Demonstration of Size Variation of RNA Segments Between Different Isolates of Calf Rotavirus

(Accepted 7 February 1977)

**SUMMARY**

Polyacrylamide gel electrophoresis of RNA extracted from wild rotavirus isolates and cell culture-adapted virus revealed a significant variation in the molecular weight of individual RNA segments. The major differences were observed between wild isolates on the one hand and the adapted strain on the other hand. The slight variations that were observed between different wild isolates were found regularly and appeared to be related to the origin of the samples.

It has been shown in various parts of the world that a reovirus-like agent, also called rotavirus (Flewett et al. 1974) or duovirus (Davidson et al. 1975) is a major causal agent of acute non-bacterial gastroenteritis in calves (Mebus et al. 1969; Turner et al. 1973; Woode et al. 1974; Scherrer et al. 1976), children (Flewett, Bryden & Davies, 1973), mice (Much & Zajac, 1972) and piglets (Middleton, Petric & Szymanski, 1975). Although wild isolates of rotaviruses grow poorly or not at all in tissue culture, large numbers of particles are produced in the intestinal tract of infected animals and human infants. This enabled several investigators to study biophysical and biochemical properties of the particles, which are easily obtained from faeces and intestinal contents.

Some properties of calf and human rotavirus have already been characterized, particularly in that the genome consists of 11 segments of double-stranded RNA. However, it has also been argued that differences in mol. wt. of individual RNA segments exist between calf and human virus (Newman et al. 1975; Rodger, Schnagl & Holmes, 1975).

The aim of the present study was to characterize the calf rotavirus genome further by comparing wild isolates and tissue culture adapted virus with respect to RNA segments distribution. Our results demonstrate differences in mol. wt. of particular RNA segments.

Wild isolates of calf rotavirus were prepared from diarrhoeic faeces collected from calves, 1 to 15 days old, with naturally occurring diarrhoea and from gnotobiotic, colostrum-deprived calves infected experimentally. Experiments with gnotobiotic calves were performed in the Laboratoire de Microbiologie du CRZV de Theix (Dr Gouet). The tissue culture adapted strain used in this study was kindly provided by Dr Mebus, Department of Veterinary Science, University of Nebraska, Lincoln, Nebraska, and was routinely grown in primary embryonic calf kidney cells in our laboratory. Reovirus type 3 was produced in BHK cells and used as a standard for mol. wt. determinations.

To prepare purified virus, 1 vol. of faecal sample diluted with 1 vol. of TNE buffer (0.01 M-tris-HCl, 0.1 M-NaCl, 1 mM-EDTA, pH 7.4) or 1 vol. of tissue culture fluid was mixed with 2 vol. of Freon 113 and homogenized in a Polytron mixer. The suspension was then clarified at 6300 g for 10 min and the virus pelleted at 113,200 g for 90 min through a 1 cm column of caesium chloride adjusted to a density of 1.30. The pellet containing the virus was resuspended in 2.5 ml of TNE buffer, mixed with 2.5 ml of a solution of caesium chloride adjusted to a density of 1.74 and centrifuged for 17 h at 40,000 rev/min in a Spinco SW 50 rotor. Fractions were collected from the top of the tube; extinction was
Short communications

Fig. 1. Slab gel electrophoresis of RNA from reovirus type III (R), cell culture-adapted rotavirus (A) and wild isolate 1071 (W). The mol. wt. of each segment are 2-2, 1-73, 1-60, 0-94, 0-78, 0-53, 0-50, 0-50, 0-26, 0-20 × 10⁶ for the wild isolate no. 1071. For the adapted strain they are 2-2, 1-85, 1-70, 1-55, 1-00, 0-82, 0-51, 0-51, 0-51, 0-26, 0-20 × 10⁶. Each indicated mol. wt is the average of three separate determinations.

monitored at 254 nm with an ISCO U A₅ spectrometer and the presence of virus particles checked under the electron microscope. When this procedure was applied to virus grown in tissue culture, we regularly observed a single virus band having a density of 1-376 ± 0-003 (four determinations). For gel electrophoresis the 200 µl fractions containing the virions were diluted in 5 ml TNE buffer, centrifuged at 120000 g for 60 min and resuspended in 0-1 M-sodium acetate, pH 5. Virus particles were disrupted by heating at 37 °C for 30 min in the presence of 2 % SDS and 0-5 M-urea. This treatment, as for reovirus (Bellamy et al. 1967), yielded an RNA moiety that was suitable for polyacrylamide gel analysis. The RNA was then electrophoresed for 20 to 26 h in slab gels of polyacrylamide (5 % acrylamide, 0-13 % bis acrylamide) according to Loening (1967). After electrophoresis the slab gels were stained with ethidium bromide (Sigma Chem. Co.), examined under u.v. light, and photographed with an orange filter (Kodak, Wratten no. 22). Densitometer tracings were done with a Unicam 1800 u.v. spectrometer. Mol. wt. of rotavirus RNA segments were finally determined by comparison with reovirus RNA segments migrating as markers in the same gel (Wood, 1973). As demonstrated in Fig. 1, the RNA obtained from a field isolate
(no. 1071) of calf rotavirus was separated into eleven distinct bands by electrophoresis. The mol. wt. of each band and densitometer tracing of the gel indicate that the molar ratio of each segment is close to 1.

When we performed electrophoresis of RNA extracted from the cell culture-adapted strain and the wild isolate no. 1071 in the same slab gel (Fig. 1), a significant difference in migration of almost all segments was always observed. Segments 3 and 4 from the adapted strain moved faster than segments 3 and 4 from the field isolate. Conversely segments 2, 5 and 6 migrated more slowly for adapted strain than for wild isolate. The most important differences in the mol. wt. – of the order 6% – are observed for bands 3, 4, 5 and 6 (Fig. 1).

These results lead us to compare the RNAs of different wild isolates of calf rotavirus by polyacrylamide slab gel electrophoresis. For this purpose, we examined 3 groups of isolates: the first group consisted of five samples collected in 1976, at the ‘Domaine expérimental du Pin’ (Orne); the second consisted of 2 samples collected in 1975 at ‘Domaines expérimentaux de Marcenat’ (Cantal); and the third one of two samples obtained from gnotobiotic calves that had been infected experimentally with an isolate made in 1975 at ‘Domaine expérimental des Fumades’ (Puy-de-Dôme). The results (Fig. 2) indicate that the RNA segment

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**Fig. 2.** Densitometer tracings of the three kinds of band pattern obtained with RNA extracted from different wild isolates. (a) RNA extracted from a faecal sample collected at ‘Le Pin-aux-Haras’ (Orne – as sample no. 1071). (b) RNA extracted from a faecal sample collected at Marcenat (Cantal). (c) RNA extracted from a faecal sample collected from a calf infected experimentally with an isolate done at ‘Domaine Expérimental des Fumades’ (Puy-de-Dôme). The mol. wt. of all segments are identical to those listed in Fig. 1 (isolate no. 1071) except component 5' in (c) and components 7, 8 and 9 in bands (b) and (c).
distribution is characteristic for each group. All isolates of the first group exhibited a pattern identical to that described above for isolate ro7I. Both viruses in the second group showed an inversion of the band pattern between segments 7, 8 and 9. In the third group band 5 was split into two distinct components; in addition, bands 7, 8 and 9 were never resolved. The splitting of band 5 and the intensity of its two components suggest strongly that the two isolates contain a mixture of two types of rotaviruses.

Previous results have shown that differences exist between human and calf rotavirus genome (Rodger et al. 1975) and it was claimed that differences between distinct isolates of human rotavirus may well exist (Schnagl & Holmes, 1976). Our results establish that detectable variations in the mol. wt. of individual RNA segments occur among different wild isolates of calf rotavirus. The differences are small but they are observed regularly and appear to be significant. Additionally, it is shown that a more significant variation exists between the cell culture-adapted virus used in this study and other wild isolates. To date, we have no evidence that this difference is a consequence of the adaptation process; it should be of interest to compare by similar methods, a wild isolate and its derivative strain which has adapted to cell culture.

The present results underline the great variability of the rotavirus genome and emphasizes the necessity of analysing more isolates from different origins and species in order to evaluate the extent of this variability and to correlate these variations with biological properties.

**REFERENCES**


(Received 29 November 1976)