

Electron microscopy of neurotropic African horse-sickness virus

BY A. POLSON AND D. DEEKS

*C.S.I.R. and U.C.T. Virus Research Unit, Medical School,
University of Cape Town, South Africa*

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INTRODUCTION

African horse-sickness virus is an important member of the arthropod-borne viruses and the disease it causes is endemic on the African continent and in the Middle East. It affects all equines and the mortality rate in horses may be high. The virus was first attenuated by Alexander (1935) by intracerebral passage in mice and such attenuated strains form the basis of the polyvalent live virus vaccine which is widely used today.

Ultracentrifugation studies have shown that the infectivity is associated with components of two different sedimentation constants but that most of this activity is associated with particles having a sedimentation constant of 476 Svedberg units (Polson & Madsen, 1954).

In this communication electron micrographs of the virus are presented.

MATERIAL AND METHODS

Virus

The A501 strain of horse-sickness virus was used in this work, and was kindly supplied by Dr R. A. Alexander, former director of Veterinary Services. The virus was injected intracerebrally into 3- to 6-day-old suckling mice and the brains harvested when the animals showed definite signs of involvement of the central nervous system 2-3 days after infection.

Thirty to sixty brains were emulsified in 0.066 M phosphate buffer of pH 7.0 for each experiment. Thirty suckling brains per 10 ml. buffer were used. After clarification of the emulsion in the model L Spinco ultracentrifuge at 10,000 rev./min. for 10 min., the supernatant fluid was stored at 21° C. before fractionation.

Virus and protein precipitant

Polyethylene glycol (p.e.g.) M.W. 6000 was found to be a suitable precipitating agent. A 32% solution of polyethylene glycol in 0.066 M phosphate buffer pH 7.0 was prepared as stock virus precipitant (Polson, to be published).

Zone electrophoresis

The apparatus and methods for zone electrophoresis were similar to those used by Polson & Cramer (1958). Borate buffer of pH 8.6 and 40% Analar sucrose

dissolved in borate buffer, the pH of which was finally adjusted to 8.6, were used in the zone electrophoresis column and in the formation of the sucrose concentration gradient.

Infectivity titrations

The titration of virus infectivity was carried out in mice 3–5 weeks old, using tenfold dilutions and six mice per dilution. The dose was 0.03 ml. and given by the intracerebral route.

Experimental

In preliminary tests on the most suitable concentration of polyethylene glycol for precipitation of horse-sickness virus, it was found that the maximum amount of virus was precipitated at a concentration of polymer between 3 and 4%.

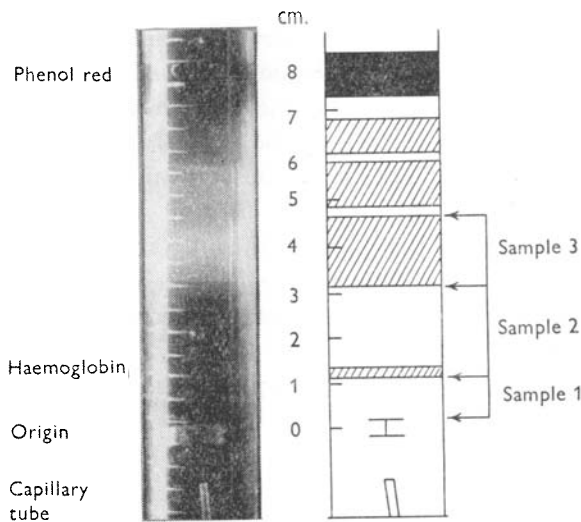


Fig. 1. Section of the zone electrophoresis column after completion of the electrophoresis run. On the left-hand side is a photograph of the column showing the different bands of opalescence and the position of the indicator substances, phenol red and haemoglobin. On the right is a diagrammatic presentation indicating the positions where the samples were taken for electron-microscopy relative to the opalescent bands (oblique lines).

To the virus suspension polyethylene glycol solution (32%) was added to yield a final polymer concentration of 3% and the mixture was kept in a waterbath at 21° C. A fairly heavy precipitate which formed was centrifuged off and discarded. The polyethylene glycol concentration was increased to 4% by further addition of the 32% solution, the reaction again being carried out at 21° C. The precipitate which formed was sedimented by centrifugation, dispersed in 10 ml. phosphate buffer and clarified at 10,000 rev./min. for 10 min. The virus in the S.N.F. was concentrated into a pellet by centrifugation at 30,000 rev./min. for 75 min. and re-dispersed in 2.2 ml. 35% sucrose–borate–buffer mixture containing a trace of phenol red and rabbit haemoglobin. After clarification at 10,000 rev./min. for 10 min., the mixture was subjected to zone electrophoresis for 8 hr. By the end of this period the phenol red had migrated 8 cm. in the concentration gradient column.

In Text-fig. 1 the bands of opalescent material are clearly visible in a photograph of the gradient column after completion of the electrophoresis run. In Text-fig. 2 the diagram indicates the distribution of virus activity relative to the bands of opalescence. It is obvious that the virus activity is not confined to a single narrow zone of the gradient column, but that it is spread over a wide region. When comparing this diagram with Text-fig. 1, it is evident that a considerable portion of the virus activity is contained in the clear zone below the regions of the opalescence. Identical opalescent regions were also found in normal uninfected brain extracts treated in the same way as the infected material. These bands must therefore be regarded as due to normal brain components.

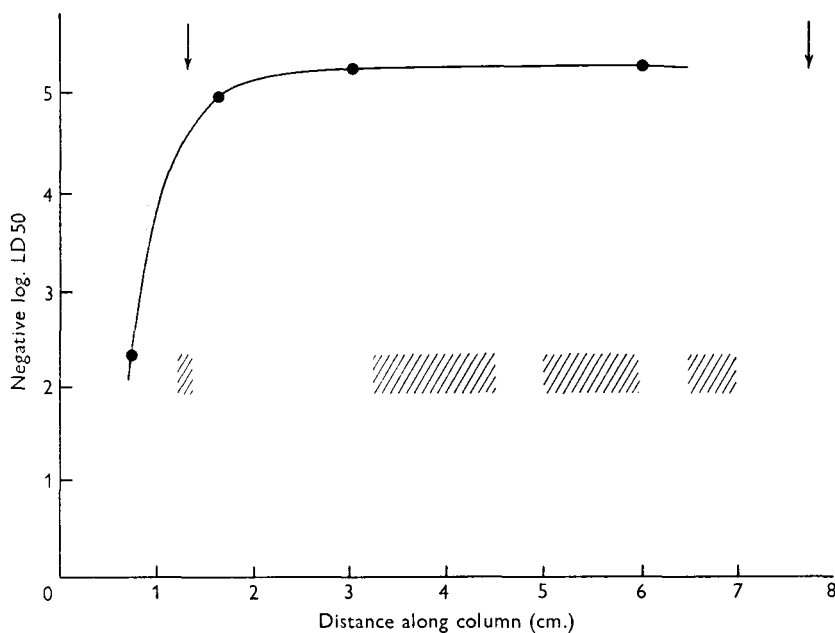


Fig. 2. Activity zone electrophoresis diagrams indicating the distribution of virus activity relative to the bands of opalescence (oblique lines). The arrows indicate the positions of the reference substances haemoglobin (left) and phenol red (right).

Each fraction taken from the column was freed of sugar by dilution with distilled water and ultracentrifugation. Two cycles of centrifugation at 33,000 rev./min. for 70 min. were adequate to reduce the concentration of the sugar to such a level that it did not interfere with electron microscopy. The final virus pellet was dispersed in 0.2 ml. of distilled water and negatively strained with an equal volume of neutral phosphotungstic acid. It was then placed on carbon films and examined in the Metropolitan Vickers EM 3A electron microscope.

RESULTS

Electron micrographs of the infected and the normal control material derived from fraction (2) (Text-fig. 1) taken at corresponding positions in the columns containing the infected and normal extracts respectively are shown in Pl. 1(a)

and (b). Virus-like particles resembling those of *Herpes simplex* virus photographed by Horne, Brenner, Waterson & Wildy (1959) are present in Pl. 1 (a). These do not appear on the electron micrograph of the control material Pl. 1 (b). Both micrographs contain a large number of spherical bodies of varying diameter which are all smaller than the virus particles. These are regarded as normal brain constituents not removable by the present technique.

Judging from the size and shape alone, the virus particles appearing in the electron micrographs correspond with particles of sedimentation constant 476S reported for the main horse-sickness virus infective component (Polson & Madsen, 1954). It was not possible to identify virus particles with sedimentation constants lower than this in the electron micrographs on account of the presence of particles in the normal material.

Counting the number of subunits on the surface of the virus gave a figure of approximately 40. As the number of subunits visible on a negatively stained virus particle represents slightly fewer than half the actual number, it may be assumed that the particle of the A501 horse-sickness virus strain has 92 subunits on its surface by the classification of Horne & Wildy (1961). Little detail can be seen of the units, but they appear to be short rods which lie on the radii of a spherical body.

Using the polyethylene glycol precipitation technique in combination with ultracentrifugation and zone electrophoresis, successful electron micrographs of the virus have been obtained on three out of a total of five attempts. An essential in the purification procedure is to have as little delay as possible in the preparation of the electron microscope grid due to the instability of the virus in albumin-free media.

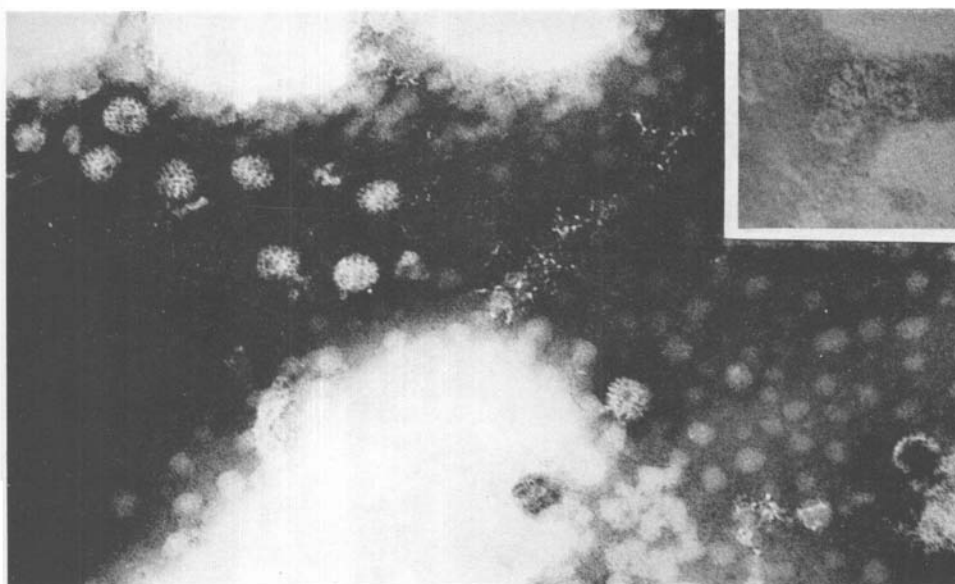
Electron micrographs of the virus have also been obtained from material collected from the fast-migrating opalescent zones (Pl. 2). The virus particles had the same appearance as those obtained from the slower-migrating clear zone of the column, but the background material was quite different.

Further attempts at purification of horse-sickness virus

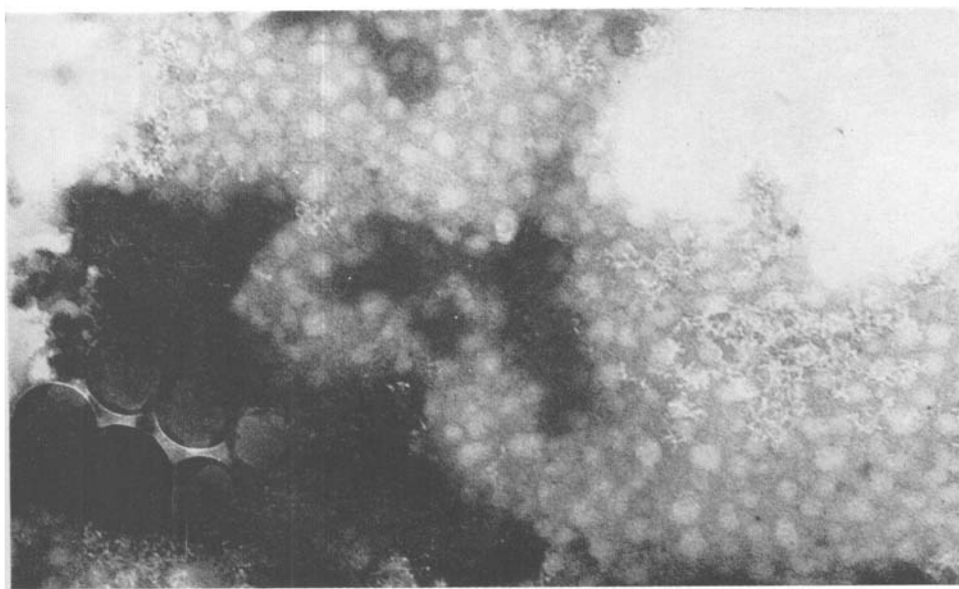
Various other methods coupled with zone electrophoresis have been tried for removal of extraneous proteins from infected brain extracts. These were treatments of the crude brain extracts with ether, chloroform and fluorocarbon, digestion of the associated proteins with trypsin and by precipitation of the virus with protamine sulphate. None of these methods was successful, as the virus was either inactivated or it was not recovered from the precipitate in any form suitable for electron-microscopy.

DISCUSSION AND SUMMARY

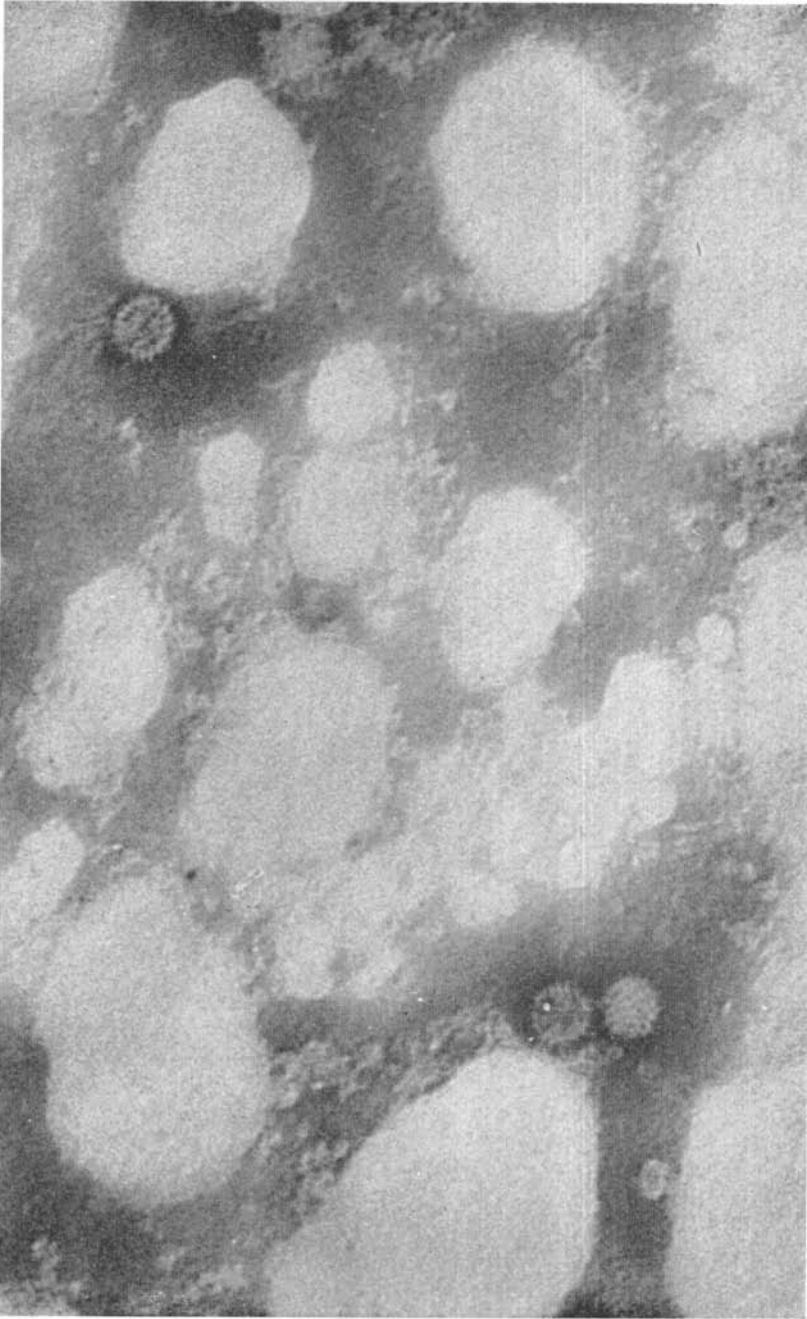
During attempts at purification of horse-sickness virus for electron-microscopy from infected mouse brain extracts, it was found that a combination of polyethylene glycol precipitation of the virus, ultracentrifugation and zone electrophoresis gave promising results. The final purified material could not be regarded as pure on account of the presence of considerable normal brain components, but



(a)



(b)



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it contained particles not present in normal brain treated identically, which could be recognized as virus particles. The virus particles appear to have diameters of 70–80 $m\mu$ and are estimated to have 92 rod-shaped subunits radiating from a spherical body.

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EXPLANATION OF PLATES

PLATE 1

- (a) Infected material showing virus particles and a virus capsid lower right-hand corner.
(b) Normal brain extracts subjected to same treatment as infected material. Note the occurrence of macromolecular material in both electron micrographs. The spherical bodies in lower left-hand corner of (b) are polystyrene latex particles of 138 $m\mu$ diameter. Inset in (a) probably virus capsids in a state of disruption at slightly lower magnification.

PLATE 2

Electron micrograph of material collected in the region of heavy opalescence showing virus-like bodies. The typical macromolecular components observed in the previous micrographs are absent in this region of the gradient column.