Relationship of a Virus from *Tellina tenuis* to Infectious Pancreatic Necrosis Virus

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**SUMMARY**

The physicochemical and serological properties of a virus isolated from the bivalve mollusc, *Tellina tenuis*, have been examined. The virus has a diam. of 59 nm, sediments at 430S in sucrose gradients and bands at a density of 1.32 g/ml in CsCl. The virus contains RNA with a mol. wt. about $2.8 \times 10^6$ as estimated by polyacrylamide gel electrophoresis but in sucrose gradients the RNA sediments at 14S. The virus RNA is resistant to ribonuclease under conditions in which ribosomal RNA and the single stranded Mengo virus RNA are completely hydrolysed. Two major polypeptides, mol. wt. 67 and $40 \times 10^3$, and one minor polypeptide, mol. wt. $110 \times 10^3$, are present in the virus particle. These properties are similar to those found for different serotypes of infectious pancreatic necrosis (IPN) virus. Although there was only a very low level of cross-neutralization between *Tellina* virus and IPN virus, there was some cross-reaction in immune electron microscopy tests and in immunofluorescence tests with infected tissue culture cells. This cross reaction, together with the close similarity in morphology and physicochemical properties, suggests that *Tellina* virus and IPN virus belong to the same virus group.

**INTRODUCTION**

During an examination of a population of the sand dwelling marine bivalve mollusc *Tellina tenuis*, Buchanan (1973) noticed that in one population the shells were markedly thinner and chalky in nature and the digestive gland was pale yellow compared with the usual dark brown colour. A high proportion of the cells in the digestive gland, which in this species manufacture shell precursor material, were necrotic and the cytoplasm contained membrane-bound inclusion bodies which, in turn, contained icosahedral particles, 70 nm in diam. and resembling reovirus. Similar particles were not found in apparently healthy populations of *Tellina tenuis*.

Hill (1976a) described the isolation of a virus from affected *Tellina* in fish tissue cultures. This virus was observed to be morphologically similar to infectious pancreatic necrosis (IPN) virus of fish but preliminary studies showed that the virus was not neutralized by polyvalent antiserum active against all known serotypes of IPN virus. However, the same serum did promote fluorescence of *Tellina* virus infected cells in an indirect fluorescent antibody test, revealing some antigenic relationship with IPN virus. In addition, several other serologically related viruses have now been isolated from a variety of marine molluscs (Hill, 1976b) indicating that the distribution of this group of viruses is widespread.
In view of the seriousness of IPN in hatchery reared fish and the serological relationship of Tellina virus to IPN virus, it was important to determine the exact relationship between them. The characterization of Tellina virus described in this paper shows that it has several physicochemical properties in common with IPN virus. Moreover, the recent characterization of the bursal disease agent of chickens (Nick, Cursiefen & Becht, 1976) has pointed to its close similarity to IPN virus and suggests that these two viruses, Tellina virus and the viruses from marine molluscs referred to above, may form a new group of viruses.

**METHODS**

**Viruses.** The Tellina virus used was the strain isolated from a population of *Tellina tenuis* showing a disease of the digestive gland and described by Hill (1976a). The IPN virus strains used were the European reference serotypes Sp and Ab provided by Dr P. E. Vestergaard Jørgensen, State Veterinary Serum Laboratory, Aarhus, Denmark and the N. American reference serotype VR 299 (A.T.C.C.) provided by Dr K. Wolf, Eastern Fish Disease Laboratory, Kearneysville, West Virginia, U.S.A.

**Cell cultures.** Monolayer cultures of BF-2 (bluegill fibroblast) and RTG-2 (rainbow trout gonad) were grown at 20 °C in MEM (Glasgow modification) buffered at pH 7·2 to 7·4 with 0·16 M-tris-HCl and supplemented with 10% tryptose phosphate broth and 10% foetal calf serum. For maintenance of cells during virus growth the serum content of the medium was reduced to 2% except for production of virus for rabbit inoculations when the serum was excluded altogether.

**Virus growth and assay of infectivity.** Tellina virus was grown in BF-2 cultures and IPN virus grown in RTG-2 cultures. Cells were infected at a multiplicity of 0·1 to 1, incubated at 15 °C and virus harvested when gross c.p.e. was observed (48 to 72 h). Infectivity titres of 10^8·5 to 10^9·5 p.f.u./ml were usually obtained. For labelling with 3H-uridine virus was inoculated in maintenance medium containing 6 µCi/ml 3H-uridine/ml and after 24 h incubation more 3H-uridine was added to the medium at a rate of 6 µCi/ml.

For assay of infectivity, 34 mm Petri dish cultures of BF-2 or RTG-2 were inoculated in triplicate with 0·2 ml virus suspension. Virus was adsorbed for 1 h at 20 °C after which the medium was removed and the cell sheets overlaid with 2 ml of 0·5% agarose in maintenance medium. Following incubation at 15 °C for 3 days dishes were covered with 2 ml of 0·01% neutral red in MEM and incubated for 3 h in the dark to reveal the plaques.

**Production of antisera.** Pooled infected cell harvests were ultrasonically treated for 10 s and cell debris removed by centrifuging at 1000 g for 30 min. Clarified virus suspensions were treated with Arkalone P (1, 1, 2-trichloro-1,1,2 trifluoroethane, ICI Ltd) and virus concentrated for rabbit inoculations as described by Finlay & Hill (1975). Approximately 1 ml of the concentrated virus suspension in PBS was blended with an equal volume of Freund’s complete adjuvant and 0·5 ml inoculated intramuscularly into each hind leg of a rabbit (New Zealand White). Three weeks later the rabbits were inoculated intravenously with 0·5 ml of virus in PBS. After a further 7 to 10 days blood was collected and serum separated. All sera were heated at 56 °C for 30 min before use.

**Neutralization tests.** Virus stocks were diluted in maintenance medium to give 100 p.f.u. per 0·1 ml and mixed with an equal volume of serial 0·5 log dilutions of antisera ranging from 10^{-1} to 10^{-8}. Controls consisted of 0·5 ml virus dilution mixed with 0·5 ml of pre-immune serum diluted 10-fold in maintenance medium. Virus-serum mixtures were incubated at 20 °C for 1 h after which 0·2 ml volumes were inoculated on to triplicate Petri dishes of BF-2 or RTG-2 cells and the residual plaque counts determined as described above.

**Immunofluorescence tests.** Monolayers of BF-2 or RTG-2 cells prepared on coverslides...
in Leighton tubes were inoculated with virus at a multiplicity of 0.1. After 24 h incubation at 15°C the cell sheets were washed in PBS and then fixed in acetone at −20°C for 10 min. Following fixation, slides were air dried and then covered for 30 min with serial twofold dilutions of rabbit antiserum ranging from 1/5 to 1/500. The antiserum treated slides were washed for 30 min in PBS and then covered with fluorescein-labelled sheep anti-rabbit immunoglobulin (Wellcome Reagents Ltd) at its optimal dilution of 1/5 (determined in a previous experiment). The anti-rabbit immunoglobulin was allowed to react for 30 min and the slides were then washed for 30 min in PBS. The stained slides were mounted with glycerol buffered at pH 8.5 and examined under a Reichert Biopan microscope equipped for incident u.v. light fluorescent microscopy with an HBO 50 lamp, an FITC 3 interference filter and an OG 515 barrier filter.

Purification of virus particles. The virus harvests were sonicated and then clarified by centrifuging at 1000 g for 5 min. The supernatants were centrifuged at 8000 g for 60 min and each pellet was then suspended in a small vol. of 0.04 M-phosphate buffer, pH 7.6. The resuspended pellets were clarified at 1000 g for 5 min. The supernatants were then treated with 1% SDS before centrifuging for 60 or 90 min at 45000 g in a 15 to 45% sucrose gradient, prepared in 0.1 M-NaCl, 0.1 M-tris, pH 7.6. One ml fractions were collected from the bottom of the tube for analysis.

Determination of sedimentation coefficient. Mixtures of 3H-Tellina virus or IPN virus and 14C-reovirus were centrifuged at 45000 g for different times in separate 15 to 45% sucrose gradients. From the distribution of the two isotopes in the different gradients the sedimentation rates of the 3H-Tellina and IPN viruses relative to that of the 14C-reovirus were calculated.

Buoyant density determination. Mixtures of 3H-Tellina virus and IPN virus and 32P-Maus-Elberfeld virus were centrifuged in pre-formed CsCl gradients at 60000 g for 6 h. The distribution of the two isotopes in the gradient was determined in fractions collected from the bottom of the tube. The refractive index of each fraction was measured in an Abbé refractometer.

Virus RNA. The different methods used for extracting the virus RNA are described in the Results section. The RNA was analysed either by centrifuging at 50000 g for 16 h (5 to 25% in 0.1 M-acetate, 0.1% SDS, pH 5), or by electrophoresing in 5% polyacrylamide gels for 24 h at 3.5 mA/gel.

Virus polypeptides. The method used for analysing the virus polypeptides was exactly as described by Cartwright, Talbot & Brown (1970) for vesicular stomatitis virus polypeptides, using SDS-phosphate, pH 7.2.

Marker viruses, RNAs and polypeptides. 32P-Maus-Elberfeld and 3P-Mengo virus were prepared according to the method described by Brown & Cartwright (1963) for foot-and-mouth disease virus. The RNA was prepared from each virus by extraction with phenol in the presence of 0.1% SDS. The 3H-uridine reovirus was a gift from Dr J. J. Skehel. 3S-methionine vesicular stomatitis virus polypeptides were prepared by the method described by Cartwright et al. (1970).

Electron microscopy and immune electron microscopy. Samples of virus were placed on carbon-formvar grids and allowed to adsorb for up to 2 h at 20°C in a humid chamber. The grids were then drained and the virus stained with either 2% phosphotungstic acid, pH 7.2, or 2% sodium silicotungstate, pH 7.0. Catalase, prepared according to the method of Wrigley (1968), was stained similarly and these grids photographed alternately with those of virus preparations. Densitometer recordings of the catalase periodicity were used to correct the measurements of the virus particles.
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Table 1. Electron microscope measurements on Tellina and IPN viruses with clearly defined hexagonal outlines

<table>
<thead>
<tr>
<th>Virus</th>
<th>Number of particles</th>
<th>Dimension across opposing edges of hexagon*</th>
<th>Dimension across apices of hexagon*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups of particles in thick stain</td>
<td>Single particles in thick stain</td>
<td>Groups of particles in thin stain</td>
</tr>
<tr>
<td>Tellina</td>
<td>46</td>
<td>56.0 ± 1.6 nm</td>
<td>61.8 ± 2.0 nm</td>
</tr>
<tr>
<td>IPN-Vr</td>
<td>51</td>
<td>55.4 ± 2.1 nm</td>
<td>62.0 ± 2.0 nm</td>
</tr>
<tr>
<td>IPN-Sp</td>
<td>43</td>
<td>57.7 ± 2.1 nm</td>
<td>61.8 ± 3.0 nm</td>
</tr>
<tr>
<td>IPN-Ab</td>
<td>61</td>
<td>57.0 ± 2.2 nm</td>
<td>61.5 ± 1.1 nm</td>
</tr>
</tbody>
</table>

* All measurements were obtained by comparison with catalase crystals and on particles stained with 2% PTA, pH 7.2. Overall mean diam. is 62.2 nm and the spread of particle diameters is 51.6 nm to 73.0 nm. Mean diam. of grouped particles supported in stain is 59.4 nm.

Table 2. Electron microscope measurements on Tellina and IPN viruses with four clearly defined edge capsomers*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Number of particles measured</th>
<th>Spacing of capsomers on one edge of hexagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tellina</td>
<td>61</td>
<td>9.2 ± 1.2 nm</td>
</tr>
<tr>
<td>IPN</td>
<td>45</td>
<td>9.1 ± 0.9 nm</td>
</tr>
</tbody>
</table>

* All measurements were obtained by comparison with catalase crystals and on particles stained with 2% PTA, pH 7.2.

For immune electron microscopy, IgG fractions were prepared from homologous and heterologous antisera by filtration through DEAE cellulose. Serial dilutions of IgG were reacted with samples of resuspended virus pellets at 4 °C overnight and then transferred to carbon-formvar grids, allowed to adsorb and stained as described above. All samples were examined in a Philips 301 electron microscope.

RESULTS

Purification of Tellina virus

The unfractionated tissue culture harvests of the virus contained hexagonal particles which apparently varied in size, those occurring in clusters in thick stain being 56 to 62 nm in diam. compared with those observed singly in thin stain which were 62 to 68 nm (Table 1 and Fig. 1a, b). Some of the particles clearly showed 4 capsomers per edge (Fig. 1d). These had a centre to centre measurement of about 9 nm (Table 2). Arrays of structural subunits similar to those seen by Cohen & Scherrer (1974) in IPN virus were also seen (see Fig. 1c).

The infectivity of the harvests was unaffected by lipid solvents and by detergents such as SDS. This indicated that the virus particles did not possess a lipid coat and enabled us to incorporate a treatment with SDS in the purification procedure.

To aid in the detection of the virus in the various fractionation procedures and at the same time to establish the nature of the nucleic acid, separate cultures grown in the presence of 3H-uridine and 3H-thymidine were sonicated, freed from cellular debris by centrifuging at 1000 g for 5 min and the virus particles pelleted at 80000 g for 90 min. The pellets were re-suspended in 2 ml 0.04 M-phosphate, pH 7.6, insoluble debris removed and the clarified supernatants treated with 1% SDS before centrifuging at 45000 g for 1 h in separate 15 to
Characterization of a virus from Tellina tenuis

Fig. 1. Electron micrographs of Tellina virus (a) groups of particles in thick stain; (b) single particles in thin stain; (c) degraded particles showing arrays of structural subunits; (d) particles showing 4 capsomers along edge of hexagon; (e) particles obtained from a sucrose gradient following treatment with 1% SDS and (f) effect of storage of purified virus at 4°C for two weeks.

45% sucrose gradients in 0.1 M-NaCl, 0.1 M-tris, pH 7.6. Each sucrose gradient was layered over a 1 ml cushion of saturated caesium chloride solution to detect any aggregation of virus particles. The distribution of radioactivity in the two gradients showed the presence of a peak of ³H-uridine at a position which a particle of 59 nm would be expected to reach under the conditions used (Fig. 2a). Examination of the ³H-uridine peak in the electron microscope showed the particles depicted in Fig. 1(e), identical to those found in the un-
fractionated harvest. In addition to the individual particles seen in the purified preparations, a sample which had been kept at 4°C for two weeks also contained groups of particles associated with what appeared to be a cellular membrane which sometimes assumed an envelope configuration enclosing several particles (Fig. 1f). Since the virus had been mixed with 1% SDS during the purification procedure it seems unlikely that the envelope is
Characterization of a virus from Tellina tenuis

Fig. 3. Co-sedimentation of (a) 3H-Tellina virus and 14C-reovirus and (b) 3H-IPN virus and 14C-reovirus in 15 to 45 % sucrose gradients at 45000 g for 30, 60 and 90 min. In (a) ○—○, reovirus; ★—★, Tellina virus; in (b) ●—●, reovirus, ○—○, IPN virus.

cellular in origin. A more likely explanation is that the envelope is composed of an aggregate of virus subunits. Titration of the individual fractions from a gradient containing a 3H-uridine labelled virus preparation which had been centrifuged for 1·5 h at 45000 g showed a co-incidence of the infective and radioactive peaks (Fig. 2b).

Overall recovery of infectivity was essentially quantitative. In the experiment shown in Fig. 2(b), the peak fraction 13 contained 4·5 × 10^10 p.f.u./ml compared with a titre of 5 × 10^6 p.f.u. for the 50 ml of original tissue culture harvest used as starting material.

These experiments provided evidence that Tellina virus contains RNA and confirmed preliminary observations in which 10^-3 M-BrdUrd was shown to have no effect on virus growth in tissue culture. This concentration of BrdUrd reduced the yield of a herpesvirus (Channel catfish virus) by more than 1000-fold.
Fig. 4. Equilibrium centrifugation of $^3$H-Tellina virus and $^{32}$P-Maus-Elberfeld virus in pre-formed caesium chloride gradient (ρ 1.30 to 1.40 g/ml). The tube was centrifuged at 60000 g for 6 h in the 6 x 16.5 ml swing-out rotor of the MSE ultracentrifuge. ●—●, $^3$H-Tellina virus; ○—○, $^{32}$P-ME virus; ▲—▲, density.

Properties of the purified virus particles

Sedimentation coefficient

Mixtures of $^3$H-Tellina virus and $^{14}$C-reovirus were centrifuged at 45000 g in 15 to 45 % sucrose gradients for 30, 60 and 90 min and the distribution of radioactivity in each gradient was measured. Plotting the distance sedimented by each virus against time gave a linear plot for each virus (Fig. 3a). Assuming an inverse relationship between S value and time taken to sediment a given distance, a value of 430S was calculated for Tellina virus relative to a value of 734S for reovirus (Farrell, Harvey & Bellamy, 1974).

Buoyant density

Co-sedimentation of $^3$H-Tellina virus and $^{32}$P-Maus-Elberfeld virus in a pre-formed CsCl gradient gave the distribution of radioactivity shown in Fig. 4. From the refractive index of the peak fractions of radioactivity the buoyant density of Tellina virus was calculated to be 1.32 g/ml.

Virus RNA

Release of RNA from purified Tellina virus particles proved to be difficult. Based on radioactive counts, the usual technique of extracting virus with phenol, whether in the presence or absence of SDS, released only about 25 % of the RNA into the aqueous phase. Including 6 M-urea in the virus suspension before extraction also failed to increase the
Characterization of a virus from *Tellina tenuis*

![Image of polyacrylamide gel electrophoresis](image)

Fig. 5. Polyacrylamide gel electrophoresis of mixtures of $^{32}$P-Mengo virus RNA (position shown by arrow) and (a) $^3$H-Tellina virus RNA; (b) IPN-Ab virus RNA; (c) IPN-Vr virus RNA and (d) IPN-Sp virus RNA. The mixtures were electrophoresed in 5 % gels for 24 h at 3.5 mA/gel.

amount of RNA released into the aqueous phase. Similarly the NaClO₄ extraction technique described by Wilcockson & Hull (1974) gave poor yields of RNA.

However, the RNA was released completely by heating the virus particles in 8 M-urea, 1.0 % SDS, 10 % mercaptoethanol at 37°C for 30 min. Samples of the disrupted preparations were used directly for polyacrylamide gel electrophoresis of the RNA and also for examination of the virus proteins (see next section). For polyacrylamide gel electrophoresis of the RNA $^{32}$P-Mengo virus RNA was first added as marker. The distribution of $^3$H and $^{32}$P in the gel (Fig. 5a) shows the presence of a heterogeneous band of Tellina virus RNA with a maximum mol. wt. of $2.8 \times 10^6$, compared with a mol. wt. of $2.6 \times 10^6$ for Mengo virus RNA.

A second sample of the disrupted virus was mixed with $^{32}$P-Mengo virus RNA and 500 µg ribosomal RNA from BHK cells and precipitated with 2 vol. ethanol. After storage at $-20^\circ$C overnight, the precipitate was washed free from SDS with 70 % ethanol and then resuspended in 2 ml 0.04 M-phosphate. One ml was mixed with 0.1 µg pancreatic ribo-
nuclease for 5 min at 20 °C and then centrifuged for 16 h at 50000 g in a 5 to 25 % sucrose gradient in 0.1 M-acetate, 0.1 % SDS, pH 5; the untreated control sample was centrifuged in a parallel gradient. The profiles in Fig. 6 show that the sedimentation coefficient of Tellina virus RNA was 14S (compared with values of 35S for Mengo virus RNA and 28S and 18S for ribosomal RNA) and that the Tellina virus RNA was unaffected by ribonuclease at a concentration of enzyme sufficient to hydrolyse the Mengo virus and ribosomal RNAs.

Fig. 6. Sucrose gradient centrifugation in 5 to 25 % gradients at 50000 g for 16 h of (a) a mixture of 3H-Tellina virus, 32P-Mengo and BHK cell RNAs; (b) the same mixture after incubation with 0.1 µg ribonuclease/ml; (c) a mixture of 3H-IPN virus, 32P-Mengo and BHK cell RNAs; (d) the same mixture after incubation with 0.1 µg ribonuclease/ml. In (a) and (b), •—•, 3H-Tellina virus; ○—○, 32P-Mengo virus; in (c) and (d) •—•, 3H-IPN virus; ○—○, 32P-Mengo virus.
Fig. 7. Polyacrylamide gel electrophoresis in 7.5% gels for 18 h at 5 mA/gel of the polypeptides of 
(a) a mixture of Tellina virus and 35S-methionine vesicular stomatitis virus; (b) Tellina virus; (c) a mixture of Tellina virus and IPN-Sp virus; (d) IPN-Sp virus; (e) IPN-Sp and IPN-Ab viruses; 
(f) IPN-Ab virus; (g) IPN-Sp and IPN-Vr viruses. The positions of the 35S peaks in (a) are shown 
by arrows.

to slowly sedimenting molecules. These results indicate that the RNA is double stranded, 
or possibly a highly ordered single stranded molecule (see Discussion).

**Virus polypeptides**

A portion of the virus that had been disrupted with 8 M-urea, 1.0% SDS, 1% mercapto-
ethanol for the RNA analysis was mixed with a small amount of 35S-methionine vesicular 
stomatitis virus polypeptides and electrophoresed in polyacrylamide gels. The gels were 
stained with Coomassie blue and the positions of the bands recorded (Fig. 7a). They were 
then cut into 1 mm slices for radioactive counting. From the positions of the 35S-labelled
Table 3. Cross-neutralization tests between IPN virus and Tellina virus

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>IPN-Sp</th>
<th>IPN-Ab</th>
<th>IPN-Vr</th>
<th>TV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPN-Sp</td>
<td>360,000*</td>
<td>3,500</td>
<td>5,400</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>IPN-Ab</td>
<td>2,600</td>
<td>120,000</td>
<td>3,300</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>IPN-Vr</td>
<td>5,800</td>
<td>3,400</td>
<td>760,000</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Tellina</td>
<td>20</td>
<td>30</td>
<td>15</td>
<td>200,000</td>
</tr>
</tbody>
</table>

* Reciprocal of antiserum dilution giving 50% plaque count reduction.

bands, indicated by arrows in Fig. 7(a), the mol. wt. of the Tellina virus polypeptides were calculated to be 110 (VP1, minor), 67 (VP2, major) and 40 (VP3, major) × 10^3. The three minor bands, each representing less than 1% of the total staining of the polypeptides, observed at positions corresponding to about 70, 45 and 25 × 10^3, were due to the added VSV polypeptides.

Comparison with IPN viruses

Physiochemical properties

The properties of Tellina virus described above suggested its close similarity to IPN virus. To provide a direct comparison within one laboratory three serotypes of IPN virus (Sp, Ab and Vr 299) were grown and purified according to the methods described for Tellina virus. Each serotype contained hexagonal particles similar in size to that of Tellina virus (Table 1) and some particles clearly showed 4 capsomers to the edge, with a centre to centre measurement of about 9 nm (Table 2) similar to those observed for Tellina virus (see above) and IPN virus (Cohen & Scherrer, 1974).

All three of the IPN viruses sedimented at about the same rate as Tellina virus. In a direct estimation of the S value in comparison with reovirus, a value of 430S was obtained for IPN virus, serotype Ab (Fig. 3b). This value is in close agreement with that obtained by Dobos et al. (1977).

The RNA profiles of the three IPN viruses in polyacrylamide gels were similar to that obtained for Tellina virus RNA (Fig. 5b, c, d). As with the Tellina virus RNA heterogeneous profiles were obtained at a position corresponding to a mol. wt. of about 2.8 × 10^6, slightly higher than that of Mengo virus RNA.

The IPN viruses contained two major and one minor polypeptide with mol. wt. similar to those of Tellina virus. To obtain a more direct comparison of their mol. wt., co-electrophoresis analyses were made of the Tellina virus and IPN virus polypeptides. In each instance the polypeptides from the two viruses had identical mobilities. A comparison of the polypeptides of Tellina virus and IPN virus, serotype Sp is shown in Fig. 7b to d. The migration of the polypeptides of the three serotypes of IPN virus (Sp, Ab and Vr) is shown in Fig. 7d to g.

Serological properties

Neutralization tests

The mean plaque count obtained at each dilution of antiserum was calculated as a percentage of the control plaque count and plotted against the logarithm of the antiserum dilution. Typical sigmoid neutralization curves were obtained and the dilutions of antisera giving 50% plaque count reduction were readily interpolated (Table 3). The degree of cross-reaction demonstrated between the three strains of IPN virus is consistent with the
Characterization of a virus from Tellina tenuis

Table 4. Cross reactions in immunofluorescent antibody tests with Tellina virus and IPN virus

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Reciprocal of highest dilution of antiserum giving fluorescence with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tellina</td>
<td></td>
</tr>
<tr>
<td>IPN</td>
<td></td>
</tr>
<tr>
<td>Tellina</td>
<td>6400</td>
</tr>
<tr>
<td>IPN</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 5. Complexing of Tellina virus and three serotypes of IPN virus with the homologous and heterologous sera in immuno-electron microscopy

<table>
<thead>
<tr>
<th>Serum*</th>
<th>Virus</th>
<th>Tellina</th>
<th>IPN-Vr</th>
<th>IPN-Ab</th>
<th>IPN-Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tellina</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IPN-Vr</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IPN-Ab</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>IPN-Sp</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

* The degree of immune complexing is expressed on a scale + to +++: + = little or no complexing; ++ = up to 40% of particles complexed; +++ = more than 95% of particles complexed.

findings of other workers (Vestergaard-Jørgensen & Kehlet, 1971; Vestergaard-Jørgensen & Grauballe, 1971; Wolf & Quimby, 1971; Vestergaard-Jørgensen, 1972; Lientz & Springer, 1973). However, there was no evidence of cross reaction between Tellina virus and any of the IPN strains except at very low dilution of Tellina virus antiserum. Since all IPN virus isolates so far tested cross-react strongly in neutralization tests with one or more of the reference strains Sp, Ab or Vr299, this finding clearly differentiates Tellina virus from the group of IPN viruses.

Immunofluorescence tests

The appearance of fluorescing Tellina virus-infected BF-2 cells was very similar to that of IPN virus-infected RTG-2 cells. Infected cells showed a bright diffuse fluorescence filling the entire cytoplasm with many cells also showing intensely staining globules as described by Piper, Nicholson & Dunn (1973) for a strain of IPN virus grown in RTG-2 cells. Background fluorescence of non-infected cells was minimal except at the lowest dilutions of antiserum and the end point dilution of antiserum giving rise to definite fluorescence of infected cells was readily determined.

A positive cross reaction between Tellina virus and IPN virus (strain Sp) was detected although the heterologous titres were considerably lower than the homologous titres (Table 4). This demonstration of a significant antigenic relationship between Tellina virus and IPN virus contrasts with the very low cross neutralization activity and could be due to the antigenic nature of the double stranded RNA.

Immune electron microscopy

Examination of the virus-antibody mixtures by electron microscopy suggested that there was some degree of cross reaction between the different IPN virus serotypes and between Tellina virus and the three IPN viruses. Table 5 gives a semi-quantitative assessment of the degree of relatedness between Tellina virus and the three IPN virus serotypes and examples of the reactions are shown in Fig. 8.
Fig. 8. Immune complexing of Tellina virus and IPN-Vr virus with homotypic and heterotypic sera.
Bar = 100 nm.
DISCUSSION

Our results show that the physicochemical properties of the virus isolated by Hill (1976a) from Tellina are very similar to those of IPN virus. The failure of Tellina virus to react with antisera to the IPN viruses in neutralization tests differentiates it from the three IPN virus serotypes. However, Tellina virus reacts with IPN virus antiserum in immune electron microscopy and immunofluorescence tests showing that there is a serological relationship between the two viruses, presumably through a group antigen.

We have found a range of particle sizes ranging from 51 to 73 nm in diam. in PTA stained preparations of Tellina and IPN viruses, which is in fair agreement with the published sizes for IPN virus, i.e. 65 to 75 nm (Moss & Gravell, 1969; Kelly & Loh, 1972; Cohen, Poinsard & Scherrer, 1973). We consider, however, that the most realistic size for Tellina virus and the three IPN viruses is 59 nm, a figure characteristic of particles found in groups in thick stain. These particles should show minimal distortion and reflect more closely their size. The value of 59 nm is more in accordance with a sedimentation coefficient of 430S, than the value of 65 to 75 nm given by other workers. Reovirus, which has a diam. of 70 nm, sediments at 734S.

The RNAs of Tellina virus and IPN virus are also similar. In several experiments with different preparations of the RNAs we consistently obtained heterogeneous profiles by polyacrylamide gel electrophoresis similar to those obtained by Cohen et al. (1973), Dobos (1976) and Macdonald & Yamamoto (1977) for IPN virus, but unlike the sharp profiles obtained by Kelly & Loh (1972). Although our results do not throw any further light on whether the RNA is double-stranded or a highly ordered single-stranded molecule, the recent evidence provided by Dobos (1976) and Macdonald & Yamamoto (1977) appears to be conclusive that the RNA is composed of two segments of double-stranded RNA with mol. wt. 2.5 and 2.3 × 10^8.

Our analyses of the polypeptides of Tellina virus and the three serotypes of IPN virus showed the presence of three bands with mol. wt. 110, 67 and 40 × 10^3. Cohen et al. (1973) also found a similar pattern for IPN virus. Although Loh, Lee & Kelly (1974) described the presence of seven polypeptides in their preparations of IPN virus, several of the bands in their polyacrylamide gels were very faint. We have also observed minor bands which individually represented less than 1% of the total staining of the polypeptides.

In addition to the close relationship between the Tellina and IPN viruses, some of the morphological features common to both viruses are remarkably similar to those described for infectious bursal disease virus (Almeida & Morris, 1973; Harkness et al. 1975). First, we have observed in a purified Tellina virus preparation which had been stored at 4°C for two weeks, groups of particles associated with a membrane-like structure, similar to that observed by Harkness et al. (1975) in purified bursal disease virus. Secondly, the small particles, diam. 18 to 20 nm, observed by Almeida & Morris (1973) and Harkness et al. (1975) in bursal disease virus preparations have also been seen occasionally in our purified preparations of Tellina and IPN viruses, but in very much smaller numbers than reported by these authors. Since we have also found large numbers of the small particle in preparations of bursal disease agent and as those examined by Harkness et al. (1975) were first purified by sucrose gradient centrifugation before examination in the electron microscope, it seems a reasonable assumption that the small particles are a breakdown product of the larger virus particle. Finally, the size of the infectious bursal disease agent is 55 to 60 nm (Almeida & Morris, 1973; Harkness et al. 1975; Nick et al. 1976) similar to the size of the Tellina and IPN viruses.
The polypeptide composition of infectious bursal disease agent is similar to but not identical with those we have found for the Tellina and IPN viruses. Moreover the infectious bursal disease agent contains two segments of RNA similar in size to those found for Tellina virus and IPN virus RNA. Although Nick et al. (1976) consider that the RNA of the bursal disease agent is single-stranded their evidence on this point is not conclusive.

The properties of Tellina and IPN viruses do not allow them to be included in any of the present virus genera. We suggest that they should be regarded as a new genus. The close morphological and biochemical similarity of the infectious bursal disease agent to the Tellina and IPN viruses suggests that this agent also should be regarded as a possible candidate for inclusion in the proposed genus although more precise characterization is required. However, we have been unable to detect any serological relationship between the bursal disease agent and IPN-Sp and Tellina virus by immuno-electron microscopy.

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REFERENCES


Characterization of a virus from Tellina tenuis


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