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EQUILIBRIUM CENTRIFUGATION OF MEASLES VIRUS

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SUMMARY : Measles virus has been centrifuged on different density gradients. It sediments at densities of 1,20 g/cm^3 in K-tartrate, of 1,18-1,21 g/cm^3 in sucrose, 1,19-1,23 g/cm^3 in CsCl and 1,19 g/cm^3 in metrizamide gradients. Metrizamide reduced measles virus infectivity. In sucrose gradients sometimes more than one infectious peak was observed. Control Vero cells produced particles of the same densities as measles virus peaks. These peaks did contain actin as the major protein. The relevance of this finding in relation to the presence of actin in measles virus is discussed.

Measles virus has attracted much interest for its possible role in subacute sclerosing panencephalitis. We and other authors have emphasized the difficulty of obtaining measles virus free of contaminating cell material (1-4).

Another feature of measles virus, shared with other paramyxoviruses, is the presence of actin in the virions (2,3). The reasons for this have never been adequately explained. The investigation presented in this paper was undertaken to clarify these points.

MATERIALS AND METHODS :

Vero cells were grown in Roux bottles in tissue culture medium 199 (Wellcome, Beckenham, England) and 7% newborn calf serum (Flow Inc. USA) (5). Maintainance medium (MM) was the medium described in (6), supplemented with 2% newborn calf serum.

Plaque purified Edmonston B measles virus was used. During virus growth, cells were maintained in MM. Viral titers were estimated by plaque formation on Vero monolayer cells.

Viruses were labelled with different isotopes : D-[1-^{3}H] glucosamine and [5,6-^{3}H] uridine were obtained from IRE, Fleurus, Belgium and were used in MM. L-[^{14}C] leucine, L-[^{14}C] valine, both from IRE, and L-[^{35}S] methionine (Radio-

Abbreviations used are : RSB : reticulocyte suspension buffer; SDS : sodium dodecyl sulfate; MM : maintenance medium.

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chemical Centre, Amersham, UK) were used in MM without calf serum and deficient in the appropriate amino acid.

Supernatant from infected cells was cleared from cell debris and centrifuged at 50.000 g during 4 hours over a 15% sucrose solution. The sediment was taken up in 1 ml buffer, either reticulocyte suspension buffer (RSB : 1,5mM Mg, 0,01M NaCl, 0,01M Tris/HCl pH 7,2) or TNE buffer (0,01M Tris/HCl pH 7,2; 0,1M NaCl; 0,02M EDTA).

This was centrifuged on discontinuous gradients with either sucrose, caesiumchloride, metrizamide or potassium tartrate in 11 ml tubes.

Polyacrylamide gel electroforesis was performed as in (7) except that sodium dodecyl sulfate (SDS) was used at a concentration of 0,2% in the gels and of 0,1% in the electroforesis buffer. After electrophoresis gels were processed for fluorography according to (8).

Measurement of radioactivity is as previously described (9).

RESULTS AND DISCUSSION :

Our results are shown in table 1, together with some data from the literature. Densities in our results are correlated with infectivity. Although differences exist, measles virus densities are mostly in the range of those reported in the literature.

In sucrose infectivity banded at δ = 1.18-1.21 g/cm^3 but in some experiments a second peak appeared at higher densities : 1,24-1,26 g/cm^3. The protein composition of this higher density peak is the same as that of the lower density peak (see below).

The density of measles virus in CsCl in our experiments was in the range of the values reported in the literature. In metrizamide however results are significantly different. But, where in other media, the infectivity peak always coincided with the major radioactive peak, this was not the case in metrizamide: most infectious material banded at a density of 1.09 g/cm^3, while the major radioactive peak was at a density of 1.19 g/cm^3 coinciding with an earlier report (19). We have shown earlier (10) that at this concentration metrizamide inactivates measles

Table 1. Density of measles virus in different density gradient materials (densities in g/cm^3)

Material	Our results	Literature
potassium tartrate	1,20	1,18 (11) 1,23 (12) 1,23 (4)
sucrose	1,18 - 1,21	1,16 - 1,20 (13) 1,17 - 1,19 (14) 1,25 - 1,26 (15)
CsCl	1,19 - 1,23	1,224 (16) 1,24 (17) 1,25 (18)
metrizamide	(1,09)	1,19 (19)

virus, but not at lower concentrations. This phenomenon could offer an explanation for the loss of infectivity of the band at $\delta = 1.19\ g/cm^3$ and recovery of infectivity at lower densities. It also illustrates that metrizamide is not absolutely inert, as was once thought.

However a great deal of variation exists between different reports. They might be due to differences in temperature of growth (11), multiplicity of infection (19) or pleomorphism of the virus.

To evaluate the amount of cell material mixed with the measles virus preparations, medium from non-infected cells was processed in an identical way. A large radioactive peak with the same density as measles virus was always found. Efforts to eliminate this material by ammoniumsulfate precipitation (2) were unsuccessful since cell material was also precipitated as evidenced by the density gradient patterns.

For study of the protein composition material from infected and non-infected cells were processed on sucrose.

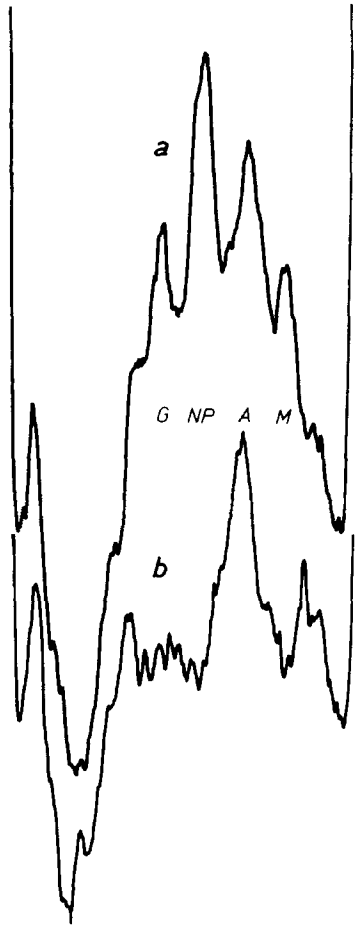

Figure 1 : Polyacrylamide gel electroforesis of peak material in sucrose. Electroforesis and fluorography as in Material and Methods. Densitometer tracing of fluorogram. a) measles virus peak b) cell control peak
(G : glycoprotein, NP : nucleoprotein, A : actin, M : membrane protein)

Radioactive peaks had densities of δ = 1.23; 1.21; 1.13; 1.10 g/cm^3 for cell material and δ = 1.25; 1.19; 1.12; 1.06 g/cm^3 for virus material. Samples from each fraction were electrophorized and autoradiographed.

Protein composition was the same for the 1.06 and 1.10 g/cm^3 bands and for the 1.12 and 1.13 g/cm^3 bands, showing no differences between material obtained from infected and non-infected cells. However, protein composition of the 2 peaks of

δ = 1.25 g/cm^3 and δ = 1.19 g/cm^3 in the virus material were identical, although less radioactivity was present in the band with δ = 1.25 g/cm^3. The 2 peaks of cell material (δ = 1.23 g/cm^3 and δ = 1.21 g/cm^3) were also identical but differed from the 2 virus peaks. In virus material the four major bands had molecular weights of 79.000 (G) 63.000 (NP) 46.000 (actin, 2,3) and 38.000 (M). In the cell material the only major protein band was found at the same level as the 46.000 MW band for measles virus. Thus, the analysis showed that the main protein band for the cell material was actin. This could mean that the presence of actin in measles virus preparations is due to contamination with cellular material with the same density as measles virus.

Nevertheless, Tyrell (3) argued that actin is present in measles virus. Treatment of measles virus with 0.1% trypsin yielded sedimentable particles containing actin. However, if measles virus preparations are mixed with actin-containing cell derived particles, it is not impossible that after treatment with trypsin, these particles sediment with the virions, resulting in preparations with a considerable amount of actin in it.

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