ISOLATION OF THE SMALL INFECTIVE PARTICLE IN ADAPTED MEF_1 POLIOMYELITIS

A. POLSON AND G. SELZER

The South African Council for Scientific and Industrial Research, and the University of Cape Town Virus Research Unit, Department of Pathology, University of Cape Town (South Africa)

The lack of homogeneity of size of infective particles has been reported to be a characteristic of suspensions of a number of viruses. It has been shown by centrifugation in the preparative Spinco ultracentrifuge that in infective mouse brain suspensions of neurotropic African horsesickness virus, particle sizes of 24, 30 and 50 mμ are demonstrable (POLSON AND MADSEN[1]; POLSON[2]). In neurotropic Rift Valley Fever virus (R.V.F.), which had had passages in eggs as well as in mouse brains during adaptation, an infective component of particle size 30 mμ occurred together with a 50 mμ component (NAUDÉ, MADSEN AND POLSON[3]). In suckling mouse adapted MEF_1 poliomyelitis virus, particle sizes of 24 and 30 mμ had been found (SELZER AND POLSON[4]).

In contrast to these findings a number of viruses which were the carriers of infectivity have been found to be monodisperse in particle diameter. Thus in Yellow Fever (POLSON[5]), Semliki Forest and West Nile viruses (HAMPTON AND POLSON[6]), infective particles of only 30 mμ have been noticed. Similarly only the 50 mμ particles were demonstrable in pantropic R.V.F. virus and neurotropic R.V.F. virus. These had received passages in mice only.

Attempts to cultivate the smaller horsesickness particles and maintain them in passage, free from the 50 mμ units, have been unsuccessful. After nine ultracentrifugal separations and limiting dilution passages in mice the virus still showed the presence of the 50 mμ infective particles (POLSON AND MADSEN[1]). Different results have, however, been obtained with the small infective particle of adapted MEF_1. In this case we have succeeded in isolating the 24 mμ form and showed that its progeny, when inoculated intracerebrally into adult as well as suckling mice, is composed of the 24 mμ particles only.

EXPERIMENTAL

The virus purified from 60 infective suckling mouse brains, by isoelectric precipitation, ultracentrifugation, and chloroform treatment, according to the method of POLSON AND SELZER[7], was suspended in 1 ml of a solution of 10% normal rabbit serum in $M/15$ phosphate buffer of pH 7.0. This was followed by the isolation of the 24 mμ particle using the agar gel technique of POLSON[2]. The agar gel extract which contained the greater portion of the 24 mμ particles was subjected to analysis for particle size using the Spinco ultracentrifuge. The sedimentation diagram obtained is shown in

References p. 600.

Fig. 1. This diagram should be compared with that in Fig. 2 which is a typical sedimentation diagram of the suckling mouse adapted MEF_1 virus as obtained from infected suckling mouse brain extracts. Although larger-sized virus particles may still be present in the virus extracted from the agar, the proportion of the smaller component is much greater, indicating that the bulk of the 30 mμ component had been removed. To further reduce the amount of the larger component in the material used for passage, a portion of the sample taken between the 2 and 2.5 cm levels (see Fig. 1) was used as inoculum for adult mice. When paralytic symptoms developed the mice were killed, their brains collected and an emulsion prepared for ultracentrifugation in the presence of *Caminella sincta* haemocyanine. As before, the sample between 2 and 2.5 cm was collected for the next passage in adult mice. This process of selection of the lighter particle was repeated three times. Brains from mice of the third passage were emulsified, and the size of the virus particle in the emulsion determined by centrifugation in the usual way. The result of the centrifugation is shown in Fig. 3. Here only the component of sedimentation constant 100 S (24 mμ

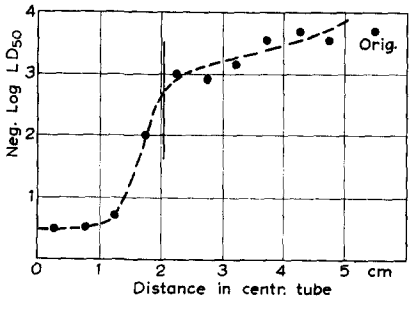

Fig. 1. Sedimentation diagram of the smaller component of MEF_1 virus separated by migration into agar gel. Centrifugation at 20,000 r.p.m. for 100 min. The vertical line marks the position of the haemocyanine boundary.

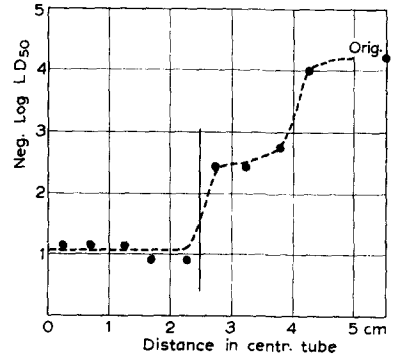

Fig. 2. Sedimentation diagram of MEF_1 virus in suckling mouse brain without preliminary selection of the smaller component. Centrifugation at 20,000 r.p.m. for 100 min. The vertical line marks the position of the haemocyanine boundary.

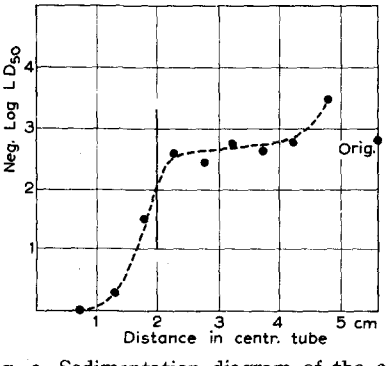

Fig. 3. Sedimentation diagram of the 24 mμ component MEF_1 grown in adult mouse brains. Centrifugation at 20,000 r.p.m. for 100 min. The vertical line marks the position of the haemocyanine boundary.

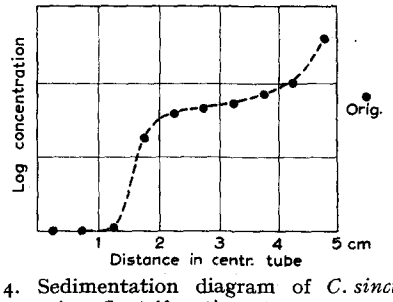

Fig. 4. Sedimentation diagram of *C. sincta* haemocyanine. Centrifugation at 20,000 r.p.m. for 100 min. The concentration in the different layers in the tube was plotted exponentially against distance in the tube.

References p. 600.

diameter) could be seen. That Fig. 3 represents a typical result with a suspension of monodisperse particles is substantiated by the results obtained with *C. sincta* haemocyanine in control experiments. The concentration of haemocyanine at various levels in a centrifuge tube which had been subjected to appropriate centrifugation, as determined by refractometric measurements, is shown in Fig. 4. It will be noticed that the distribution of the virus at various levels was very similar to that obtained with the haemocyanine, thus lending support to the conclusion that the virus was homogeneous with respect to particle size. The isolation of the 24 mμ particle in the manner described has been accomplished on three separate occasions.

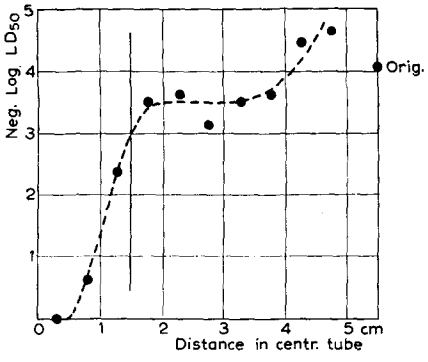

Fig. 5. Sedimentation diagram of 24 mμ MEF_1 virus grown for two generations in suckling mouse brains. Centrifugation at 21,000 r.p.m. for 60 min. The vertical line marks the position of the haemocyanine boundary.

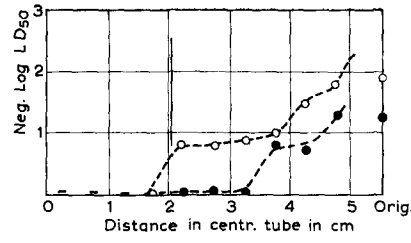

Fig. 6. Sedimentation diagrams of the MEF_1 virus before adaptation to suckling mice. In both diagrams there is evidence for the presence of the 24 mμ form of the virus. The vertical line marks the position of the haemocyanine boundary.

In Fig. 5 is given a sedimentation diagram of the virus of particle diameter 24 mμ after it had received two further passages in suckling mice. It is interesting to note that the 30 mμ form is still absent and that the titre of the unspun material is now appreciably higher than that of the material (24 mμ strain) which was grown in adult mice (see Fig. 3). The titre is also higher than that which the 24 mμ fraction had when it was present together with the 30 mμ virus in the original unfractionated suckling mouse adapted virus (Fig. 2).

The adaptation of the MEF_1 virus to suckling mice is not responsible for the appearance of the 24 mμ particles, since the two forms are present in the original unadapted strain. The sedimentation diagrams depicted in Fig. 6 show the result of two experiments performed in this material.

It can further be mentioned that no consistent results were obtained in experiments with suckling mouse adapted virus which received further passages in adult mice. The results obtained from two ultracentrifugation experiments, performed on fresh material on two different occasions, indicated that in the one experiment the 24 mμ virus was predominant and in the other experiment the 30 mμ form was present in highest titre. More experiments along these lines must be done to explain the phenomenon.

At the moment it is not known whether the 24 mμ particle will retain its homogeneity on prolonged passage in adult or suckling mice or in tissue culture, but the

References p. 600.

object of this report is to supply evidence that the small particle in adapted MEF_1 poliomyelitis is capable of being cultured independently of the larger-sized virus particle.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Professor M. VAN DEN ENDE for his interest in this work and for many helpful discussions. We are grateful to Miss D. DEEKS and Mr. T. NORCOTT for valuable technical assistance.

Additional funds were obtained from the Nkana-Kitwe and Chingola Poliomyelitis Research Funds.

SUMMARY

By means of a combined process of migration into agar gel and ultracentrifugation, the 24 mμ component of MEF_1 poliomyelitis virus adapted to sucklings has been separated from the 30 mμ particles with which it is normally associated, and grown in adult as well as in suckling mouse brains. The progeny of this separated variant in adult and suckling mice is composed of the 24 mμ particles only. This virus is probably a real variant as it "breeds truly" in the host, *i.e.* the suckling mouse.

REFERENCES

1 A. POLSON AND T. I. MADSEN, *Biochim. Biophys. Acta*, 14 (1954) 366.
2 A. POLSON, *Biochim. Biophys. Acta*, 19 (1956) 53.
3 W. DU T. NAUDÉ, T. I. MADSEN AND A. POLSON, *Nature*, 173 (1954) 1051.
4 G. SELZER AND A. POLSON, *Biochim. Biophys. Acta*, 15 (1954) 251.
5 A. POLSON, *Proc. Soc. Exptl. Biol. Med.*, 85 (1954) 613.
6 J. W. F. HAMPTON AND A. POLSON, To be published shortly.
7 A. POLSON AND G. SELZER, *Biochim. Biophys. Acta*, 14 (1954) 67.

Received November 30th, 1956

THE ELECTROPHORETIC MOBILITIES OF ADAPTED MEF_1 POLIOMYELITIS VIRUS AND ITS SOLUBLE ANTIGEN

A. POLSON, G. SELZER AND M. VAN DEN ENDE

The South African Council for Scientific and Industrial Research and the University of Cape Town Virus Research Unit, Department of Pathology, Medical School, Cape Town (South Africa)

In a previous communication it was reported that the greater part of the complement-fixing ability of an extract of MEF_1-infected suckling mouse brains was due to a substance of smaller size than that of the infective virus particles (SELZER AND POLSON[1]). From its rate of sedimentation in the preparative Spinco ultracentrifuge the diameter of the small component was estimated at 12 mμ. It was further shown that the infectivity was carried by particles of two different sizes. One to ten percent of the infectivity was associated with particles of 24 mμ and the remainder had a

References p. 603.