

Serological Relationships of the Housefly Virus and Some Members of the Family Reoviridae

Ahmed Youssef Moussa,^A Royle A. Hawkes,^B M. R. Dickson,^C E. Shipp,^A and A. Woods^A

^A School of Zoology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.

^B School of Microbiology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.

^C Biomedical Electron Microscope Unit, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.

Abstract

The use of immunodiffusion to test the serological relationships among members of the family Reoviridae has shown that the housefly virus (HFV) is highly divergent from members of other genera in the family. HFV virions gave five distinct precipitin lines, indicating that they contain five specific antigens. Two of these lines contained recognizable viral structural components when sections of them were examined in the electron microscope. No antibodies to HFV double-stranded RNA were detected in HFV antiserum, indicating that none of the five precipitin lines is a result of HFV double-stranded RNA antigen-antibody reaction.

Introduction

The housefly virus (HFV), which was isolated from laboratory colonies of adult housefly *Musca domestica* exhibiting symptoms of lethal insect virus infection, was found to multiply in haemocytes of infected flies (Moussa 1978, 1980a). Attempts to purify the virus on sucrose and Ficoll 400 density gradients failed because of the breakdown of the virus (Moussa 1980a, 1980b, 1981). Virus particles were also disrupted when treated with some clarifying reagents (viz. carbon tetrachloride and Freon 113) (Moussa 1980a, 1980b, 1981). HFV virions, with diameter of 64-80 nm, have a middle layer (a proteinaceous thread-like structure) and a nucleoprotein core, 30-35 nm in diameter, in addition to the outer and inner shells (Moussa 1980a, 1980b, 1981). HFV contains a double-stranded (ds) RNA genome, consisting of 10 segments (Moussa 1980a, 1980c), with a total molecular weight estimated at between 17×10^6 and 18×10^6 .

The family Reoviridae, as approved by the International Committee on Taxonomy of Viruses, includes all viruses of diameter 60-80 nm and which contain a segmented dsRNA genome (Joklik 1974; Fenner *et al.* 1974; Fenner 1976). Six genera, each of which has its own distinct morphological structure, are listed in this family (Matthews 1979). They are: orthoreoviruses; orbiviruses; rotaviruses; cytoplasmic polyhedrosis viruses; fijiviruses; phytoreoviruses. In the present paper the serological relationships between HFV and selected members of the six Reoviridae genera are examined by immunodiffusion tests. Some limited observations on the nature of HFV and the mammalian reovirus antigens are also presented.

Materials and Methods

HFV Antigens

HFV was extracted from infected but dead flies, pelleted and suspended in phosphate buffer [0.01 M Na₂HPO₄ + 0.01 M NaH₂PO₄, pH 7.2] as previously described (Moussa 1981). The virus suspension was mixed vigorously with carbon tetrachloride (1 : 1 v/v) for 10 min and then centrifuged at 12 100 *g* for 15 min. The aqueous phase (antigen) was removed and stored at -20°C.

Normal Fly Antigen

Uninfected flies were treated in the same way to serve as normal control antigen.

Reovirus Antigens

Reovirus types 1, 2 and 3 were propagated in L cells, strain 929, maintained in medium 199 (Morton 1970) containing 5% (v/v) foetal calf serum. Cultures, which showed widespread cytopathic effect 3-4 days after inoculation, were then frozen and thawed three times and the fluids centrifuged at 142 800 *g* for 2 h. The resulting pellets were resuspended in a small volume of phosphate buffer and shaken vigorously for 5 min with Freon 113 (4 : 1 v/v). Debris was removed by centrifuging the preparation at 3000 *g* for 15 min and the aqueous phase, containing semi-pure virus was removed and stored at -20°C. Disruption of virions was achieved by ultrasonic vibration of the suspension in an ice-bath for 5 min using an MSE ultrasonic vibrator.

Normal Tissue Culture Antigen

Normal tissue culture antigen was prepared from confluent monolayers of L cells in medium 199 and 5% (v/v) foetal calf serum by subjecting the cells to three cycles of freezing and thawing. This preparation served as an uninfected control.

Human Rotavirus

Semi-purified human rotavirus in distilled water was used as supplied.

Nucleic acids (dsRNA)

(i) HFV dsRNA, obtained by the method of Moussa (1980*c*), was suspended in sodium chloride + sodium citrate buffer (1 × SSC), pH 7.4 (Bellamy *et al.* 1967). (ii) Fiji disease virus (FDV) dsRNA (supplied dried) was suspended in 1 × SSC buffer, pH 7.4, before it was used. (iii) Polyinosinic: polycytidylic acid (poly I-poly C), a synthetic dsRNA (Calbiochem, California, U.S.A.) was suspended in sodium chloride-Tris-EDTA buffer (STE buffer) (Ikegami and Francki 1973) and was used at final concentration of 10 mg/ml.

Antisera

HFV antiserum

HFV antiserum was prepared as previously described (Moussa 1981). Host-specific (fly) antibodies were removed by absorbing the antiserum for 1 h at 37°C and then overnight at 4°C with 5 vol. of normal fly antigen. The homologous titre was more than 10 000 when tested by the ring precipitin test. (In order to detect heterologous antibodies in absorbed HFV antiserum, specific antibodies to those antigens were reacted with HFV antigen.)

Antisera to reovirus types 1, 2 and 3

Antisera to reovirus types 1, 2 and 3 were produced in rabbits by four intramuscular injections of 1 ml of semi-pure virus (prepared as described above) at intervals of 7 days; the inoculum contained approximately 10⁷ tissue culture median infectious doses (TC ID₅₀). Serum was collected 7 days after the last injection. Clear precipitin lines formed only when the reovirus antisera were concentrated threefold using polyacrylamide beads (Lyphogel, Gelman, Michigan, U.S.A.). Antibodies to normal cell culture components were removed by absorption with normal tissue culture antigen as described above for HFV antiserum.

Other antisera

Antisera to viruses belonging to other genera of the Reoviridae which were used are listed with their sources in Table 1. Antisera which reacted with either HFV or normal fly antigens were absorbed with normal fly antigen to remove all antibodies to that antigen, unless such antisera reacted only with it. Similarly, antisera reacting with HFV dsRNA or FDV dsRNA were also absorbed with FDV dsRNA to remove all antibodies to dsRNA.

Table 1. Summary of different viral antisera used in this investigation

Genus	Antisera to (virus prototype) ^A	Source	Reference
Cytoplasmic polyhedrosis virus	CPV	T. Hatta	Personal communication
Fijivirus	FDV, LAV, MRDV, PSV, RRSV, OSDV	R. Francki	Boccardo <i>et al.</i> (1980); Van Der Lubbe <i>et al.</i> (1980)
Orbivirus	Wallal, Warrego, Mitchell River, Corriparta, D'Aguilar, Tilligerry	B. Gorman	Gorman (1979)
Rotavirus	SA 11	I. Holmes	Bastardo and Holmes (1980)
Phytoreovirus	1. RDV 2. WTV	1. I. Kimura 2. American Type Culture Collection	Kimura and Miyajima (1976)

^A Abbreviations as follows:

CPV, a cytoplasmic polyhedrosis virus	RRSV, Rice ragged stunt virus
FDV, Fiji disease virus	OSDV, Oat sterile disease virus
LAV, Leafhopper A virus	SA 11, Simian rotavirus 11
MRDV, Maize rough dwarf virus	RDV, Rice dwarf virus
PSV, Pangola stunt virus	WTV, Wound tumor virus

Antiserum to poly I-poly C

Antiserum to poly I-poly C was reacted against HFV dsRNA and poly I-poly C.

Immunodiffusion Tests

Wells 6 mm in diameter and 12.5 mm apart (centre to centre) were punched in gels of 1% (w/v) agarose in 0.02 M phosphate buffer and 0.15 M NaCl (pH 7.4). Gels were left at room temperature (22 ± 2°C) for 10 days in a humidified chamber before photography. All experiments were repeated several times with differently designed patterns (see Results).

*Electron Microscopy**Sectioned materials (HFV)*

Precipitin lines were cut out, sliced, fixed in 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 3 h and then washed overnight in cacodylate buffer. The gel slices were post-fixed in 2% (w/v) osmium tetroxide for 3 h, dehydrated in a series of concentrations of alcohols and acetone and finally embedded in Spurr's epoxy resin. Sections cut with glass knives were mounted on 150-mesh grids (carbon-coated collodion) and stained with 4% (w/v) uranyl acetate for 3 h at 60°C.

Negative-stained samples (reovirus)

Gel slices were transferred to small tubes, crushed and suspended in a small volume of phosphate buffer, left for 15 min and then centrifuged at 3000 *g* for 20 min. A drop of the supernatant was mixed with a drop of 2% (w/v) sodium phosphotungstate, pH 7.0, on a clean microscope slide. Then 483-mesh grids covered with a film of carbon-coated collodion were allowed to float on the

mixture for 2 min, blotted and examined in an electron microscope. Reovirus, disrupted by ultrasonic vibration, was also examined in the same way to ensure that disruption of virions had occurred after this treatment. Negative-stained and sectioned samples were examined in a Philips EM 300 electron microscope operated at 80 kV.

Results

Interrelationships between HFV and Reovirus Types 1, 2 and 3

The absorbed HFV antiserum reacted strongly with homologous HFV antigen, consistently producing five distinct precipitin lines, but gave no reaction with the reovirus antigens or with the uninfected control fly antigen (Fig. 1*a*). Conversely, antisera to reovirus types 1, 2 and 3 did not react with HFV antigen (Fig. 1*b*).

Nature of the HFV Precipitated Antigens

HFV antiserum did not react with either HFV dsRNA or FDV dsRNA or poly I-poly C, indicating that none of the HFV-anti-HFV precipitin lines was a result of HFV dsRNA-anti-dsRNA reaction. Electron micrographs from the three precipitin lines furthest from the antigen well (Fig. 1*a*) revealed no clearly identifiable structures. However, the line next but one to the antigen well revealed numerous antibody-aggregated, electron-dense particles of diameter 30-35 nm which were identified as the virus nucleoprotein cores (Moussa 1981) (Fig. 1*d*). The line closest to the antigen cup was composed of aggregations of broken and empty particles, approximately 60 nm in diameter, which were probably disrupted HFV particles (lacking capsomeres) affected by the preparative process (Fig. 1*c*).

Relationship between HFV and Other Members of the Reoviridae

After removing all antibodies to normal fly antigen and dsRNA, no reaction occurred between HFV antigen and the antisera of other members of the Reoviridae.

Antibodies to rotavirus antigen were present in HFV antiserum, resulting in two precipitin lines, both of which were unrelated to the HFV antigen-antibody lines (Fig. 2*a*). However, there was no cross-reaction when HFV antigen was tested against rotavirus antiserum.

Other Observations on Antisera

Other observations on the antisera used in these tests were as follows:

- (i) As previously shown (Ikegami and Francki 1977), poly I-poly C reacted with its homologous antiserum, and resulted in one precipitin line which was close to the anti-poly I-poly C well. Interestingly, HFV dsRNA also produced a single precipitin line with anti-poly I-poly C, and it was close to the RNA well.
- (ii) FDV and RDV antisera contained antibodies reacting with HFV dsRNA and FDV dsRNA.
- (iii) Tests in which FDV, HFV and RDV antisera were reacted against HFV and normal fly antigens showed that a continuous line extended between the three antisera and HFV antigen. This was the extended second line of HFV antigen-antiserum reaction (Fig. 2*b*). Electron micrographs showed that this line contained clumps of agglutinated HFV nucleoprotein cores. The line still existed even after both FDV and RDV antisera were absorbed

(1 : 1) with FDV dsRNA (1 mg/ml); but the line was absent when HFV antiserum was not used in the test (Fig. 2c).

- (iv) Some antisera cross-reacted only with normal fly antigen, but not crude HFV (Fig. 2d).

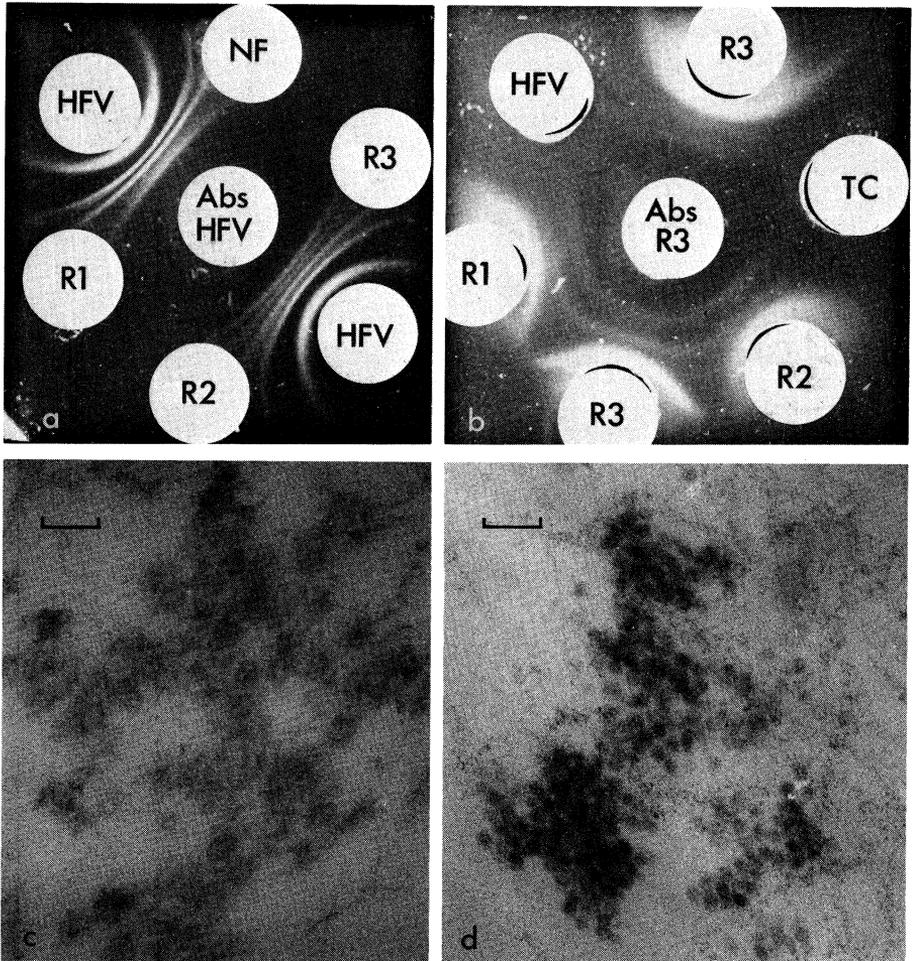


Fig. 1. (a), (b) Immunodiffusion tests of the housefly virus (HFV) and reovirus types 1, 2, and 3 (R1, R2, and R3 respectively). Antiserum placed in centre well and antigens in outer wells. In (a) HFV antiserum was used; in (b) R3 antiserum was used. Note that no cross-reaction occurred between HFV and reovirus types. *Abs*, prior absorption with normal fly antigen (NF); TC, tissue culture antigen. (c), (d) Electron micrographs of thin sections of agarose gel of first (c) and second (d) HFV precipitin lines (cf. Fig. 1a). The section of the first line, closest to the virus antigen well, contained HFV particles, most of which were empty or broken but a few contained small, dense nucleoprotein cores. The section of the second precipitin line showed clumps of electron-dense nucleoprotein cores agglutinated with viral antibodies. Bar in (c) and (d) = 100 nm.

- (v) Each of the three absorbed reovirus antisera, when reacted against ultrasonically disrupted reovirus antigens, showed a high degree of homologous and heterologous reactivity (see representative pattern in Fig. 1b). However, as stated above, no reactivity with HFV antigen occurred with any of the

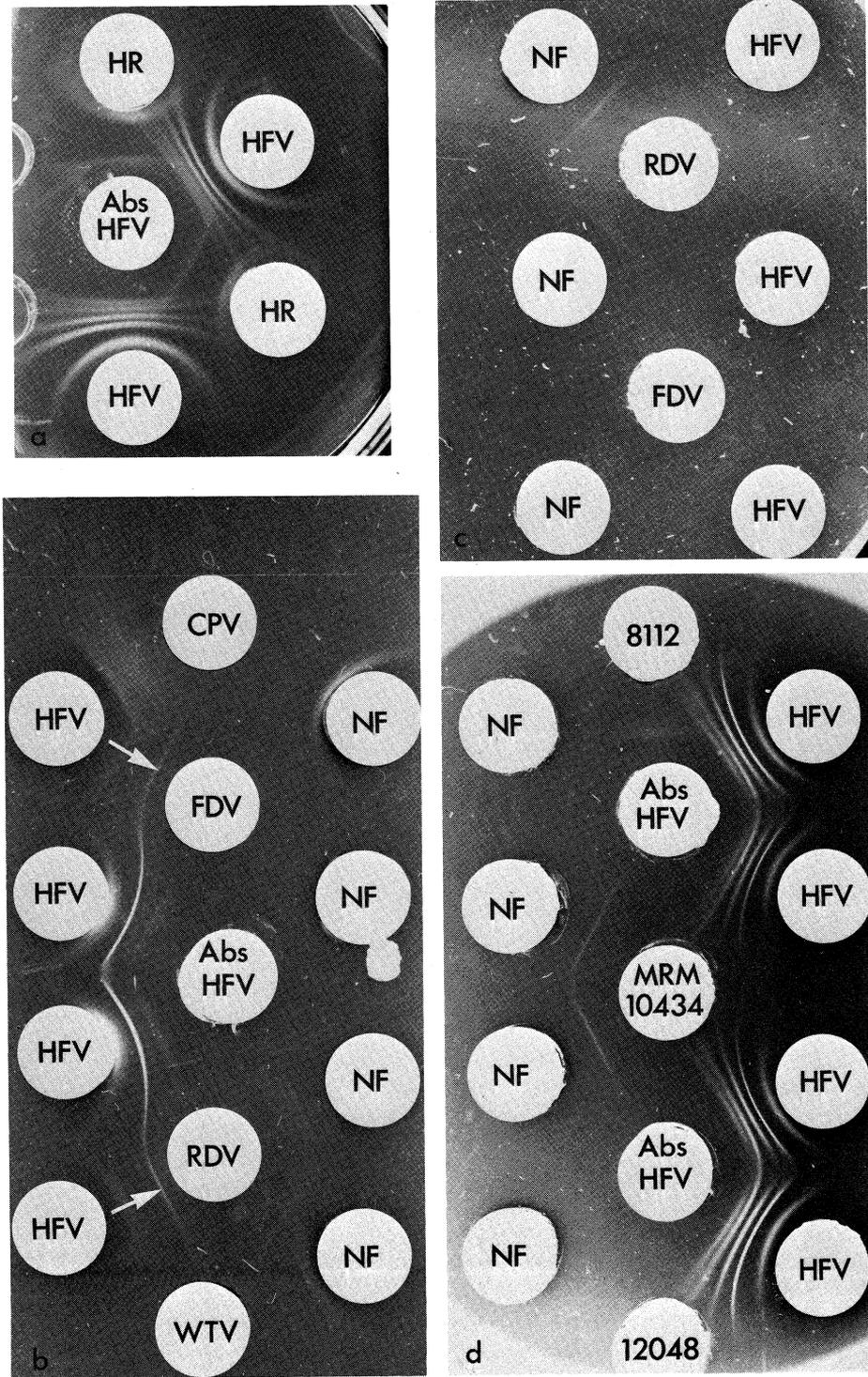


Fig. 2

types 1, 2 and 3 antisera. When these reovirus antisera were reacted with non-disrupted reovirus antigens, only one line was seen; this was close to the antigen well and exhibited cross-reactivity amongst the three reovirus

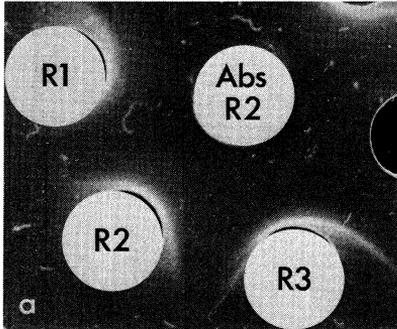
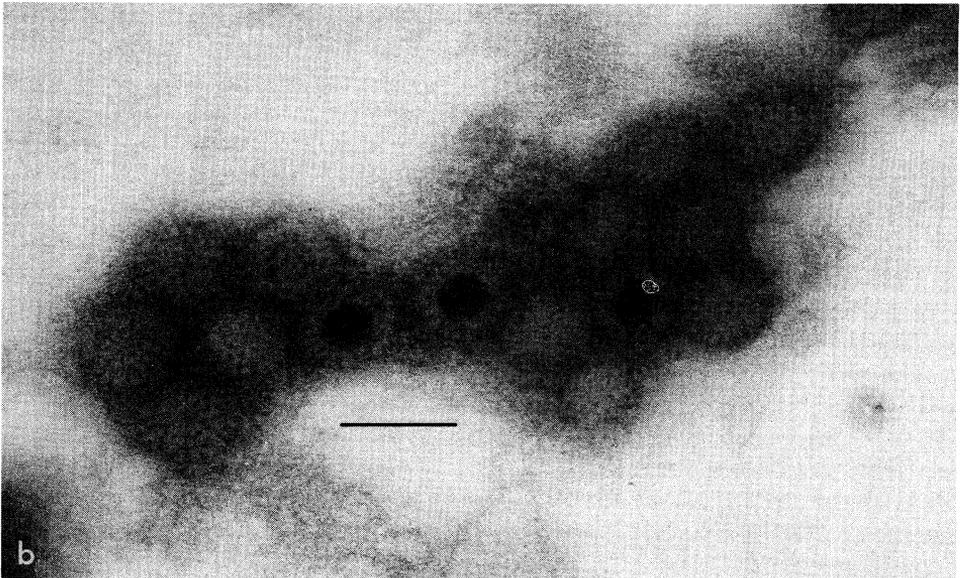


Fig. 3. (a) Immunodiffusion test with undisrupted reovirus types 1, 2 and 3 (R1, R2, and R3 respectively) reacted against R2 absorbed antiserum. (b) Electron micrograph of negatively stained (2% w/v sodium phosphotungstate, pH 7.0) reovirus particles recovered from the only precipitin line in (a). Note that the recovered particles are mostly intact and are surrounded by viral antibodies, indicating that the reovirus types share a common antigen. Bar = 100 nm.



types (Fig. 3a). These cross-reacting antigens were evidently on the surface of the virions, because suspensions of the precipitin line (in Fig. 3a) were found to contain aggregated reovirus virions, most of which were intact (Fig. 3b).

Fig. 2. (a)–(d) Immunodiffusion tests of housefly virus (HFV), using homologous and heterologous antisera. In all figures, *Abs* indicates prior absorption with normal fly antigen (NF), otherwise antisera were not absorbed. (a) Absorbed HFV antiserum reacted against HFV and human rotavirus (HR). Note two precipitin lines which are unrelated to HFV antigen–antibody lines, indicating that HFV antiserum contains antibodies to HR. (b) The second precipitin line of HFV antigen–antibody complex extended between Fiji disease virus (FDV), HFV, and rice dwarf virus (RDV) antisera. This line is still apparent even after absorbing FDV and RDV antisera with NF and FDV dsRNA antigens (see text). No such line appears between HFV antigens and FDV and RDV antisera when the HFV antiserum was excluded from this test (c). (d) Showing reaction between orbivirus antiserum (Mitchell River virus, MRM 10434) and NF antigen only and the specific precipitin lines of HFV. CPV, cytoplasmic polyhedrosis virus; WTV, wound tumour virus; 8112, D’Aguilar virus; 12048, Wallal virus.

Discussion

The results presented show that HFV is serologically unrelated to any of the members of Reoviridae which were tested. HFV produces its own pattern, resulting in five specific antigens, two of which are structurally identifiable (Moussa 1980a, 1982). The HFV, as a member of Reoviridae, is structurally different from any known existing member in this family (Moussa 1980a, 1980b, 1981) and its distinctive characters are the possession of a middle layer and a nucleoprotein core, which are not possessed by members of other genera of Reoviridae, and the tendency to fragment during certain treatments (Moussa 1980a, 1980b, 1980c, 1981, 1982)*

The possession of common antigens by the three mammalian reoviruses has been reported previously (Sabin 1959; Rosen 1960, Leers *et al.* 1968) and this study extends the number of these common antigens to three. Electron-micrographic study showed that at least one of these common antigens was on the external surface of virions. It was necessary to disrupt the reovirus virions ultrasonically before more antigens could be seen, and no attempts were made to ascribe a structural identity to these antigens.

Like other members of the Reoviridae and, in particular, those of the genus fivirus, HFV dsRNA reacted with anti-poly I-poly C. Furthermore, antibodies to viral dsRNA were found in the sera of rabbits immunized with viruses belonging to the above genus (Ikegami and Francki 1973, 1977). However, there were no antibodies to either HFV or FDV dsRNA present in the HFV antisera, despite the high concentration of HFV injected in rabbits and the large amount of antibody produced. This is perhaps because of the breakdown of the HFV during purification (Moussa 1980b, 1981), resulting in many of the nucleoprotein cores being empty in virus fraction used to immunize the rabbits.

The results have shown that FDV and RDV antisera cross-reacted with HFV dsRNA (resulting in a line of antigenic identity), which reveals that FDV and RDV antisera contain antibodies to FDV dsRNA and RDV dsRNA respectively (this was also shown when FDV and RDV antisera were tested against FDV dsRNA). These observations confirm and extend our knowledge of the presence of a common antigen to dsRNA among these viruses as already demonstrated by Ikegami and Francki (1977).

The electron-microscopic examinations of the precipitin line have shown that the line second from the HFV wells contains the agglutinated HFV nucleoprotein cores (Moussa 1980a, 1982). The application of this technique to the puzzling results (see Fig. 2b) gave a very helpful clue to the contents of that line (as it was thought that this line between FDV and RDV antisera and HFV antigen was a result of dsRNA antigen-antibody reaction from possibly fragmented nucleoprotein cores). This precipitin line still appeared even after absorbing all dsRNA antibodies (with FDV dsRNA), but when the design of the test was altered by not including HFV antiserum in the test, the line did not appear. It is believed that the high titre of HFV antibodies, as well as their rapid diffusion in every direction, are the cause of

* Application has been made to the International Committee on Taxonomy of Viruses to consider HFV as a representative of a new genus of the family Reoviridae with the name 'Muscareovirus'. The siglum 'Musca' refers to the housefly *Musca domestica* from which HFV was isolated and the siglum 'reo' indicates that the genus has the general characteristics of other members of the Reoviridae, namely segmented dsRNA, size range, site of replication, morphology, etc.

this phenomenon. This particular result emphasizes that care must be taken in interpreting such false results.

It was also found that pre-immunization serum from one rabbit, after threefold concentration, reacted with HFV antigen even after it was absorbed with normal fly antigen (Moussa 1980a), resulting in only a single precipitin line. It is possible that rabbits could pick up HFV-infected dead flies or other related virus in their diet, or by other means. This may also show that HFV is present in locally collected strains of the housefly (Moussa 1978) because there is no such report on the occurrence of HFV elsewhere. The presence of antibodies to rotavirus in pre-immunization sera from rabbits subsequently immunized with HFV indicates how widespread rotavirus infections are among mammals (Holmes 1979).

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