Viruses with Double-stranded RNA Genomes

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During the past decade 16 viruses which possess double-stranded ribonucleic acid (ds-RNA) genomes have been found in vertebrate, invertebrate, bacterial, higher plant and fungal hosts. Although once considered a small unique class of viruses, it is now apparent that ds-RNA viruses may be of common occurrence especially among the mycoviruses (viruses of fungi) and arboviruses. Besides the viruses discussed herein, there are presently more than 50 additional viruses which are considered as possibly containing ds-RNA genomes.

A comparison of these viruses with disregard to host specificities shows that many ds-RNA viruses share common properties and are similar enough to be considered as distinct groups of ds-RNA viruses. The purpose of this article is to present the salient chemical, physical and biological properties of these viruses as currently described, and to discuss the similarities and dissimilarities both within and between groups of these viruses.

REOVIRUS

The reoviruses, serotypes 1, 2 and 3, are ubiquitous in vertebrates. They have been reported in horses, kangaroos, cattle, pigs, fowl, mice, sheep and trout, as well as humans (Rosen, 1962; Stanley & Leak, 1963; Stanley et al. 1964). Although the virus has been isolated following clinical disorders such as Burkitt’s lymphoma (Bell et al. 1964), because of their broad distribution in nature, it has not been possible to assess the role of reoviruses pathologically.

Purified reovirus particles have a buoyant density in caesium chloride (CsCl) of 1.38 g/cm³ and by chemical measurement contain 14.6% RNA. The particles have a sedimentation coefficient of 630 S and diffusion coefficient of 8.3 x 10⁻⁸ cm²/s, corresponding to a minimum particle mol. wt. of 70 x 10⁶ (Gomatos & Tamm, 1963a).

The particles are constructed of a nucleic acid core surrounded by two distinct protein shells. The outer capsid is icosahedral (5:3:2 symmetry) with an overall diameter of 60 to 75 nm (Fig. 1B) (Gomatos et al. 1962; Vasquez & Tournier, 1962; Loh, Hohl & Soergel, 1965; Mayor et al. 1965; Luftig et al. 1972). Three interpretations of the outer capsid structure have been proposed. The first, illustrated in Fig. 2A, considers that there are 60 hexagonal and 12 pentagonal (92) elongated hollow capsomeres measuring 10 nm long and 8 nm wide with a hole about 4 nm in diameter (Vasquez & Tournier, 1962; Loh et al. 1965; Mayor et al. 1965). An alternate suggestion (Vasquez & Tournier, 1964; Amano et al. 1971) is that the outer capsid is composed of 180 solid capsomeres which are truncated pyramids of shared structural subunits (Fig. 2B). Twelve of the 92 holes on the surface are surrounded by five structural subunits and the remainder by six structural subunits. A third proposed structure (Luftig et al. 1972) is that the outer capsid is composed of approximately 127 capsomeres which are truncated pyramids.

The outer capsid can be removed in vitro by treatment with trypsin, sodium pyrophosphate, sonication or chymotrypsin (Dales, Gomatos & Hsu, 1965; Mayor et al. 1965; Gomatos, 1967; Shatkin & Sipe, 1968). The inner capsid measures 40 to 45 nm in diameter
Fig. 1. Electron micrographs of wound tumour virus (A), reovirus (B) and subviral reovirus particles (C). (From Streissle & Granados, 1968.)

Fig. 2. Proposed structures of reovirus outer capsid. (A) 80 hexagonal and 12 pentagonal hollow prisms. (From Vasquez & Tournier, 1962.) (B) 180 truncated pyramids. (From Vasquez & Tournier, 1964.)

(Fig. 1 C) and may be constructed of 42 (Müller, Schneider & Peters, 1966) or 127 (Luftig et al. 1972) capsomers. The nucleic acid core encapsulated by the inner shell is referred to as a subviral particle (SVP).

The virus particles are constructed of seven protein subunits ranging in mol. wt. from 155,000 to 34,000 (Table 1) (Loh & Shatkin, 1968; Smith, Zweerink & Joklik, 1969; Zweerink & Joklik, 1970). Two noncapsid virus proteins have been identified in infected L cells (Zweerink, McDowell & Joklik, 1971). The μ2 polypeptide has been shown to arise by cleavage of μ1 protein (Zweerink & Joklik, 1970). Thus, a total of 8 primary gene products has been identified.

The reovirus ds-RNA exists as ten segments with mol. wts. ranging from 2.5 to 0.61 x 10^6 (Fig. 3) (Shatkin, Sipe & Loh, 1968). A possible coding relationship between these RNAs and virus proteins is illustrated in Table 1. Each ds-RNA segment has been shown to be specific and from each is transcribed a single piece of messenger (m) RNA (Bellamy & Joklik, 1967; Shatkin & Rada, 1967; Watanabe & Graham, 1967; Watanabe, Prevec & Graham, 1967). Electron microscopy of degraded particles indicates that each virion contains 10 ds-RNA segments (Vasquez & Kleinschmidt, 1968). From the molar ratios an estimate of 1.47 x 10^6 ds-RNA per virion was made.

Chemical studies have shown that the encapsulated and extracted reovirus ds-RNAs have the same number of nonphosphorylated 3' terminal nucleotides and therefore the segments do not arise by specific breakage of RNA molecules during extraction (Millward
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Table 1. Possible relationship between reovirus proteins and ds-RNA segments*

<table>
<thead>
<tr>
<th>Protein class</th>
<th>Protein mol. wts.</th>
<th>RNA segment</th>
<th>Mol. wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer shell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu 1 )</td>
<td>80000</td>
<td>5</td>
<td>1.6 x 10^6</td>
</tr>
<tr>
<td>( \mu 2 ) (from ( \mu 1 ))</td>
<td>72000</td>
<td>5</td>
<td>1.6 x 10^6</td>
</tr>
<tr>
<td>( \sigma 1 )</td>
<td>42000</td>
<td>7</td>
<td>0.92 x 10^6</td>
</tr>
<tr>
<td>( \sigma 3 )</td>
<td>34000</td>
<td>10</td>
<td>0.61 x 10^6</td>
</tr>
<tr>
<td>Inner capsid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \lambda 1 )</td>
<td>155000</td>
<td>1</td>
<td>2.5 x 10^6</td>
</tr>
<tr>
<td>( \lambda 2 )</td>
<td>140000</td>
<td>3</td>
<td>2.3 x 10^6</td>
</tr>
<tr>
<td>( \sigma 2 )</td>
<td>38000</td>
<td>8</td>
<td>0.76 x 10^6</td>
</tr>
<tr>
<td>Non-capsid protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu 0 )</td>
<td>88000</td>
<td>4</td>
<td>1.6 x 10^6</td>
</tr>
<tr>
<td>( \sigma 2A )</td>
<td>36000</td>
<td>9</td>
<td>0.64 x 10^6</td>
</tr>
<tr>
<td>Not described</td>
<td></td>
<td>2</td>
<td>2.4 x 10^6</td>
</tr>
<tr>
<td>Not described</td>
<td></td>
<td>6</td>
<td>1.4 x 10^6</td>
</tr>
</tbody>
</table>

† Shatkin, Sipe & Loh (1968).

Fig. 3. Electrophoretogram of reovirus ds-RNA following electrophoresis in polyacrylamide gel columns. (From Fujii-Kawata, Miura & Fuke, 1970.) For mol. wts. see Table 1.

& Graham, 1970). The 3' and 5' termini of the ds-RNA and virus m-RNA segments are cytosine and ppGp-pyrimidine, respectively (Bellamy, Hole & Baguley, 1970; Banerjee & Shatkin, 1971; Banerjee, Ward & Shatkin, 1970). It has been suggested that the 5' termini but not the 3' termini of these ds-RNA strands may have 6 to 8 unpaired bases (Millward & Nonoyama, 1970).

Besides ds-RNA the reovirus particles also contain heterogenous single-stranded (ss) RNA which has a high adenine content (Bellamy et al. 1967). The adenine-rich RNA constitutes 25% of the total encapsulated nucleic acid and has a maximum chain length of 12 nucleotides (Bellamy et al. 1967; Shatkin & Sipe, 1968; Bellamy & Hole, 1970) but as it is not required for infectivity (Krug & Gomatos, 1969), its significance is unknown.
In close association with the reovirus ds-RNA is a transcriptase which is only active in the SVP following removal of the outer shell. The product of the ss-RNA transcriptase hybridizes specifically with the virus RNA (Borsa & Graham, 1968; Shatkin & Sipe, 1968; Skehel & Joklik, 1969; Levin et al. 1970). The ss-product is synthesized in a conservative manner and only one strand of each ds-RNA segment is copied (Skehel & Joklik, 1969; Banerjee & Shatkin, 1970; Levin et al. 1970). Annealing and chemical analyses have indicated that this in vitro product is identical to in vivo m-RNA (Banerjee et al. 1971; Hay & Joklik, 1971). This was confirmed when McDowell et al. (1972) synthesized in vitro the eight virus proteins using the transcriptase product as m-RNA.

Four nucleotide-5'-triphosphatases have also been found associated with SVP of reovirus (Borsa, Grover & Chapman, 1970; Kapuler et al. 1970). The function of these enzymes is not known.

The replication of reoviruses in L mouse cells has been studied extensively and a proposed replicative scheme has evolved. The virus particles enter the cells by phagocytosis (Dales et al. 1965). Within an hour 80% of the particles are enclosed in lysosomes and shortly thereafter the outer shells are stripped away leaving SVP (Silverstein & Dales, 1968). Although these SVP are similar in appearance and function to those produced in vitro, they have a lower density and differ in a single protein from the in vitro produced SVP (Silverstein et al. 1972). Messenger RNA is transcribed from the ds-RNA segments in a conservative manner and becomes associated with ribosomes (Bellamy & Joklik, 1967; Shatkin & Rada, 1967; Watanabe et al. 1967). Following protein synthesis (Kudo & Graham, 1966) the m-RNA is used as a template for the production of ds-RNA segments (Schonberg et al. 1971). Therefore ds-RNA replication is asynchronous. Among the progeny particles are masses of filaments and mitotic spindle tubules which apparently are coated with virus proteins (Dales, 1963; Dales et al. 1965).

The polymerization of progeny protein and nucleic acid has been difficult to follow because of the non-synchronous production of progeny particles. The parental SVP particles are conserved in the lysosomes and in vitro encapsulation with the outer capsid proteins can occur following cell disruption (Chang & Zweerink, 1971; Astell et al. 1972).

WOUND TUMOUR VIRUS

The wound tumour virus (WTV) of plants and insects closely resembles the reoviruses. This insect transmitted virus has a host range of 43 plant species in 20 different families and at least three species of the leafhopper, Agallia (Black, 1945; Black & Brakke, 1952; Black, 1965). The most common symptoms of infected plants are an enlargement of veins, shortening of internodes, distortion of leaves and tumour development in roots (Black, 1945, 1965). Wounding has generally been associated with the origin of tumour development (Black, 1946). Systemically infected insects do not appear to be affected by the virus (Shikata & Maramorosch, 1965; Maramorosch, Shikata & Granados, 1969a) although degeneration of ganglion, intestinal and fat body cells has been reported (Hirumi, Granados & Maramorosch, 1967; Maramorosch, Shikata & Granados, 1969b). The virus can be transmitted transovarially to a low percentage of insect progeny (Black, 1953; Sinha & Shelley, 1965). Following repeated vegetative propagation of infected sweet clover plants, a vectorless strain of WTV was obtained which lost its ability to replicate in insects (Black, Wolever & Whitcomb, 1958).

The WTV particles have a sedimentation coefficient of 514 ± 14 S, contain 20 to 23% ds-RNA and are estimated to have a particle mol. wt. of 68 to 75 × 10^6 (Black & Markham,
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1963; Kalmakoff, Lewandowski & Black, 1969). The particles have a buoyant density of 1.411 g/cm³ in CsCl (Gomatos & Tamm, 1963b).

The virions contain only ds-RNA, which can be fractionated into eight size classes (Fig. 4) ranging from 2.55 to 0.34 x 10⁶ (Kalmakoff et al. 1969; Wood & Streissle, 1970). Molar ratios of 12 and 15 segments of RNA have been reported, resulting in estimates of 15.5 and 16.5 x 10⁶ daltons of ds-RNA per genome. The GC content of WTV-RNA is approximately 38% (Gomatos & Tamm, 1963b). Double-stranded RNAs purified from infected tumour tissue and preparations of the vectorial or vectorless strains of WTV are identical in distribution and mol. wts. (H. A. Wood & G. Streissle, unpublished data).

The virus particles measure 60 to 80 nm in diameter (Brakke, Vatter & Black, 1954; Bils & Hall, 1962) and are composed of two protein shells (Fig. 1A) (Streissle & Granados, 1968). The outer shell is icosahedral and contains 92 capsomeres measuring approximately 7.5 nm in diameter (Bils & Hall, 1962). As with the reoviruses, following treatment with trypsin, sodium pyrophosphate or urea, the outer capsid is removed leaving an inner shell measuring approximately 44 nm in diameter (Streissle & Granados, 1968).

Associated with purified virions is an RNA polymerase which transcribes ss-RNA from the viral genome (Black & Knight, 1970). The product anneals specifically with the virus genome and pretreatment of the particles is not required to activate the enzyme.

Infection of insect cells apparently occurs by phagocytosis since virions are initially found in phagocytic vesicles and lysosome-like structures (Shikata & Maramorosch, 1967; Granados, 1973). Some of these particles appear to have lost their outer protein shell and abnormal tubular structures are commonly found in both infected plant and insect cells (Fig. 5) (Shikata & Maramorosch, 1965, 1966; Granados, 1973). Although phagocytic vesicles containing virions have not been reported in plants, replication of the virus in the cytoplasm in both plant and insect hosts appears identical (Shikata et al. 1964; Granados, 1973).

RICE DWARF VIRUS

The rice dwarf virus (RDV) is a leafhopper-borne virus of rice and other members of the Gramineae (Fukushi, 1934, 1965; Shikata, 1965). The virus is transmitted by Nephotettix cincticeps and Inazuma dorsalis following replication (Fukushi, 1935; Fukushi et al. 1960). From 60 to 85% of the progeny from viruliferous females are infected with RDV (Fukushi, 1933, 1935, 1940). Infected females generally have reduced egg production and adult life spans (Nasu, 1963).

The RDV particles are approximately 70 nm in diameter and are icosahedral in appear-
Fig. 5. Wound tumour virus particles within virus-associated cytoplasmic tubules. Nerve cell of *Agalli~ constricta*. (Courtesy of R. R. Granados.)

ance (Fukushi *et al.* 1960; Fukushi, Shikata & Kimura, 1962; Fukushi & Shikata, 1963a). The protein shell is composed of 32 hollow capsomeres which are 6 nm in diameter and 9.5 nm long (Kimura & Shikata, 1968). Although not observed, RDV may have two protein shells which are tightly packed. Purified virus particles generally are encapsulated in a membranous structure which can be removed without loss of infectivity (Suzuki, Kimura & Miura, 1965; Toyoda, Kimura & Suzuki, 1965). The virus sediments at 510 S and contains approximately 11.7% ds-RNA (Suzuki & Kimura, 1969). The RNA contains 44% GC (Miura, Kimura & Suzuki, 1966). RDV has been reported to contain only ds-RNA (Miura *et al.* 1966) which has been fractionated into 9 mol. wt. classes with molar ratios indicating the presence of 12 genomic segments (Fig. 6A) (Fujii-Kawata, Miura & Fuke, 1970).

Replication in plant and insect hosts appears to be very similar (Fukushi *et al.* 1962; Fukushi & Shikata, 1963b; Shikata, 1965). Virus maturation occurs in a cytoplasmic matrix. The particles appear to be enveloped in a membrane and the formation of tubules with or without internal viral particles has been observed.

**BLUETONGUE VIRUS**

The bluetongue virus (BTV) causes a fatal disease of sheep and is prevalent mainly in Africa. The virus is transmitted by biting flies of the genus *Culicoides* (DuToit, 1944; Foster, Jones & McCrory, 1963). Replication of BTV occurs in the insect host (Bowne & Jones, 1966) but there is no evidence for transovarial transmission (Jones & Foster, 1971).

The BTV particles have a bouyant density in CsCl of 1.38 to 1.36 g/cm³, sedimentation
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Fig. 6. Electrophoretograms of ds-RNA from rice dwarf virus (A) and silkworm cytoplasmic polyhedrosis virus (B). Mol. wts. of RDV-RNA classes 1 to 10, respectively, are 2.8, 2.35, 2.05, 1.75, 1.72, 1.03, 1.03, 0.77, 0.71, and 0.44 \times 10^6. CPV-RNA mol. wts. 1 to 10, respectively, are 2.55, 2.42, 2.32, 2.03, 1.82, 1.12, 0.84, 0.62, 0.56 and 0.35 \times 10^6. (From Fujii-Kawata et al. 1970.)

The virions are icosahedral in appearance and diameters of 53 to 68 nm have been reported (Owen & Munz, 1966; Studdert, Pangborn & Addison, 1966; Cromack, Blue & Gratzek, 1971; Murphy et al. 1971; Els & Verwoerd, 1969). Recent studies (Martin & Zweerink, 1972; Verwoerd et al. 1972) indicate that unlike reovirus and WTV, the BTV has a diffuse outer coat which is removed following exposure to CsCl (Fig. 7A).

The outer coat may contain 92 capsomeres (Owen & Munz, 1966; Studdert et al. 1966). The inner shell has a diameter of 54 to 64 nm and contains 32 capsomeres (Els & Verwoerd, 1969; Murphy et al. 1971; Martin & Zweerink, 1972). Each capsomere appears to be an elongated hollow column 8 nm long, 10 to 12 nm wide with a hole 4 nm in diameter (Fig. 7B) (Els & Verwoerd, 1969; Cromack et al. 1971).

The BTV particles are constructed of four major and three minor protein subunits with mol. wts. from 155 to 29 \times 10^3 (Martin & Zweerink, 1972; Verwoerd et al. 1972). The outer coat is composed of two major proteins. It is not known if these are primary gene products.

The ds-RNA of BTV exists as ten segments ranging from 2.5 to 0.3 \times 10^6 (Fig. 8) (Verwoerd, 1969; Verwoerd et al. 1970; Martin & Zweerink, 1972; Verwoerd et al. 1972). The equal molar ratio of the segments suggests that each particle contains 12 \times 10^6 daltons of ds-RNA which agrees with other chemical and physical data. Single-stranded RNA which sediments at approximately 4 S has also been found associated with BTV (Verwoerd,
Fig. 7. Bluetongue virus particles. (A) Virions with diffuse outer shell. (B) Inner capsid following exposure to CsCl. (From Verwoerd et al. 1972.)

Fig. 8. Fractionation of bluetongue virus ds-RNA by electrophoresis in polyacrylamide gels. RNA classes 1 to 10 have mol. wts. of 2.5, 1.99, 1.82, 1.31, 1.16, 1.08, 0.6, 0.54, 0.50 and 0.3 × 10^6 respectively. (From Verwoerd et al. 1970.)

1969; Verwoerd et al. 1970). It is not known if this RNA is a contaminant or is encapsulated similar to the ss-RNA of reovirus.


In insects and mammalian tissue cultures, BTV particles appear to enter cells by phagocytosis and are associated with phagocytic vesicles and lysosomes (Bowne & Jones, 1966; Lecatsas, 1968; Cromack et al. 1971). Subviral structures have been observed in lysosomes and accumulations of cytoplasmic tubules have been associated with viral synthesis in the cytoplasm. BTV is serologically related to the EHDNJ, IbAr 22619, Eubenangel and B 1327 viruses (Borden, Shope & Murphy, 1971).
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AFRICAN HORSE-SICKNESS VIRUS

The African horse-sickness virus (AHSV) is an arthropod-borne virus similar to BTV. It is transmitted by insects in the genus Culicoides (DuToit, 1944) and has a host range which includes horses, mules, donkeys, goats, dogs and ferrets (Howell, 1963). The AHSV particles measure 55 to 80 nm in diameter. They are icosahedral in appearance and are described as being constructed of 32 (Oellermann, Els & Erasmus, 1970) or 92 capsomeres (Polson & Deeks, 1963). The measurement range suggests the presence of an outer protein shell which is labile, similar to that of BTV. The virus has a sedimentation coefficient of approximately 476 S (Polson & Deeks, 1963). The particles contain only ds-RNA, which has been fractionated by polyacrylamide gel electrophoresis into six components (Oellermann et al. 1970). The distribution of these components is similar to those found with BTV (Verwoerd et al. 1970). The infection and replication of AHSV as reported by Lecatsas & Erasmus (1967) are also similar to that described for BTV. Like reovirus (Lerner, Cherry & Finland, 1963), AHSV hemagglutinates red blood cells (Pavri, 1961).

COLORADO TICK FEVER VIRUS

Another arthropod-borne virus containing ds-RNA is the Colorado tick fever virus (CTFV). The virus is transmitted by Dermacentor andersoni (Florio, Stewart & Mugrage, 1944) and contains ds-RNA which sediments at approximately 14 S (Green, 1970). Available evidence suggests that the virus may also contain ss-RNA. The particles are constructed of two protein shells measuring 80 and 50 nm in diameter. The outer coat is considered to contain 92 capsomeres. The virus is cytoplasmic in nature and replication is accompanied by the appearance of tightly packed filaments (Murphy et al. 1968).

CYTOPLASMIC POLYHEDROSIS VIRUS

The cytoplasmic polyhedrosis viruses (CPV), like reoviruses and WTV, are ubiquitous. A host list of 80 insect species was compiled by Smith (1963). The CPVs are restricted to insects and following replication in midgut cells are occluded in a crystalline protein matrix. These inclusion bodies vary in size and may be hexagonal, pentagonal or triangular polyhedrons. The shape of the polyhedrons is characteristic of a particular CPV and therefore the inclusion protein may be a virus-coded product (Hukuhara & Hashimoto, 1966; Hukuhara, 1967). Virus can be extracted from insect midgut tissue prior to occlusion or from inclusions following treatment with dilute alkali solutions (Hills & Smith, 1959).

The majority of CPV-type viruses of insects have not been shown to contain ds-RNA and little is known about the relationships between the CPVs described in different hosts. The CPV of silkworm has been studied extensively. The particles sediment at 415 to 440 S and have a buoyant density in caesium chloride of 1.37 g/cm³. The virions contain 23 to 30 % RNA and have an estimated mol. wt. of 54 ± 4 x 10⁶ (Kalmakoff et al. 1969; Miyajima, Kimura & Kawase, 1969; Nishimura & Hosaka, 1969).

The particles consist of two concentric icosahedral capsids. The outer capsid has an overall diameter of 60 to 69 nm and the inner shell a diameter of approximately 45 nm. The model proposed for the structure of silkworm CPV (Hosaka & Aizawa, 1964) agrees well with the structural interpretations of CPVs in other insects such as the white marked tussock moth (Bird, 1965), noctuid larva (Smith, 1967) and the monarch butterfly (Arnott, Smith & Fullilove, 1968).
The outer shell of CPV consists of 12 pentagonal capsomeres, localized at the vertices of the icosahedron (Fig. 9). The capsomeres are in the form of hollow discs with an outer diameter of 20 nm and inner diameter of 5 nm. Each capsomere has a protruding hollow segmented projection. At the end of the projections spherical structures measuring 12 nm in diameter have been described (Fig. 10) (Asai, Kawamoto & Kawase, 1972). The inner shell is viewed as being connected to the outer shell by the tubular structure at its 12 vertices (Fig. 9). Similar to the reoviruses and AHSV, the CPV of silkworms agglutinate mammalian erythrocytes (Miyajima & Kawase, 1969).

The ds-RNA from the silkworm CPV can be fractionated into ten components by polyacrylamide gel electrophoresis (Fig. 6B) (Fujii-Kawata et al. 1970; Lewandowski & Millward, 1971). The approximately equal molar ratio of these components suggests that each
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A double-stranded RNA virus particle contains a complete complement of ten RNA components. The silkworm CPV-RNA has a 43% GC content (Miura et al. 1968). Using a periodate-tritium borohydride procedure, it was found that, as with reovirus, the number of 3' terminal bases is the same before and after RNA extraction, therefore precluding the possibility of breakage of a large ds-RNA molecule during extraction. The 3' terminal bases are half cytidine and half uridine, and neither is phosphorylated (Furuichi & Miura, 1972). No single-stranded RNA has been reported in CPV particles.

The CPV particles of silkworm also contain a virus specific RNA transcriptase. Removal or alteration of the outer protein shell, however, is not necessary for activation of the enzyme (Lewandowski, Kalmakoff & Tanada, 1969; Donagheue & Hayashi, 1972).

Only preliminary studies have been made concerning the replication of CPV. Following ingestion of occluded virus, the inclusion bodies are disrupted by the alkalinity of the gut fluids. Infection of the gut cells has been hypothesized to occur by the release of virus core materials into the cells through the tubular structures at the vertices (Hosaka, 1964; Kobayashi, 1971). The evidence for this is that soon after ingestion, virus particles which appeared empty were often observed attached to cell membranes at the vertices.

Several investigations have indicated that transcription of the parental RNA occurs in the nucleus. Using autoradiographic procedures Watanabe (1967) observed increased uptake of (H)-uridine, but not thymidine, in the nucleolar region of the nucleus even at late stages of infection. Host RNA synthesis, however, is not significantly altered following infection (Hayashi, 1970). In the presence of actinomycin D host RNA synthesis can be halted but in infected nuclei RNA synthesis proceeds (Hayashi, Kawarabata & Bird, 1970). In vitro synthesis studies with cell fractions from infected cells indicate that the nuclei and a 10,000 g pellet comprised of mitochondria and large particles contain a virus-induced RNA polymerase similar to that found in the virion (Hayashi et al. 1970). These investigations indicate that virus transcription occurs in the nuclei, followed by protein and ds-RNA synthesis, virus assembly and occlusion in the cytoplasm.

Maize Rough Dwarf Virus

The maize rough dwarf virus (MRDV) is a planthopper-borne virus which has been transmitted to 16 species of plants in the Gramineae (Lovisolo, 1971) by members of the Delphacidae, Laodelphax striatellus (Harpaz, 1961), Javesella pellucida (Harpaz et al. 1965), Delphacodes propinquus (Harpaz & Klein, 1969) and Sogatella vibix (Harpaz & Klein, 1969). The virus is transmitted transovarially (Harpaz & Klein, 1969); however, viruliferous females of L. striatellus lay few eggs and only a small percentage of the progeny reach the adult stage (Harpaz, 1972). Infected corn develops small galls on the veins on the under surface of leaves and the plants are dwarfed. Root system development is reduced and atrophy of the ears may occur.

Recent investigations by Lesemann (1972) and R. G. Milne, M. Conti & V. Lisa (personal communication) have shown that purified MRDV particles have two capsid shells which are similar in structure to the silkworm CPV. The outer capsid measures 70 nm in diameter, is composed of 92 capsomeres and has 12 spikes on its 5-fold vertices (Fig. 11). The inner shell has a diameter of 50 to 55 nm with similarly positioned spikes which appear to project into the core (Fig. 11). The particle cores have been shown to contain only ds-RNA and have a sedimentation coefficient of 400 S (Redolfi & Pennazio, 1972).

The intracellular appearance of MRDV in insects and plant neoplasma is similar. The virus cores appear encapsulated in lysosome-like structures; abundant cytoplasmic micro-
tubules are observed; and mature particles are often found aligned in these tubular structures (Gerola et al. 1966a, b; Vidano, 1970). The particles were not observed outside of the cytoplasm.

Lovisolo (1970) has reported that in cooperation with E. Shikata and co-workers the rice black-streaked dwarf virus (RBSDV) was found to be serologically related to MRDV. The RBSDV is transmitted by and replicates in *Laodelphax striatellus, Delphacodes albi-fascia* and *Unkanodes sapporonus*. It is restricted to Gramineae hosts such as corn, wheat, barley and rice (Hirao, 1968; Iida, 1969). Galls are produced on the veins of the underside of the leaves, the plants are stunted and late in the development of the disease dark streaks are observed on rice leaves. In thin section the RBSDV particles appear 75 to 85 nm in diameter with core particles measuring 50 to 55 nm (Shikata, 1969). Tubules similar to those produced by MRDV are produced and the particles are often observed within them. Therefore the RBSDV and MRDV are considered to be strains of the same virus.

**FIJI DISEASE VIRUS**

The Fiji disease virus (FDV) of sugar cane also stimulates gall formation and is similar to the RBSDV and MRDV. It is transmitted by members of the genus *Perkinsiella*, also a member of the *Delphacidae*. Electron-microscopic studies of leaf and insect tissue, as well as purified preparations, indicate that the virus is restricted to the cytoplasm and has a double protein shell. The outer and inner capsids have diameters of 70 and 40 nm, respectively (Teakle & Steindl, 1969; Francki & Grivell, 1972). Using antiserum to ds-RNA, Francki & Jackson (1972) immunologically detected ds-RNA in infected but not healthy sugar-cane tissue. Since they were unable to detect ds-RNA prior to phenol extraction, it was concluded that the ds-RNA was encapsulated in the virus particles.
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Fig. 12. Electron micrograph of *Penicillium chrysogenum* (ATCC 9480) virus particles. (From Wood & Bozarth, 1972.)

**PENICILLIUM CHRYSOGENUM VIRUS**

The virus in the ATCC 9480 isolate of *Penicillium chrysogenum* and derived isolates (Banks *et al.* 1969) is the most thoroughly described of the mycoviruses. The virus is spore transmitted and purified preparations will infect host protoplasts (Pallett, 1972). The *P. chrysogenum* virus (PcV) particles are single-capsid icosahedrons measuring 35 to 40 nm in diameter (Banks *et al.* 1969; Buck, Chain & Himmelweit, 1971; Wood & Bozarth, 1972) (Fig. 12). The virus sediments at 150 S and has a buoyant density of 1.35 g/cm³ in CsCl. A diffusion constant of $1.03 \times 10^{-7}$ cm²/s as well as chemical measurements, indicate an RNA content of 11 to 15 % and a particle mol. wt. of approximately $13 \times 10^6$ (Wood & Bozarth, 1972).

The PcV particles contain only ds-RNA with a GC content of 56 % (Lemke & Ness, 1970). The RNA can be fractionated into three species with mol. wts. of 1.89, 1.99 and $2.18 \times 10^6$ (Wood & Bozarth, 1972). The biophysical and chemical data indicate that each PcV particle contains a single piece of ds-RNA. A virus similar in its properties has been isolated from *Penicillium brevi-compactum* NRRL 5260 (Wood, Bozarth & Mislivec, 1972) and shown to be serologically related to PcV (Wood & Bozarth, 1972).

Nash *et al.* (1973) have reported that the PcV particles contain an RNA-dependent RNA polymerase. Its activity can be increased following treatment of the virus with Triton X-100. They also reported an indication of RNA-dependent DNA polymerase activity in their preparations.

Thin-section electron-microscopic investigations of PcV indicate that the particles replicate in the cytoplasm. They are only associated with organelles after cell degeneration occurs (Fig. 13) (Yamashita, Doi & Yora, 1973).

**PENICILLIUM STOLONIFERUM VIRUSES**

The viruses in the ATCC 14586 isolate of *Penicillium stoloniferum* were the first viruses found in the *Fungi Imperfecti* (Ellis & Kleinschmidt, 1967). Two serologically distinct viruses, PsV-f and PsV-s, are present in this isolate (Buck & Kempson-Jones, 1970; Bozarth, Wood & Mandelbrot, 1971). The PsV-f has the highest electrophoretic mobility at basic pHs. The viruses are morphologically indistinguishable with diameters of 30 to 34 nm (Fig. 14).
The PsV-f and -s are component viruses with a minimum of 7 and 4 density species, respectively, ranging from 1.299 to 1.376 g/cm³ in CsCl. Both viruses have three centrifugal components which sediment between 104 and 61 S. PsV-f particles contain a small amount of ss-RNA plus three ds-RNAs with mol. wts. of 0.99, 0.89 and 0.23 x 10⁶. The purified PsV-s particles contain 1.01 and 0.94 x 10⁶ mol. wt. ds-RNAs (Bozarth et al. 1971).

Lhoas (1971a) has demonstrated transmission of both viruses through heterokaryosis between two isolates of Penicillium stoloniferum. Only PsV-s was transmitted when virus extracts were mixed with host cell protoplasts (Lhoas, 1971b). A virus serologically related to PsV-s has been found in an isolate of Diplocarpon rosae (Bozarth, Wood & Goenaga, 1972). The PsV-s thus appear capable of replicating in the absence of PsV-f.

Both viruses are present in the single-celled spores of Penicillium stoloniferum and replicate concurrently without evidence of genotypic or phenotypic mixing (Bozarth et al. 1971). Replication and maturation of these viruses may occur at different sites in the cell since mixing of the high molecular weight ds-RNA does not occur. Adler & MacKenzie (1972) have studied these viruses in vivo using fluorescent-antibody techniques and observed that both viruses occur in the cytoplasm; however, no compartmentalization of the PsV-s and -f was found.

Lapierrre, Astier-Manifacier & Cornuet (1971) reported that preparations of PsV contain RNA polymerase activity which was inhibited by ethidium bromide but not by actinomycin D.
**Double-stranded RNA viruses**

Two viruses have also been found in the IMI 41871 isolate of *Aspergillus foetidus*, AfV-s and AfV-f (Banks *et al.* 1970). They are serologically distinct polyhedrons with diameters of 40 to 42 nm. Electrophoresis or differential solubility in 0.03 M-phosphate buffer, pH 7, can be used to purify the individual viruses (Ratti & Buck, 1972). Virus particles found in the IMI 46891 isolate of *A. niger* have a serological relationship to at least one of the *A. foetidus* viruses (Banks *et al.* 1970). The virus from *A. niger*, with the highest electrophoretic mobility at a basic pH, has been transmitted to *Saccharomyces cerevisiae* during mating of the yeast cells (Border, 1972; Lhoas, 1972).

Ratti & Buck (1972) reported that the AfV-f particles have four buoyant density components ranging from 1.351 to 1.381 g/cm³ in CsCl and three centrifugal components with sedimentation coefficients from 145 to 158 S. From AfV-f preparations they isolated four ds-RNA components with mol. wts. of 2.13, 1.87, 1.70 and 1.44 x 10⁷.

The AfV-s could be fractionated into four density components ranging from 1.396 to 1.435 g/cm³ in CsCl and two centrifugal components of 146 and 172 S. The virus contained ds-RNA with mol. wts. of 2.76 and 2.24 x 10⁷. From their data, it was concluded that like the PcV and PbV, the AfV-s and -f virus particles contain only a single segment of ds-RNA.

**Ustilago maydis Virus**

Mycoviruses have also been isolated from certain strains of *Ustilago maydis* (i.e. ATCC isolates 22898 and 22903) (Wood & Bozarth, 1973). The particles are spherical and measure 41 nm in diameter. From partially purified preparations, five sedimenting components were observed which sedimented at 110 to 160 S. The purified virus ds-RNA exists as five components with mol. wts. of 2.87, 2.52, 0.93, 0.44 and 0.06 x 10⁶ (Fig. 15). From the distribution of virus centrifugal and RNA components, it appears that each particle probably contains a single piece of ds-RNA.
Fig. 16. Particles of 06 bacteriophage isolated from Pseudomonas phaseolicola HB10Y. Membrane encapsulating polyhedral head. (By M. K. Brakke from A. K. Vidaver, R. K. Koski & J. L. Van Etten, personal communication.)

Indirect evidence suggests that the virus may be a cytoplasmically inherited genetic factor of Ustilago maydis (Day & Anagnostakis, 1973). The virus was found only in U. maydis isolates that contained a cytoplasmically inherited factor resulting in non-sensitivity of the cells to a toxin produced by certain strains of U. maydis. Following mating and haploidization procedures, the virus and this cytoplasmic factor are transmitted together.

**06 BACTERIOPHAGE**

The most distinct ds-RNA virus reported to date is the 06 bacteriophage of Pseudomonas phaseolicola. The 06 particles consist of a polyhedral head 60 nm in diameter which is encapsulated in a membranous structure that is required for infectivity (Fig. 16). This envelope appears to elongate, reminiscent of a phage tail, upon attachment to pili. The particles are composed of 25% lipid, 13% RNA and 62% protein and have a buoyant density of 1.27 g/cm³ in CsCl (A. K. Vidaver, R. K. Koski & J. L. Van Etten, personal communication).

The particles contain only ds-RNA with a GC content of 56%. The nucleic acid can be fractionated into three mol. wt. species using density-gradient or polyacrylamide-gel electrophoresis techniques. From the sedimentation values of $14.7$, $15.5$ and $17.2$ S and electron-microscopic length measurements of $0.85$, $1.2$ and $2.0$ μm, mol. wt. values of $1.9$, $2.8$ and $4.6 \times 10^6$ were obtained for the three RNA species (Semancik, Van Etten & Vidaver, 1972).

**DISCUSSION**

A comparison and classification of ds-RNA viruses can most meaningfully be achieved on the basis of particle morphology and the amount of the genome which is encapsulated per virus particle. These criteria are complementary in organizing the information which is presently available concerning these viruses (Table 2) and present a coherent scheme as opposed to secondary properties such as host specificity.

On the basis of particle morphology, these viruses can be classified into three major groups, double-capsid, single-capsid and membrane-bound single-capsid viruses. From existing information the first group can be subdivided according to morphological types.
Double-stranded RNA viruses

Table 2. Summary of chemical and physical properties of double-stranded RNA viruses

<table>
<thead>
<tr>
<th>Virus₁</th>
<th>Host range</th>
<th>Diameter (nm) of capsid shell(s)</th>
<th>No. of ds-RNA segments</th>
<th>Mol. wt. range of ds-RNA (× 10⁻⁶)</th>
<th>RNA/ particle (%)</th>
<th>Particle mol. wt. (× 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roovirus</td>
<td>Vertebrates</td>
<td>70.45b</td>
<td>10⁰</td>
<td>2.5 to 0.61⁰</td>
<td>15⁰</td>
<td>70⁰</td>
</tr>
<tr>
<td>WTV</td>
<td>Plants and insects</td>
<td>70⁴ , 44¹</td>
<td>15⁸</td>
<td>2.55 to 0.34⁴</td>
<td>23⁸</td>
<td>68b</td>
</tr>
<tr>
<td>RDV</td>
<td>Plants and insects</td>
<td>70¹</td>
<td>12¹</td>
<td>2.8 to 0.44¹</td>
<td>12¹</td>
<td>--</td>
</tr>
<tr>
<td>BTV¹</td>
<td>Mammals and insects</td>
<td>c. 64</td>
<td>10</td>
<td>2.5 to 0.3</td>
<td>20</td>
<td>59</td>
</tr>
<tr>
<td>AHHSV²</td>
<td>Mammals and insects</td>
<td>55 to 80</td>
<td>Minimum</td>
<td>of 6</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CTFV</td>
<td>Mammals and insects</td>
<td>80, 50a</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CPV</td>
<td>Insects</td>
<td>65, 45°</td>
<td>10¹</td>
<td>2.5 to 0.35²</td>
<td>23²</td>
<td>54²</td>
</tr>
<tr>
<td>MRDV</td>
<td>Plants and insects</td>
<td>70, 50⁰</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>FDV</td>
<td>Plants and insects</td>
<td>70, 40⁰</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>φ 6 b</td>
<td>Bacteria</td>
<td>60⁴</td>
<td>3⁸</td>
<td>4.6 to 1.9⁸</td>
<td>13⁸</td>
<td>--</td>
</tr>
<tr>
<td>PeV¹</td>
<td>Fungi</td>
<td>40⁴</td>
<td>3³</td>
<td>2.18 to 1.89</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>PsV-s⁸</td>
<td>Fungi</td>
<td>34</td>
<td>2³</td>
<td>1.01 to 0.94</td>
<td>Component virus</td>
<td>Component virus</td>
</tr>
<tr>
<td>PsV-f⁹</td>
<td>Fungi</td>
<td>34</td>
<td>3³</td>
<td>0.99 to 0.23</td>
<td>Component virus</td>
<td>Component virus</td>
</tr>
<tr>
<td>AfV-s¹</td>
<td>Fungi</td>
<td>41</td>
<td>2³</td>
<td>2.76 to 2.24</td>
<td>Component virus</td>
<td>Component virus</td>
</tr>
<tr>
<td>AfV-f¹</td>
<td>Fungi</td>
<td>41</td>
<td>4³</td>
<td>2.13 to 1.44</td>
<td>Component virus</td>
<td>Component virus</td>
</tr>
<tr>
<td>UmV²</td>
<td>Fungi</td>
<td>40</td>
<td>5³</td>
<td>2.87 to 0.06</td>
<td>Component virus</td>
<td>Component virus</td>
</tr>
</tbody>
</table>

¹ WTV, wound tumour virus; RDV, rice dwarf virus; BTV, bluetongue virus; AHHSV, African horse-sickness virus; CTFV, Colorado tick fever virus; CPV, cytoplasty polydorosis virus of silkworms; MRDV, maize rough dwarf virus; FDV, Fiji disease virus; φ 6, bacteriophage; PeV, Penicillium chrysogenum (ATCC 9480) virus; PsV-s and -f, P. stoloniferum (ATCC 14586) -s and -f viruses; AfV-s and -f, Aspergillus foetidus (IMI18711) -s and -f viruses; UmV, Ustilago maydis (ATCC 22898 & 22903) virus.

² Luftig et al. 1972.
³ Shatkin et al. 1968.
⁴ Komatos & Tamm, 1963a.
⁵ Bills & Hall, 1962.
⁶ Streissle & Granados, 1968.
⁸ Kalmakoff et al. 1969.
¹¹ Verwoerd et al. 1972.
¹² Oellermann et al. 1970.

The viruses within these subgroups differ in the number and sizes of the ds-RNA segments which they contain. These differences probably represent the number and size of gene products, which, at present, do not lend themselves as workable classification criteria.

Viruses with two protein shells can be separated into three categories. The first includes the reovirus and WTV. They are similar in size and both have distinct double-capsid shells which are similar if not identical in symmetry. The outer capsid can be removed under similar conditions. Following infection, both viruses are encapsulated in lysosomes and their outer shells are removed. However, in vitro the WTV transcriptase does not require removal of the outer shell for activation. The RDV may belong in this group. Although unsubstantiated, the properties of RDV suggest that the particles are composed of a double protein shell. These viruses contain from 10 to 15 RNA segments/particle with similar mol. wt. ranges.

A second subgroup includes the BTV, AHHSV and probably CTFV. They also have double-
capsid shells; however, the outer capsid is diffuse with little discernible structure. The outer capsid protein apparently is easily dissociated. With BTV at least partial removal of the outer coat is required for in vitro transcriptase activation. These viruses are all arthropod-borne and replicate in their insect hosts. There are several arboviruses which are similar in physical, morphological and replicative properties to these viruses. In addition they are also not serologically related to the togaviruses. A list of 21 of these viruses in 7 serologically unrelated groups have been proposed as belonging to the BTV group (Borden et al. 1971; Murphy et al. 1971). However, only preliminary data have been accumulated concerning the possibility that these viruses have ds-RNA genomes. Therefore, they have not been considered here.

A third subgroup of viruses with double-capsid shells consists of the silkworm CPV and MRDV. The outer capsid of the particles has projections which appear as spikes or knobs depending on staining conditions. There are 12 projections/particle each at the 5-fold vertices. The CPV is unique among the ds-RNA viruses discussed in that following maturation it is occluded in a proteinaceous matrix which may be viral in origin.

The single-capsid mycoviruses represent a completely different group of ds-RNA viruses. Morphologically they are similar to the inner capsids of the higher plant, vertebrate and invertebrate viruses with diameters of 34 to 41 nm. They apparently contain smaller genomes than the other viruses, and each of the RNA genomic segments may be encapsulated in individual particles. Aside from the UmV, the presence of these viruses does not appear to affect the gross biological properties of their hosts. Thirty-three additional isometric virus-like particles have been described in fungi (Bozarth, 1972). It is not known if they contain ds-RNA or are actually viruses. Many of these virus-like particles have been examined and found to contain several centrifugal components (H. A. Wood & R. F. Bozarth, unpublished data). Therefore, if they do contain ds-RNA they will probably also have particles containing single ds-RNA segments.

The viruses which have double- and single-capsid type protein shells therefore also differ in their encapsulation of RNA segments, each of which is probably monocistronic. The double-capsid viruses have complete genetic complements encapsulated in each particle. The single-capsid viruses generally contain single segments per particle, and therefore require a full complement of particles for infection. This allows for extracellular genetic mixing. Intracellularly, this monocistronic phenomenon allows for genetic reassortment without recombination, thereby providing an effective means of genetic variation. This may partially account for the extremely wide host range of many of the ds-RNA viruses. It should be noted, however, that the mechanism of intracellular reassortment with these two types of genomic assembly may differ. With the single-capsid PsV-s and -f viruses, there is no indication of genetic reassortment despite the fact that the genomic segments of both viruses fall within the size ranges of ds-RNA which each can encapsulate. A comparison of the mechanism of replication and maturation with these two types of ds-RNA viruses is currently of great interest.

The ø6 bacteriophage is unique among the ds-RNA viruses and bacteriophage. It apparently has a small genome similar to the mycoviruses; however, its largest RNA segment may not be monocistronic. It is the only ds-RNA virus described which is encapsulated in a host-like membrane that is required for infection and therefore represents the only ds-RNA virus which has been shown to contain lipid. Certain of the other viruses vary in their resistance to lipid solvents; however, this is probably a measure of protein denaturation rather than depletion of lipids.

It should be noted that none of the 16 viruses listed in Table 2 have been shown to be
Double-stranded RNA viruses

serologically related, and RNA hybridization studies have not indicated relationships between these viruses.

The ds-RNA viruses discussed have several biological properties in common. Replication appears to be restricted to the cytoplasm of host cells. Organelles such as lysosomes or vacuoles as well as other membranous structures appear to be involved in virus replication and maturation. During replication large accumulations of microtubules, spindle tubules and/or filaments develop. The significance of these structures is uncertain but they appear to be a general phenomenon during the replication of ds-RNA viruses.

The CPV, WTV, BTV, PcV, PsV and reovirus particles contain an RNA polymerase. It is expected that all ds-RNA viruses have a transcriptase associated with them since known host enzymes do not transcribe ds-RNA.

More than half of the viruses discussed are associated with insects. They not only transmit these viruses but can act as primary or intermediate hosts. The only vertebrate virus which has not been shown to be insect-borne is the reovirus.

Within the subgroups of double-capsid viruses, there is no suggestion of host specificity as a criterion of classification. This raises the question of evolutionary relationships between these viruses. It is easily envisioned that insects may have played a central role as a common host through which different evolutionary lines could arise. This could explain why these groups of viruses bridge the host specificity barrier commonly found amongst morphologically similar viruses.


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