Characterization of Two Particle Types of Calf Rotavirus

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SUMMARY

Two distinct types of rotavirus particle were isolated in caesium chloride density gradients. The higher density particle banded at 1.38 g/ml and measured 55 ± 0.4 nm in diameter while the less dense particle banded at 1.36 g/ml, measured 66 ± 0.4 nm in diameter and appeared to possess an extra outer capsid layer. Some forms intermediate between the two types were seen. Since the two particles had a similar but not identical morphology and polypeptide composition, they were considered to be different forms of the same virus. Infectivity was associated with the larger particle which contained one more polypeptide than the smaller particle.

Viruses with a distinct morphology have been described recently in association with diarrhoea of calves (Mebus et al. 1969; Turner et al. 1973; Woode et al. 1974), children (Flewett, Bryden & Davies, 1973; Bishop et al. 1974; Middleton et al. 1974), mice (Much & Zajac, 1972; Kapikian et al. 1974), piglets (Woode & Bridger, 1975) and foals (Flewett, Bryden & Davies, 1975). The names 'rotavirus' (Flewett et al. 1974) and 'duovirus' (Davidson et al. 1975) have been proposed for these viruses which are morphologically similar and related antigenically. Several of the above reports have described two forms of the virus particle, one of which appears to be derived from the other by loss of an outer layer. The present report describes some of the properties of the two forms.

Virus suspensions were prepared from either diarrhoeic faeces or tissue culture fluid. Diarrhoeic faeces were collected from colostrum-deprived calves infected experimentally with a faecal filtrate containing calf rotavirus (Woode et al. 1974). Secondary calf kidney monolayers in 40 oz roller bottles were used to cultivate the tissue culture adapted strain of calf rotavirus (Bridger & Woode, 1975). To label the virus RNA, 100 µCi of 3H-uridine (The Radiochemical Centre, Amersham, England) were added daily to each culture. After 3 days incubation at 37 °C, the infected monolayers and supernatant fluids were frozen and thawed three times; these preparations contained 10^4.5 to 10^6.0 TCD50 per 0.2 ml.

To prepare purified virus suspensions, 40 ml of diarrhoeic faeces, diluted with an equal volume of phosphate-buffered saline (PBS, containing 170 mm-NaCl, 3.4 mm-KCl, 9.5 mm-Na2HPO4, 1.6 mm-KH2PO4, pH 7.3, which was used throughout as diluent), or 80 to 100 ml. of infected tissue culture fluid were centrifuged at 8000 g for 30 min; the supernatant fluid was re-centrifuged at the same speed. The supernatant fluid from the second centrifugation was centrifuged further at 56000 g for 3 h at 4 °C in an MSE 8 × 50 ml angle-head rotor. The pellet was resuspended and layered on top of 10 ml of a 40 % sucrose solution. After centrifugation at 82000 g for 2 h at 4 °C, the pellet of material which had sedimented through the sucrose solution was resuspended in 0.1 to 0.5 ml of PBS and examined in the electron microscope. This virus suspension was then added to 10 or 25 ml of a solution of Analar caesium chloride (CsCl) in PBS, pH 7.3, with a refractive index of 1.37 and centrifuged at 155000 g for 17 h at 4 °C in an MSE 8 × 25 ml angle-head rotor; fractions were collected from the bottom of the centrifuge tube. Extinction measurements were made at 260 nm and the density of each fraction was determined from its refractive index; the
density of the fractions containing virus were also determined by pycnometry. Fractions were then dialysed for 4 h against three changes of PBS.

To examine fractions for virus particles, a drop of each fraction was placed on a carbon-collodion grid for about 30 s, stained with 3% (w/v) phosphotungstic acid, pH 6.0, and examined in a Philips 300 electron microscope at 80 kV. The microscope was calibrated with beef liver catalase crystals (Boehringer Ltd., Mannheim) and calculations of the true microscope magnification were made taking the period of the crystals as 84.4 Å (Cox & Horne, 1968). Virus particles were counted by the modification of the loop drop method (Watson & Wildy, 1963). A drop of a mixture of 0.05 ml of virus suspension, 0.05 ml. of a standard latex suspension (109 nm diam. Dow latex particles, Micro Bio Laboratories Ltd., London) and 0.01 ml of a 0.5% solution of bovine serum albumin was allowed to dry on a grid, stained with 3% (w/v) potassium phosphotungstate and the ratio of latex particles to virus particles determined. Infectivity was measured by inoculating primary calf kidney monolayers on flying coverslips with 0.2 ml. of serial tenfold dilutions of the samples to be assayed. Three coverslips were used per dilution and endpoints were read by the indirect immunofluorescence technique after 5 days incubation at 37 °C (Bridger & Woode, 1975). For polyacrylamide gel electrophoresis, fractions from caesium chloride gradients were either dialysed against 0.01 m-phosphate buffer or diluted to 5 ml with PBS, centrifuged at 123000 g for 1 h and the pellets resuspended in 0.01 m-phosphate buffer. Virus particles were disrupted by boiling in the presence of 1% (w/v) SDS and 1% (v/v) 2-mercaptopethanol. After electrophoresis through 5% (w/v) polyacrylamide gels for 4 h at 10 mA/gel, in 0.1 m-phosphate buffer, pH 7.2, and 0.1% SDS, the separated polypeptides were stained with 0.1% (w/v) Coomassie blue. Solutions containing 50 µg of bovine serum albumin, pepsin, trypsin and haemoglobin were prepared similarly and electrophoresed in parallel gels.

Preparations of virus from both faeces and tissue culture fluid usually gave two peaks of u.v.-absorbing material after centrifugation in caesium chloride gradients (Fig. 1 a). The larger peak, A, was found at a density of 1.38 g/ml with a second smaller peak, B, at 1.36 g/ml (an average of four determinations by pycnometry for each value). The relative size of these two peaks varied with different virus preparations; in some preparations peak B was represented only as a shoulder on peak A while, in the occasional faecal preparation only peak B at a density of 1.36 g/ml was present.

Virus particles were located mainly in these two peaks with only small numbers of damaged particles at the top of the gradients. The predominant particles in peak A had a mean diam. of 55.0 ± 0.4 nm (determined from measurements of 100 particles) and showed capsomeres projecting from their periphery (Fig. 2 a); they resembled the particles seen in preliminary studies (Woode et al. 1974). Although similar to the particles in peak A, the majority of particles in peak B (density of 1.36 g/ml) appeared to possess an extra capsid layer which conferred a smooth edge to these particles (Fig. 2 b); they had a larger mean diam. of 66 ± 0.4 nm. This value of 1.36 g/ml for the buoyant density agrees with that for calf rotavirus found by Welch (1971). A ‘spoke-like’ arrangement of the capsomeres was often visible on the surface of the 66 nm particles and some particles of both types showed ‘doughnut-shaped’ structures on their surfaces, which were 10 to 14 nm wide. This latter characteristic has been described as a property of the orbiviruses (Borden, Shope & Murphy, 1971). When penetrated by the negative stain, both the 55 and 66 nm particles revealed an inner electron-dense area, 38 to 40 nm in diam. (Fig. 2 c). Both particle types were shown to contain RNA by density-gradient centrifugation of 3H-uridine labelled tissue culture virus.

Because of the similarity between the 55 and 66 nm particles, it seemed likely that they
Fig. 1. (a) Distribution of u.v.-absorbing material (●—●) and infectivity (▽—▽) after centrifugation of calf rotavirus in a caesium chloride gradient; △—△, density of fractions. (b) Polyacrylamide gel electrophoresis of virus particles in peaks A and B of a caesium chloride gradient. Polypeptides are indicated by numbers; polypeptide 3 was detected only occasionally.
Fig. 2. Negatively stained virus particles. (a) Particles found in peak A of caesium chloride gradients. 'D' indicates 'doughnut-shaped' structures. (b) Particles found in peak B of caesium chloride gradients. 'D' indicates 'doughnut-shaped' structures and 'S' the 'spoke-like' arrangement of the capsomeres. (c) Virus particles before centrifugation through caesium chloride. Arrows indicate some of the particles penetrated by the negative stain. (d) Virus particles before centrifugation through caesium chloride. Arrows indicate particles which have lost only part of their outer capsid layer.
were of a common origin and that the outer capsid layer was missing from the smaller particles. This view was substantiated by the observation that some particles in peak B appeared to have only a part of the outer capsid layer (Fig. 2d). Further evidence for a relationship between the 55 and 66 nm particles was provided by polyacrylamide gel electrophoresis of virus prepared from faeces and infected tissue culture fluid. Newman et al. (1975) showed that calf rotavirus particles contain two major and three minor polypeptides. In the study reported here, electrophoresis of the particles from peaks A and B showed that both particle types contained the 2 major polypeptides described previously; the mol. wt. were 103 x 10^3 for polypeptide 2 and 44 x 10^3 for polypeptide 5 (Fig. 1b). However, the 66 nm particles also possessed a third major polypeptide, 4, with a mol. wt. of 63 x 10^3 which was absent from the 55 nm particles. Two minor polypeptides, 1 and 3, with mol. wt. of 125 and 98 x 10^3 were occasionally found in polyacrylamide gels of both particle types. Thus, the similar polypeptide patterns and morphology with the observation of intermediate forms between the two types suggest that the 55 and 66 nm particles are different forms of the same virus. The presence of two particle types likens the calf rotavirus to bluetongue virus, the type species of the orbiviruses, which also has two particle types, one of which is morphologically similar to the 55 nm rotavirus particle (Martin & Zweerink, 1972; Verwoerd et al. 1972).

Infectivity assays of the fractions around peaks A and B showed that, in three separate experiments, the greatest infectivity coincided with the 66 nm particles in peak B although the largest numbers of virus particles were found in peak A (Fig. 1a). In a typical experiment, peak B contained 0.2 x 10^10 virus particles/0.2 ml with an infectivity of 10^6.5 TCD50/0.2 ml while peak A contained 0.5 x 10^10 particles/0.2 ml and had an infectivity of 10^5.7 TCD50/0.2 ml.; the ratios of particle number to infectivity were 7 x 10^2:1 for peak B and 1 x 10^6:1 for peak A. As 1 to 2% of particles in peak A could not be distinguished from the 66 nm particles of peak B, it seems likely that the infectivity in peak A was due to an overlap of particle types between the two peaks. Thus, infectivity was associated with the 66 nm particles with the outer capsid layer and additional polypeptide.

Centrifugation through sucrose or caesium chloride did not appear to be responsible for the two types of virus particle. Both types could be found when only differential centrifugation was employed and no loss of infectivity was observed when tissue culture adapted rotavirus was exposed to CsCl at a density of 1.36 g/ml for 48 h at 4 °C. However, we do not know whether the 55 nm particles without the outer capsid layer are degraded forms of the 66 nm particles or incomplete forms produced during virus multiplication.

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**REFERENCES**


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