Heterogeneity of Virus Particles in Measles Virus

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SUMMARY

A heterogeneous population of virions is generated by measles virus-infected cells. These particles are partially separable by sucrose density centrifugation into three peaks. Each population is stable and contains infectious particles. The particles of all three populations contain at least six polypeptide species that differ between particle populations only in quantity. All three populations contain a 50S RNA species, and the heaviest density peak also contains an additional species of 43S RNA. The difference between these results and previous studies with measles virions will be discussed.

INTRODUCTION

Measles is classified as a paramyxovirus containing a non-segmented single-stranded RNA molecule with a mol. wt. of approx. 6 × 10⁶ (Schleuderberg, 1971). The infectious virion consists of a helical nucleocapsid which is membrane bound. The purified virion has haemagglutinating-haemadsorbing and haemolytic activities (Rapp et al. 1960; Baker et al. 1965). Although preliminary biochemical studies have been performed (Hall & Martin, 1973, 1974a; Waters & Bussell, 1973; Bussell et al. 1974; Mountcastle & Choppin, 1977; Tyrrell & Norrby, 1978), it has been difficult to obtain purified preparations in sufficient quantities to accurately discriminate between virus and host cell components. From our observations, the virus buds from the host membrane in a heteromorphic fashion. While the most abundant particle corresponds to that classically described in the literature, we have also noted particles of both heavier and lighter densities than the ‘standard’ particle. This study describes the structure and composition of these particles compared with standard virus.

METHODS

Cells. Clonal cell lines of CV-1, HeLa and mouse L cells were grown in monolayer cultures in Eagle’s minimal essential medium (MEM; Grand Island Biological Co.) with 10%, foetal calf serum at 37 °C. Cells were monitored for evidence of mycoplasma infection (Levine, 1972). The wild-type Edmonston measles virus was used unless otherwise specified. After an initial passage in CV-1 cells, the virus was cloned twice in the same cells. Virus

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used was from low passage, low multiplicity infections (< 0.05 p.f.u./cell). Stock virus was routinely harvested by scraping infected cells into the supernatant fluid with a rubber policeman when c.p.e. involved over 80% of the cell monolayer. After collection, virus was frozen at -90 °C. Maximum yields were obtained without initial freeze-thawing or sonication of harvested cultures.

**Infectivity assay.** The semi-microtitre method of Rager-Zisman & Merigan (1973) was used for titration of infectivity. The assay wells containing a confluent monolayer of CV-1 cells were exposed to virus for 1 h at 37 °C, overlaid with carboxymethylcellulose (CMC) in Eagle's MEM plus 10% foetal calf serum and then incubated at 37 °C. Six days p.i. the cells were fixed with formol-saline, stained with crystal violet and the plaques numbered.

**Isotopic labelling of virus.** For studies of virus polypeptides, roller bottles (750 cm² surface area) containing a confluent layer of CV-1 cells were infected at a m.o.i. of 0.01 to 0.05 and then labelled continuously at 26 h p.i. with 10 to 15 Ci/ml of 35S-L-methionine (New England Nuclear or Amersham; sp. act. 239 to 436 Ci/mM) in 10 ml of MEM plus 10% foetal calf serum. Companion cultures analysed for glycoproteins were labelled in the same manner with 1 to 2 Ci/ml of 14C-fucose or glucosamine (New England Nuclear; sp. act. 50 mCi/mM and 220 Ci/mM, respectively). Supernatant fluids were usually harvested at 42 to 48 h p.i., clarified by centrifugation at 1500 g for 5 min at 4 °C and either used immediately or stored at -90 °C. No significant differences in physical properties or infectivity were noted after storage.

For RNA analysis, infected cultures were labelled with 100 μCi/ml of 3H-uridine (Amersham, Arlington Heights, Ill.; 45 Ci/mmol) in 5 ml MEM per roller bottle at 21 h p.i. An additional 100 μCi/ml in 5 ml were added at 28 h p.i. Supernatant fluids from the cultures were harvested at 48 h p.i., clarified and stored at -90 °C.

**Virus particle purification.** The method of Stewart et al. (1973) was modified as follows: clarified supernatant fluids (containing approx. 3 x 10⁷ total p.f.u.) were incubated for 2 to 4 h at 4 °C with 40 μg/ml concanavalin A (Con A) [Miles-Yeda, Elkart, Ind.] in saturated NaCl or lyophilized (Calbiochem, La Jolla, Calif.) The Con A was always freshly prepared from the stock solution by diluting with phosphate-buffered saline (PBS) just before use. After incubation, the samples were centrifuged at 10500 g for 10 min. Con A was eluted from the virus pellet by the addition of α-methylmannoside to a final concentration of 0.4 M in NET buffer (0.1 NaCl, 0.001 M-EDTA, 0.01 M-tris; pH 7.3). The sample was layered over a 15 to 60% sucrose–NET gradient and centrifuged for at least 180 min at 55000 g in a SW27 rotor at 4 °C. One ml samples were then collected from the bottom of the gradient and 20 μl portions of each sample were precipitated with 5% trichloroacetic acid (TCA), solubilized in Liquifluor (Beckman Instruments, Fullerton, Calif.) and counted in a liquid scintillation spectrometer. Radioactive peaks were pooled, pelleted in an SW41 rotor at 27500 g for 15 min, resuspended and recentrifuged to equilibrium as above. Fractions were collected and peaks analysed for radioactivity and infectivity. Using this method more than 70% of total initial infectivity was recovered. Preparations in which α-methylmannoside was omitted or added in amounts insufficient to uncouple Con A from virus resulted in up to 99% reduction of virus infectivity.

**Analysis of virus polypeptides.** Polyacrylamide gel analysis was performed by the method of Laemmli (1970) on purified virions labelled with either 35S-methionine, 14C-glucosamine or 14C-fucose. Samples of approx. 30 μg protein each were applied to 10% polyacrylamide slab gels with a 3% spacer gel using a tris-glycine buffer and electrophoresed for 4 h at 80 mV. Gels were stained with 0.1% Coomassie brilliant blue, dried and exposed for autoradiography on Kodak NS2T safety screen X-ray film. Polypeptide bands were quantified with a Joyce-Loebl or an RFT scanning densitometer (Transidyne General Corp.
Ann Arbor Mich., U.S.A.). The concentration of individual virion proteins was determined by normalizing the concentration of the nucleocapsid-associated protein (NP) to 100. The relative concentrations of the other virion proteins and actin are expressed relative to NP concentration. To compare migration of polypeptides using other buffer conditions, slab gels were also used with either a 10% running gel with a 4% spacer according to Maizel (1969) and run at 80 mV for 4 h or a 7.5% phosphate gel, run for 4.75 h at 25 mV. Mol. wt. markers of 5 µg each included rabbit muscle myosin heavy chain (mol. wt. 200,000), lactoperoxidase (mol. wt. 78,000), bovine serum albumin (mol. wt. 67,000), rabbit skeletal muscle actin (mol. wt. 45,000) and myoglobin (mol. wt. 16,500).

Analysis of virion RNA. Virus grown in the presence of 3H-uridine was purified on two successive sucrose gradients as described above. Samples were pooled in three groups a, b and c as indicated in Fig. 2. Pelleted samples were solubilized in 2% SDS–NET buffer containing 0.3 ml of heparin sulphate (100 mg/ml) and applied to a 15 to 30% linear sucrose NET gradient (RNase-free sucrose obtained from Schwarz-Mann) containing 0.2% SDS and centrifuged in an SW27 rotor for 17 h at 55,000 g at 20 °C. One ml samples were collected and analysed for radioactivity by precipitation with 5% TCA; the precipitate was solubilized in Liquifluor and counted in a liquid scintillation spectrometer. During the course of collection, samples were also analysed for RNA at 254 nm by a Guilford Recording Spectrophotometer. Gradients also contained internal markers of 18 and 28S ribosomal RNA and 14C-uridine-labelled 45S ribosomal precursor RNA prepared from HeLa cells (Penman, 1969).

Electron microscopy. Pelleted samples were fixed in phosphate-buffered 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in ethyl alcohol and embedded in Epon for electron microscopy. Grids were double stained with uranyl acetate and lead citrate, coated with carbon and scanned in a Siemens 101 at 50 kV. The diameters of over 60 particles from each density were measured on coded micrographs. Average diameters were correlated with each density.

Infected cell monolayers were fixed with glutaraldehyde, and then post-fixed with osmium tetroxide. Cells were scraped from the surface with a rubber policeman pelleted at 1500 g for 5 min and processed in an identical fashion.

RESULTS

Electron microscopy

At 42 h p.i. over 80% of the cell monolayer was fused into a syncytium. Electron microscopic studies of these cells (Fig. 1) showed focal areas of the plasma membrane containing virus spike material. In close proximity to these modified areas of the membrane were fuzzy-coated nucleocapsids typical of those seen in purified virions. At this time, released particles were seen in the extracellular space. Most of these were uniform in size and shape, approx. 0.5 μm in diam., enclosed by a unit membrane (the lipid containing envelope) and were externally coated with electron-dense, spike material (Fig. 1b). In some areas, a minor population of virions both larger and smaller in diameter than the main virion population were present (Fig. 1a).

Sucrose density analysis

First density gradient centrifugation

A heterogeneous pattern of radioactive material was routinely obtained after sucrose density centrifugation of measles virions purified as described in Methods. The major peak of radioactivity banded at a density of 1.18 g/ml (Fig. 2). Minor peaks of radioactive material were also found at a density of 1.21 g/ml and 1.15 g/ml. Although the peak infectivity was
Fig. 1. Measles-infected CV-1 cells harvested 42 h p.i. (a) Released virions (V) within the extracellular space are generally circular, with a minor population of pleomorphic forms of variable size. Virion budding sites occur along the plasma membrane (arrows) which has been focally modified by an external lining of dense material. (b) A slightly higher magnification of another representative area of the same cell; released virions in the extracellular space are externally coated by the dense material (arrows) and contain nucleocapsids just beneath the enclosing membrane.

found to coincide with the major peak of radioactivity, both minor peaks contained infectious virus separate from the major peak.

Second sucrose gradient centrifugation

The peak fractions of the heavy density range, a (1.20 to 1.21 g/ml), middle density, b (1.17 to 1.18 g/ml) and light density range, c (1.14 to 1.15 g/ml) as indicated in Fig. 2, were collected, pooled and analysed by a second sucrose density gradient centrifugation.
Fig. 2. Sucrose density gradient purification of measles virus supernatant fluids harvested at 42 h p.i. The virus produced three density peaks of radioactivity (●—●) and infectivity (△—△), a major peak at 1.18 g/ml and two minor peaks at 1.21 and 1.15 g/ml.

Fig. 3. Sucrose density gradient purification of individual density peaks. Samples from first gradient were pooled at indicated densities and recentrifuged on individual sucrose gradients. Each peak maintained a stable density pattern. ●—●, Radioactivity; △—△, Infectivity.
Table 1. Infectivity of different density fractions of measles virus*

<table>
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<tr>
<th>Density (g/ml)</th>
<th>log&lt;sub&gt;10&lt;/sub&gt; Dilution/ml</th>
<th>Average number of plaques</th>
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<tbody>
<tr>
<td>1.21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
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<tr>
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<td></td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1.18</td>
<td>0</td>
<td>&gt; 100</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>&gt; 100</td>
</tr>
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<td>2</td>
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</tr>
<tr>
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<td>3</td>
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<tr>
<td></td>
<td>4</td>
<td>0</td>
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<td>1.15</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Samples were assayed for infectivity on CV-1 cells and plaques counted after 6 days incubation at 37 °C. Note reduction of plaques in samples of low dilution in 1.21 and 1.15 g/ml peaks.

As seen in Fig. 3, each peak consistently banded again at its original density. All three particle populations contained infectious particles which banded coincident with the major peak of radioactivity. Only minor amounts of spillover into adjacent densities were observed.

To compare different virus strains, hamster neurotropic strain of measles virus (HNT) originally derived from the Philadelphia 26 measles isolate (Burnstein et al. 1964) was grown in CV-1 cells and purified in an identical fashion to the Edmonston strain. Yields of released particles were at least 3 logs less than the Edmonston virus, but the same particle distribution was observed on sucrose gradients, with the major peak at 1.18 g/ml. Similarly, the density profile of particles purified from measles-infected HeLa or mouse L cell cultures was identical to that derived from CV-1 infections, although total infectious virus produced was 1 to 3 logs less, respectively (not shown). To examine the stability of each particle population, virus pooled from each density population was individually pelleted by centrifugation at 100,000 g for 1 h. Pellets were resuspended in NET buffer and examined by an additional sucrose gradient centrifugation. No significant alterations in density of each population were observed although the 1.18 g/ml fraction appeared more susceptible to breakdown with a reduction in radioactive counts (not shown). Samples from each density population were also sonicated with a Branson Sonifier for two 30 s intervals at a setting of 4. The densities of each peak remained constant on subsequent sucrose density gradient analysis of three individual preparations.

**Biological properties of the particle populations**

Infectivity of the three populations was examined by plaque assay of pooled samples of each density range following the second sucrose density gradient. Samples from each population were tested at 33, 37 and 39 °C. Only slightly greater total infectivity (1 to 2 logs) was found in each fraction at 33 and 37 °C although the maximum titres appeared about 24 h later than in virus grown at 39 °C. Auto-interference indicated by an absolute reduction in the numbers of plaques in undiluted samples compared to those of 1 or 2 log dilutions were observed in both the 1.21 g/ml and 1.15 g/ml densities incubated at all three temperatures. In contrast, a normal dilution-dependent curve was seen in the 1.18 g/ml peak sample (Table 1). No significant temperature sensitivity was observed in the original harvested supernatant before purification, nor, as indicated, in the individual particle populations.
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Fig. 4. Purified virions from each density peak contained spike material externally coating the encapsulating membrane that enclosed the fuzzy-coated nucleocapsid within. (a), (b) 1.18 g/ml peak; (c) 1.21 g/ml peak; (d) 1.15 g/ml peak. Magnification ×100000.

In plaque assays incubated at 37 °C and observed 6 days after infection, plaque morphology of the 1.15 and 1.21 g/ml fractions grown at 33 and 39 °C differed significantly from the 1.18 g/ml peak. These fractions produced abundant, small, non-lytic plaques (less than 1 mm diam.) while the 1.18 g/ml peak produced, primarily, the larger and lytic forms (greater than 2 mm diam.) and only a few of the smaller foci. However, similar to the findings of Rapp (1964), propagation of five individual clones of both large, lytic and small, non-lytic forms resulted in heterogeneous plaque progeny.

Ultrastructure of purified particles

Similar preparations of measles grown in the absence of radioisotope were purified and examined by electron microscopy (Fig. 4). The virions found at a density of 1.18 g/ml (Fig. 4b) were similar in appearance and size to measles virus previously described (Raine et al. 1969), as well as the major particle observed in the infected cultures. The larger sized particles (Fig. 4c) banded at 1.20 g/ml and the smaller (Fig. 4d) at 1.15 g/ml; each closely resembled particles released into the culture supernatant fluids as described above. Each contained more abundant nucleocapsid material within the central portion of the virion.
Table 2. Average diameter of virions pooled from each density peak

<table>
<thead>
<tr>
<th>Density peak (g/ml)</th>
<th>1.21</th>
<th>1.18</th>
<th>1.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size range (nm)</td>
<td>280-1260</td>
<td>200-710</td>
<td>170-650</td>
</tr>
<tr>
<td>Mean diam. (nm)</td>
<td>770 (±270)*</td>
<td>465 (±110)</td>
<td>410 (±120)</td>
</tr>
</tbody>
</table>

* Standard deviation of mean.

Fig. 5. Polyacrylamide gel electrophoresis of each pooled peak indicated by density range from second sucrose density purification. Seven major polypeptides were found which differed only in quantity. Note the reduction of H in the heaviest density sample (a) 1.21 g/ml peak; (b) 1.18 g/ml; (c) 1.15 g/ml.

rather than primarily under the enclosing membrane. The 1.18 g/ml fraction contained primarily the standard measles material but also a minor amount of particles characteristic of the other fractions (Fig. 4a). Each of the 1.21 g/ml and 1.15 g/ml fractions which also contained a minor amount of the other particle types and were less pure, with some contamination by cellular structures. The particles in each density peak showed significant heterogeneity of size (Table 2). The largest particles were observed at the 1.21 g/ml density, with mean diam. of 770 nm. The lightest density peak 1.15 g/ml primarily contained particles with a smaller mean diam. of 410 nm and the major peak at 1.18 g/ml contained particles of 465 nm.
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Table 3. Quantitative analysis of virion polypeptides

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Density g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.21</td>
</tr>
<tr>
<td>H</td>
<td>28.4 (±3.2)*</td>
</tr>
<tr>
<td>P</td>
<td>10.5 (±2.1)</td>
</tr>
<tr>
<td>NP</td>
<td>100.0</td>
</tr>
<tr>
<td>Actin</td>
<td>31.6 (±2.1)</td>
</tr>
<tr>
<td>F1</td>
<td>44.2 (±6.4)</td>
</tr>
<tr>
<td>M</td>
<td>5.3 (±2.8)</td>
</tr>
</tbody>
</table>

* Standard deviation of mean.

Polypeptide analysis of purified particles

Purified populations of 35S-methionine-labelled measles virus were examined by polyacrylamide gel electrophoresis (Laemmli, 1970). As seen in Fig. 5 all of the polypeptides were represented in the three particle populations, and neither unique large mol. wt. precursors nor any minor difference in migration of individual polypeptides of each particle species could be detected. Like other investigators (Waters & Bussell, 1973; Mountcastle & Choppin, 1977; Graves et al. 1978), we identified at least six virion polypeptides. Using the polypeptide designations of Graves et al. (1978): H, a glycoprotein of mol. wt. 78,000; P, a phosphoprotein of mol. wt. 69,000; NP, the major virion polypeptide of mol. wt. 60,000 which corresponds to the nucleocapsid associated species; a polypeptide of mol. wt. 45,000, co-migrating with rabbit skeletal muscle actin; F1, a 41,000 mol. wt. protein thought to carry haemolysin properties (Hardwicke & Bussell, 1978) and M, of mol. wt. 37,000.

A polypeptide of mol. wt. 53,000 was inconsistently present in all fractions but more abundant in the heaviest density fraction and thought to be of cellular origin. It is important to note that in Maizel (1969) or standard phosphate gels, the mol. wt. of all of the polypeptides could be assigned as indicated above except P which consistently migrated slightly more rapidly in both of these systems (not shown).

Quantitative differences (Table 3) were noted in several of the polypeptides in all three particle species. When normalized to NP protein, the higher density particles (1.21 g/ml) contained significantly less H, M and F1 polypeptides. When normalized to actin, similar results were noted in the H, M and F1 polypeptides (data not shown) and both NP and P polypeptides were nearly twofold more abundant in the 1.21 g/ml population. In contrast, the 1.15 g/ml peak contained significantly more H, which we as well as others (Mountcastle & Choppin, 1977) find to be the sole glycosylated polypeptide species under reducing conditions as determined by radioactive glucosamine or fucose label. This density peak also contains more F1 and M than the major particle peak of 1.18 g/ml.

All three particle species occasionally contained a large minor polypeptide of mol. wt. 200,000 that co-migrated with myosin heavy chain. This polypeptide has been noted by Waters & Bussell (1973) and Graves et al. (1978) and designated L, and remains unchanged in migration even under reducing conditions.

RNA analysis of virions

To see if the populations contained any unique RNA species, supernatant fluids of 3H-uridine-labelled, infected cultures were prepared as above by two successive sucrose density gradient purifications. Each density range in the second sucrose gradient was individually pooled, solubilized in 0.2% SDS and prepared for RNA sucrose density gradient analysis as described in Methods. The preparation was repeated on four separate occasions with identical results. As seen in Fig. 6, virion RNA species in each of the three populations
Fig. 6. RNA analysis of density peaks. Samples were pooled from the second sucrose density gradient at indicated densities and RNA extracted as described in Methods and co-centrifuged with 18, 28 and 45S HeLa RNA (arrows). Each density peak contained RNA of 50 to 53S; the heavier density virion contains an additional 43S peak.

were composed of two components consistent with those noted in other studies (Schleuderberg, 1971; Hall & ter Meulen, 1977) which have sedimentation values of 50S and 52S, respectively. The 1.21 g/ml population also contained a single radioactive species of approx. 43S value. Uninfected cells labelled in the same way did not yield significant amounts of 45S ribosomal RNA precursor. To exclude the possibility that this species associated with purified virus was of host cell origin, 14C-uridine-labelled 45S CV-1 ribosomal precursor RNA was prepared and included in the gradients. The RNA peak derived from the purified virus clearly migrated more rapidly than the 45S marker. While this 43S species was clearly demonstrable in the 1.21 g/ml density fraction, it is possible that this species might not be apparent if all three particle populations were pooled and analysed together. All three preparations contained a minor amount of smaller RNA species not as clearly resolved in the gradients that migrate in the range of 4S.

**DISCUSSION**

Heterogeneity of measles particle populations has been observed by several investigators. Chiarini & Norrby (1970) first described more than one particle population in HeLa cultures infected with serially passed, undiluted measles virus. Two particle species were noted on caesium chloride gradients that banded in equilibrium conditions, at 1.20 to 1.21 g/ml and 1.24 g/ml. The lighter species possessed haemagglutinating, haemolytic and complement-fixing abilities but contained significantly less infectivity as compared to the denser particle. In contrast to our observations, electron microscopy of the lighter particle popu-
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lation revealed larger and more pleomorphic particles than the 1.24 g/ml species. In cultures infected with serially passed but diluted stocks, only the denser particle was present in significant quantities. Therefore, the variability in appearance of particles of different density was produced by simply altering the dilution of the infecting stock.

Hall & Martin (1974b) identified two particle populations by velocity centrifugation of virus released into the supernatant fluids from infected cultures. Using a plaque reduction assay, they demonstrated that the more slowly migrating population interferes with the ability of the heavier species to produce infectious particles.

In the purification method using Con A described in Methods these particles with densities of 1.20 to 1.21 and 1.15 g/ml remained in the supernatant sample after centrifugation at 1500 g and continue to be observed throughout the subsequent purification procedure. In several other methods of preparation of purified virus, a more vigorous clarification step is initially employed and it is possible that the larger and denser particles are removed at that time (Hall & Martin, 1974b; Mountcastle & Choppin, 1977).

The host cell type or strain of virus does not appear to alter the particle density distribution. A similar particle distribution was observed in our laboratories after infection of both HeLa and mouse L cells although total infectious virus produced was 1 to 3 logs less, respectively, than in CV-1 cell hosts.

The mechanism for particle heterogeneity is unclear. Roman & Simon (1976) noted similar morphological variation in plaque purified Newcastle disease virus (NDV) grown in embryonated egg, chick chorioallantoic membrane and a variety of tissue culture cells. The size differences were not altered by differences in growth media, sonication, time of harvest or source of host cell. The particles had linear u.v. inactivation kinetics although the larger particles were observed to contain more RNA. Particles of the same sedimentation value on velocity gradients were observed to have different densities on isopycnic sucrose gradient centrifugation and were thought more likely to contain defective particles, possibly in multiploid form.

Further observations on the ability of NDV to generate heterogeneous particle populations were made by Granoff (1959) in single-cycle infections of cultures infected at very low multiplicities. Bratt & Hightower (1977) indicated that such cultures may contain potentially infectious but non-plaque forming virus.

In our studies, the polypeptide species of all three particle populations are qualitatively identical and are similar in mol. wt. to those described by Bussell et al. (1974); and Mountcastle & Choppin (1977). Our quantitative data indicates that the heaviest density range species (1.20 to 1.21 g/ml) contain smaller amounts of membrane-associated polypeptides, H, and F1 and the M protein relative to NP. Variation in migration of the P polypeptide was noted in gels using different buffer systems indicating that caution must be taken in assigning mol. wt. values to individual polypeptides examined under different gel conditions.

Ultrastructural observations reveal that the larger particle may also contain entrapped cellular components. Thus it may be impossible to obtain a totally 'pure' preparation of virus free of host cellular contaminants.

A variety of measles virion RNA species have been identified in several laboratories (Schleuderberg, 1971; Hall & ter Meulen, 1977). In our experiments, the virion RNA appears as a broad band with a prominent peak of 50S value when analysed on sucrose density gradients. A second major RNA species slightly larger than 50S was observed in all three density regions but was most abundant in the 1.20 g/ml peak. A similar peak was seen by Hall & ter Meulen (1977) in sucrose density gradient RNA analysis of measles-infected cells. Some of the RNA in this region contained poly(A) sequences, under dissociating conditions rebanded in sucrose in the 12 to 36S range and was therefore considered possibly to be a base-paired messenger RNA species.
Most significant is that in our studies the 1.20 g/ml density contains a 43S RNA species as well as the 50S virion RNA. We are unsure of the role of this species in virus replication, but we are convinced that it is not a 45S cell RNA contaminant. After undiluted passage of the virus, Kiley et al. (1974) observed RNase-resistant nucleocapsids of 170S, but the corresponding RNA composition was not indicated in their studies. Hall & Martin (1974b) observed a second population of nucleocapsids of 130 to 150S that contained a single peak of 18S RNA. The 43S RNA species we found in the 1.20 density particle may form a subgenomic sized nucleocapsid.

We have shown that CV-1 cells infected with measles virus release a heterogeneous virion population. The major particle population (1.18 g/ml in a productive infection) corresponds in ultrastructural appearance and in polypeptide and RNA composition to the virion described throughout the measles literature. Particles of this density form lytic plaques and maintain the highest specific infectivity on sucrose gradient analysis. The two minor particle populations (1.20 to 1.15 g/ml) are also observed both in cultures by electron microscopy and after gradient purification, as a spectrum of virions which are larger and smaller in diameter respectively than particles that form small, non-lytic plaques and interfere with growth of lytic particles. Lipid analysis by Ledeen et al. (1976) indicates that the 1.20 density species has a lower lipid to protein ratio than its 1.18 g/ml counterpart.

From our data, it is not known whether the three populations of released particles may be formed following breakdown or by merging of particles after release or simply as variations in the active maturational, budding process. However, the fact that particles appear stable in density during multiple purification steps and even after sonication strongly suggests that they are not an artefact of preparation. Five subacute sclerosing panencephalitis (SSPE) viruses grown in our laboratories manifested even greater heterogeneity with relatively more of the 1.21 or 1.15 particles generated even when grown in the same host cell line at low multiplicities of infection from low passage stocks. Both measles and SSPE generate relatively more 1.21 and 1.15 g/ml peaks in high multiplicity conditions after multiple passages (C. A. Miller & C. S. Raine, in preparation).

The ability of each particle population to interfere with the growth of the others is also of primary interest. More clearly defined separation of each particle species would permit evaluation of the biological and structural properties of this virus.

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