduced until a pH of approximately 4.3 is reached whereafter the titres again increase. The reverse occurs in the case of the deposit where the maximum titre is reached at approximately pH 4.3.

In the second experiment carried out at higher salt concentration and without dialysis it was observed that the supernatant fluid was not as clear as that obtained against dilute buffer of pH 4.3. Moreover there was considerably more virus in the supernatant fluid ($LD_{50} = 10^{-4.5}$) than in that from the dialysed material ($LD_{50} = 10^{-2.0}$) indicating that a low electrolyte content is a necessary factor in the removal of the virus from the supernatant fluid.

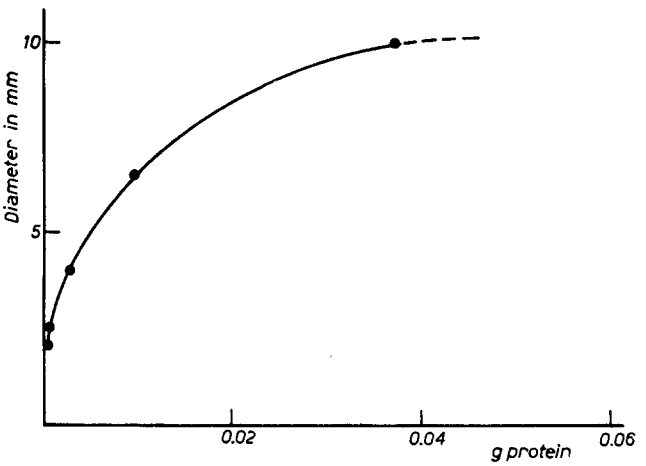


Fig. 2. Curve showing the relationship between amount of protein and the diameter of the pellet. The protein spun was the haemocyanine of Caminella sincta with sedimentation constant 100 S. It was subjected to centrifugation sufficient to deposit all the protein present.

In the third experiment the $LD_{50}$ of the original suspension (1400 ml) was $10^{-4.5}$ and that of the resuspended pellet (1 ml) $10^{-7.8}$ indicating a quantitative recovery of the virus within the bounds of experimental error.

By the indirect method we have described it could be shown that centrifugation and chloroform extraction has resulted in a significant degree of purification. Thus virus was recovered almost completely from 1400 ml of crude suspension containing 13.7 g of non-dialysable solid material but in the final pellet containing all the virus there was only 1.1 mg or 0.008% of the total non-dialysable solid material of the original emulsion.

## DISCUSSION

It would appear from these experiments that extracts of the brains of suckling mice infected with $MEF_1$ strain of poliomyelitis virus contain one or more substances which can be used in the removal of virus from solution. The substances precipitate during dialysis against dilute buffer at pH 4.3 apparently adsorbing the virus from

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solution. On resuspending the precipitate at pH 8.2 the virus is brought back into suspension while at least one of the brain substances remains undissolved. The virus can be further freed from protein by chloroform treatment without detectable loss of its titre, and can be easily concentrated by ultracentrifugation producing a very small pellet. The purity of this final product still needs confirmation by electron microscopy.

It is known that nuclear proteins remain in solution after chloroform treatment. The fact that the poliomyelitis virus does the same may be an indication of its chemical constitution.

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SUMMARY

1. It was shown that on dialysing infected brain suspensions against buffers of different pH values the virus comes down in the precipitate at pH 4.3 leaving little virus in the supernatant fluid.

2. The virus in the deposit can now be redispersed in a small volume of phosphate buffer of pH 8.2.

3. The substances responsible for the adsorption of the virus to the deposit can be removed by shaking with chloroform without loss of titre of the virus.

4. The virus in a relatively pure state can now be recovered quantitatively by ultracentrifugation.

RÉSUMÉ

1. Lorsque l'on dialyse des suspensions de cerveau infectées contre des tampons de pH différents, le virus se trouve dans le précipité formé à pH 4.3, tandis que le liquide surnageant n'en renferme que peu.

2. Le virus précipité peut ensuite être dispersé dans un petit volume de tampon phosphate à pH 8.2.

3. Les substances responsables de l'absorption du virus sur le précipité peuvent être éliminées par extraction au chloroforme sans perte de titre du virus.

4. Le virus, dans un état de pureté avancée, peut alors être récupéré quantitativement par ultracentrifugation.

ZUSAMMENFASSUNG

1. Es wurde gezeigt, dass bei der Dialyse infizieter Gehirnsuspensionen gegen Puffer mit verschiedenen pH-Wert das Virus bei pH 4.3 in den Niederschlag geht und wenig Virus in der überstehenden Flüssigkeit verbleibt.

2. Das Virus im Niederschlag kann nun in einer kleinen Menge Phosphatpuffer vom pH 8.2 wieder dispergiert werden.

3. Die für die Absorption des Virus an den Niederschlag verantwortlichen Substanzen können ohne Verlust des Titers des Virus durch Schütteln mit Chloroform entfernt werden.

4. Das Virus in relativ reinen Zustand kann nun quantitativ durch Ultrazentrifugation erhalten werden.

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