Physico-chemical Evidence for the Re-classification of the Caliciviruses

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SUMMARY

The caliciviruses (feline picornavirus and vesicular exanthema virus) have a diam. of 35 to 40 nm and possess a distinctive morphology which clearly distinguishes them from the other members of the family Picornaviridae. They also differ in having an $E_{260/280}$ ratio of about 1.52 compared with 1.65 to 1.70 for the picornaviruses. More significantly, they are unique among the mammalian viruses in containing only one major polypeptide, mol. wt. about $65 \times 10^3$. Feline picornavirus and vesicular exanthema virus differ from each other in their behaviour in caesium chloride gradients and in the base composition of their RNAs. Our evidence suggests that the two viruses are not typical picornaviruses but are in fact distinct members of a new virus family, the Caliciviridae.

Although the International Committee for the Nomenclature of Viruses (Wildy, 1971) classify the caliciviruses – feline picornavirus (FPV) and vesicular exanthema virus (VEV) – as members of the family Picornaviridae, these viruses differ from other members of the family in several of their physico-chemical properties:

1. The morphology is quite distinct, the particles having a fringe-like appearance and an easily recognizable pattern of staining (Zwillenberg & Büri, 1966; Almeida et al. 1968; Wawrzkiewicz, Smale & Brown, 1968). In contrast, the other members of the group show little or no structural detail (a comparison is shown in Newman, Rowlands & Brown, 1973, fig. 2).

2. The diam. of 35 to 40 nm is significantly greater than the values (24 to 30 nm) for the other members.

3. Published values for the sedimentation coefficient of VEV range from 160S to 207S (Wawrzkiewicz et al. 1968; Oglesby, Madin & Schaffer, 1971; Newman et al. 1973) and are greater than those for other members (146S to 160S, Newman et al. 1973).

4. The ratio of extinction coefficients at 260 and 280 nm of VEV is lower, which may be due to a smaller proportion of RNA (Oglesby et al. 1971). In fact, Oglesby et al. (1971) found an RNA content of 22% in VEV compared with about 30% for the picornaviruses (Rueckert, 1971).

We have now shown that the polypeptide composition of both caliciviruses differs from that of the other members in consisting of only one major polypeptide, mol. wt. 60 to $65 \times 10^3$, compared with the four polypeptides found in the entero-, cardio-, human rhino- and foot-and-mouth disease viruses. For these experiments, VEV (types A, D and E) grown in IB-RS-2 cells (de Castro, 1964) in the presence of $[^{14}\text{C}]-$ or $[^{3}\text{H}]-$leucine was purified by the method described by Wawrzkiewicz et al. (1968). FPV (strain K1) was grown in feline embryo lung cells, also in the presence of $[^{14}\text{C}]-$ or $[^{3}\text{H}]-$leucine, and purified by the same method. Samples of the viruses were disrupted with 1% SDS, 8 M-urea, 1% mercaptoethanol at 37 °C for 1 h and examined by SDS-polyacrylamide gel electrophoresis. Each type of VEV contained one major polypeptide, mol. wt. 60 to $65 \times 10^3$, and co-electrophoresis analyses showed
Fig. 1. Polyacrylamide gel electrophoresis of FPV and VEV in 7% gels for 6 h at 7 mA/gel. (a) mixture of VEV (type A) labelled with $[^{14}C]$-leucine and VEV (type E) labelled with $[^{3}H]$-leucine; (b) mixture of FPV (strain K1) labelled with $[^{3}H]$-leucine and VEV (type E) labelled with $[^{14}C]$-leucine; (c) VEV (type E) labelled with $[^{14}C]$-leucine. ●—●, $^{14}C$; ○—○, $^{3}H$.

that the mol. wt. of the polypeptide of each type was very similar (Fig. 1a). FPV also contained only one major polypeptide but this had a slightly lower mol. wt. (Fig. 1b). Similar polypeptide patterns were also obtained with viruses which had been labelled with $[^{14}C]$-aspartic acid or $[^{14}C]$-Chlorella protein hydrolysate. In addition to the major polypeptide, minor bands were also found by polyacrylamide gel electrophoresis (Fig. 1c), but only the smallest polypeptide (approx. mol. wt. $15 \times 10^3$) was found consistently. It is not known
Table 1. Comparison of some of the physico-chemical properties of the caliciviruses and picornaviruses

<table>
<thead>
<tr>
<th>Property</th>
<th>Caliciviruses</th>
<th>Picornaviruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Fringe-like appearance; distinctive pattern of staining.</td>
<td>No structural detail</td>
</tr>
<tr>
<td>Diam. (nm)</td>
<td>35 to 40</td>
<td>24 to 30</td>
</tr>
<tr>
<td>Sedimentation coefficient</td>
<td>170 to 180S</td>
<td>146 to 160S</td>
</tr>
<tr>
<td>$E_{260/280}$</td>
<td>1.50 to 1.54</td>
<td>1.65 to 1.70</td>
</tr>
<tr>
<td>%RNA</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>Number of polypeptides</td>
<td>One major</td>
<td>Four major</td>
</tr>
<tr>
<td>Mol. wt. of polypeptides</td>
<td>$6.0 \times 10^3$ (major)</td>
<td>$3.0 \times 10^3$ (3 species)</td>
</tr>
<tr>
<td></td>
<td>$1.5 \times 10^3$ (minor)</td>
<td>$1.0 \times 10^3$</td>
</tr>
</tbody>
</table>

whether the slower migrating minor polypeptides which are sometimes found are cleavage products of the major polypeptide or cellular contaminants. However, the presence of a polypeptide of mol. wt. $1.5 \times 10^3$ in all the preparations suggests that this is a structural component of the virus.

The sedimentation coefficient of each virus was greater than the values published for the picornaviruses. Mixtures of $[^{35}S]$-methionine labelled poliovirus ($S = 160$; Rueckert, 1971) and either $[^{3}H]$-leucine labelled FPV or $[^{3}H]$-leucine labelled VEV were centrifuged at 25,000 rev/min in 15 to 45% sucrose gradients for 40, 80 and 120 min and the distribution of the radioactivity in each gradient was measured. The distance sedimented by each virus was plotted against time. Linear plots were obtained for each virus. Assuming an inverse relationship between S value and the time taken to sediment a given distance, values of 170S for FPV and 180S for VEV (type E) were calculated.

The ratio of $E_{260}/E_{280}$ for FPV was 1.53 to 1.54, which is similar to the values reported by Oglesby et al. (1971) for two types of VEV and obtained in the present work with types A, D and E (Table 1). This provides further evidence that FPV and VEV are distinct from the picornaviruses.

The identical morphology of the two viruses and their similar polypeptide composition, which are quite distinct from those described for other mammalian viruses, suggested that FPV and VEV may be very closely related. However, the viruses differed from each other in some important respects. The buoyant density of the caliciviruses in caesium chloride has been variously reported to be 1.36 to 1.39 g/ml (references in Newman et al. 1973). This wide range of values may be ascribed to the different conditions used by the various groups of workers. In this laboratory we obtained values of 1.36 g/ml for VEV when the virus was centrifuged for 6 h in a preformed gradient (Rowlands, Sangar & Brown, 1971) and 1.37 g/ml when the virus was centrifuged overnight (Wawrzkiewicz et al. 1968). In a more extended study we have carried out co-sedimentation experiments with mixtures of VEV and FPV and have confirmed that the density of VEV increases from 1.36 to 1.37 g/ml as the time of centrifuging is increased from 6 to 24 h. However, the density of FPV was 1.38 g/ml when the virus was centrifuged for 6 h and did not increase after 24 h (Fig. 2).

The two viruses also differed in their reaction with glutaraldehyde. Mixing each virus with 4% glutaraldehyde immediately before centrifuging in caesium chloride gradient increased the buoyant density of VEV to 1.38 g/ml when centrifuged for 6 h and to 1.39 g/ml when centrifuged for 24 h, whereas the buoyant density of FPV was unaltered at 1.38 g/ml in
Fig. 2. Isopycnic banding of mixtures of [$^{14}$C]-leucine FPV (strain K1) and [$^{3}$H]-leucine VEV (type E) in preformed gradients for different periods of time. The samples, before or after mixing with glutaraldehyde, were centrifuged at 20 °C for 6 h or 24 h at 24,000 rev/min in the six-bucket rotor no. 59108 of the MSE 65 Ultracentrifuge.

both experiments (Fig. 2). Foot-and-mouth disease virus showed a similar increase in density on treatment with glutaraldehyde, whereas poliovirus was unaffected (Sangar et al. 1973). Likewise, foot-and-mouth disease virus increases in density on prolonged centrifuging in caesium chloride, whereas poliovirus is unaffected (Rowlands et al. 1971). These parallel observations point to a greater degree of interaction of vesicular exanthema virus with caesium ions and glutaraldehyde and imply that there is a basic difference in the structure of the two caliciviruses.

The sedimentation coefficients and base compositions of the two virus RNAs also differ (Table 2). Newman et al. (1973) gave a value of 32S for FPV (strain BF) compared with 37S for VEV. Analyses for three serotypes of VEV were given by Wawrzkiewicz et al. (1968) and Newman et al. (1973). These were similar to each other but differed from that of FPV, strain BF (Newman et al. 1973). The value obtained for FPV (strain K1) in the present work also differed markedly from those for the three strains of VEV. It is interesting that both the feline picornavirus strains have a lower cytidylic acid content than VEV and that they contain equal amounts of adenylic acid and uridylic acid, in sharp contrast to VEV.
Table 2. Base composition of FPV and VEV ribonucleic acids

<table>
<thead>
<tr>
<th>Virus</th>
<th>Base composition (%)</th>
<th>Reference</th>
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<tbody>
<tr>
<td></td>
<td>Adenylic acid</td>
<td>Cytidylic acid</td>
</tr>
<tr>
<td>FPV, strain BF</td>
<td>29.3</td>
<td>19.9</td>
</tr>
<tr>
<td>KI</td>
<td>27.9</td>
<td>20.9</td>
</tr>
<tr>
<td>VEV, type A</td>
<td>29.8</td>
<td>24.9</td>
</tr>
<tr>
<td>D</td>
<td>29.1</td>
<td>25.0</td>
</tr>
<tr>
<td>E</td>
<td>28.2</td>
<td>25.4</td>
</tr>
<tr>
<td>E</td>
<td>29.8</td>
<td>23.2</td>
</tr>
</tbody>
</table>

Ref. 1, Newman et al. (1973); 2, present work; 3, Wawrzkiewicz et al. (1968).

We have shown above that the polypeptides of the two caliciviruses differ slightly in mol. wt. Co-chromatography of the polypeptides of VEV (type E) and FPV on hydroxyapatite columns (Moss & Rosenblum, 1972), a method in which separations are dependent on factors other than mol. wt., also showed differences between the polypeptides of the viruses. The two viruses, FPV (strain K1) labelled with [14C]-leucine and VEV (type E) labelled with [3H]-leucine, were disrupted with 1% SDS and 1% mercaptoethanol at 100 °C for 2 min, diluted tenfold with 0.01 M-phosphate, pH 6.6, containing 0.1% SDS and 1 mM-dithiothreitol and applied to a 15 x 1.5 cm column equilibrated with the same buffer. The polypeptides were then eluted with a gradient ranging from 0.35 M to 0.55 M-sodium phosphate, pH 6.6, containing 0.1% SDS and 1 mM-dithiothreitol. One ml fractions were collected for counting and the fractions were monitored by refractometry to measure the molarity. The polypeptides of VEV eluted at 0.39 M-phosphate, whereas those of FPV eluted at 0.42 M-phosphate.

These results show that physico-chemical methods can be used to distinguish between three of the serotypes of VEV on the one hand and one strain of FPV on the other. Considerably more work is necessary to determine whether these differences extend throughout the entire range of the 13 serotypes of VEV and numerous strains of FPV. It should then be possible to demonstrate more convincingly the degree of relationship to VEV or FPV of new caliciviruses such as San Miguel sea lion virus (Smith et al. 1973). Irrespective of this problem, however, the most important finding to emerge from our work is that FPV and VEV should not be classified as picornaviruses but should be regarded as members of a separate family, the Caliciviridae.

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REFERENCES


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