Comparison of a 22 nm Virus from Human Faeces with
Animal Paroviruses

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SUMMARY

The 22 nm virus seen in the faeces of people with and without gastroenteritis has been shown to have the electron microscopic appearance of porcine parovirus (PPV) and of mink enteritis virus (MEV). In CsCl gradients the density of the human virus (1.34 to 1.42 g/ml) was similar to that of PPV (1.31 to 1.39 g/ml).

During an investigation of transmissible gastroenteritis in volunteers (Clarke et al. 1972) a small virus 22 nm in diam. was seen in the faeces of such volunteers and in those of people without gastroenteritis (Paver et al. 1973). The virus could be detected by electron microscopy only after mixing the clarified faeces with certain human sera, when the virus appeared in clumps with antibody-like material between the particles.

The virus was thought not to be a phage, for electron microscopy (without antiserum) (Fig. 1a, b) of representatives of each of the two groups of small spherical phages showed that their appearance was quite different from that of the faecal viruses.

Since the 22 nm particles were similar in size to paroviruses, two known paroviruses, PPV and MEV, were examined in order to compare their electron microscopic appearances and densities.

Dr R. C. Povey of the Department of Veterinary Medicine, University of Bristol, kindly supplied the seventh pig kidney tissue culture passage of the 3060 strain of PPV (Johnson & Collings, 1969), a rabbit antiserum to the 3060 strain, the fourth kitten kidney tissue culture passage of the MV-3 strain of MEV (Johnson, 1971), and a rabbit antiserum to the BW strain of feline panleucopenia virus. Four-tenths of a ml of each of these infected tissue culture fluids were mixed with 0.2 ml of their homologous antiserum diluted 1 in 10 in phosphate-buffered saline (PBS). The mixtures were incubated for 1 h at room temperature then kept for 18 h at 4 °C. They were then centrifuged at 35 000 g in the SM 24 rotor of a Sorvall RC2B centrifuge for 1 h. The resuspended deposit was inoculated onto a 400 mesh carbon/formvar-coated ‘Athene’ grid, stained with 1.5% (w/v) sodium phosphotungstate, pH 6.5, and examined in an AEI 801 electron microscope at a magnification of ×40 000 (Fig. 1c, d).

Faeces from subject A and volunteer O (Paver et al. 1973) were emulsified as a 50% (by vol.) suspension in Earle’s saline and clarified by centrifuging. Then 0.4 ml of the supernatant fluid was mixed with 0.2 ml of a 1 in 10 dilution of serum (taken from the same subject at the same time as the faeces), incubated, centrifuged, and examined as for PPV and MEV (Fig. 1e, f).

Repeated examinations of all four viruses gave a similar appearance, with particle diam. of 20 to 23 nm, and all showed occasional empty particles. Faecal viruses from three other human subjects (S, K, and M) were similar. This measurement accords with that found for paroviruses by others (Tinsley & Longworth, 1973).

The viruses were examined for buoyant density in CsCl gradients. Four-tenths of a ml of tissue culture fluid (for PPV) or clarified, concentrated faecal emulsion from subject A were
layered on to a 3.9 ml discontinuous CsCl density gradient (1.1 to 1.7 g/ml) and centrifuged at 325000 g for 48 h in an MSE 75 ultracentrifuge at 18 °C. Fractions were collected from the bottom of the tube by needle puncture and 10 fractions of 0.36 ml were collected. Three-tenths of a ml of each fraction was mixed with 0.15 ml of a 1 in 10 dilution of homologous serum and incubated at room temperature for 1 h. To dilute the CsCl, 3 ml of PBS was added to each fraction, which was then centrifuged and examined by electron microscopy as for the unfractionated viruses. Three grids were made from each fraction and each was examined ‘blind’ to eliminate biased interpretation, the 22 nm human faecal virus for 45 min and the PPV for 10 min. The number of clumps and the number of particles in each clump was recorded. Where the clumps contained hundreds of particles, this last figure was

Fig. 1. Particles of (a) phage φX 174 and (b) phage MS2 (no antibody). (c) PPV and (d) MEV clumped with homologous antiserum. (e) 22 nm particles from the faeces of subject A clumped with serum from subject A, and (f) from subject O clumped with serum from subject O. (c), (d), (e) and (f) show antibody between the particles.
only approximate. The results are shown in Fig. 2 where both the number of clumps and the total number of particles (clumps multiplied by particles per clump) are recorded. The buoyant density of both viruses were similar; that of PPV was 1.31 to 1.39 g/ml (peak: 1.35 g/ml), that of the virus from subject A was 1.34 to 1.42 g/ml (peak: 1.38 g/ml). Similar results were obtained on several occasions with these two viruses and with MEV, and the virus from volunteer O. This measurement agrees with that found by others for parvoviruses (Tinsley & Longworth, 1973).

Thus in size, appearance, and density the 22 nm human faecal virus resembles these two animal parvoviruses. It is suggested that the 22 nm human faecal virus may be a parvovirus, although more work is needed to establish this.

Kapikian et al. (1972) have described virus particles in the faeces of a volunteer given their Norwalk gastroenteritis agent, but these particles were larger than those described here – 27 nm in diam. In CsCl gradients, the 27 nm particles had a density of 1.38 to 1.41 g/ml (Kapikian et al. 1973) and they suggest that it may be a parvovirus. They have shown (personal communication) that the fraction containing the particles produces gastroenteritis in volunteers. We have not yet demonstrated a link between our 22 nm virus and gastroenteritis.

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