Adaptation of Two Avian Rotaviruses to Mammalian Cells and Characterization by Haemagglutination and RNA Electrophoresis

BY KATHY HANCOCK,† G. WILLIAM GARY, JR*‡ AND ERSKINE L. PALMER

Division of Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333, U.S.A.

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SUMMARY

Turkey and chicken rotaviruses were successfully adapted to replicate in a rhesus monkey embryo kidney (MA104) cell line. Trypsin treatment of virus and cells was essential for serial passage of the viruses. Polyacrylamide gel electrophoresis separated the viral RNA into 11 segments with clear resolution of segments 10 and 11. Segment 5 migrated with size class I, and this characteristic appeared to be a unique feature of the avian rotavirus genome. The ability of the avian rotaviruses to haemagglutinate a variety of erythrocytes was demonstrated. A type-specific antigen was detected by haemagglutination-inhibition assays and a group or subgroup antigen by enzyme immunoassays. Treatment of serum with heparin–MnCl₂ was shown to be the best method for removing non-specific inhibitors of haemagglutination.

INTRODUCTION

The importance of rotaviruses in causing enteritis in avian species is well-documented (McNulty et al., 1980). Bergeland et al. (1977) in the United States first reported finding rotavirus in faeces from turkey poults with enteritis. Later, McNulty et al. (1978) in Northern Ireland detected rotavirus in faeces from scouring turkey poults, and Jones et al. (1979) demonstrated rotavirus in the faeces of chickens with diarrhoea in England. Since then, McNulty et al. (1979b) have demonstrated rotavirus particles in the faeces and intestinal contents of turkeys with diarrhoea and in asymptomatic chickens, and have isolated and adapted turkey and chicken rotaviruses to serial replication in primary chick cell cultures (McNulty et al., 1979a). Serological evidence of rotavirus infection has been demonstrated in chickens in the United States (Bartz et al., 1980) and in pigeons in Belgium (Vindevogel et al., 1981). Nine avian rotaviruses have been isolated from turkeys (Ty) and chickens (Ch) (McNulty et al., 1980, 1981). On the basis of serum neutralization tests, seven of these isolates have been grouped into three serotypes: Ty 1, Ty 3, and Ch 1. The Ty 3 serotype includes Ch 2, Ty 4, Ty 5, and Ty 6 viruses. The eighth isolate, Ty 2, appears to be a strain intermediate between Ty 1 and Ch 1 (McNulty et al., 1980). The ninth isolate, apparently a chicken rotavirus, lacks the rotavirus group antigen (McNulty et al., 1981).

The ability to propagate the viruses in a continuous cell line is a distinct advantage for biochemical and serological characterization of avian rotaviruses. This study describes the adaptation of two serotypes of avian rotaviruses to replication in a monkey kidney cell line, shows RNA electropherograms of avian rotaviruses which differ from those previously reported (Todd et al., 1980; McNulty et al., 1980), and presents data on the development of haemagglutination.
glutination (HA) and haemagglutination-inhibition (HI) test procedures using the avian viruses as HA antigens.

METHODS

Viruses. Ty 1, a turkey rotavirus, and Ch 2, a chicken rotavirus, both propagated in primary chick embryo liver (PCEL) cells, were provided by Dr S. McNulty, Department of Agriculture, Veterinary Research Laboratories, Belfast, Northern Ireland. Simian rotavirus SA-11 was obtained from Dr H. H. Malherbe, University of Texas Health Science Center, San Antonio, Tex., U.S.A. and human rotavirus (HRV) strain Wa, subgroup 2 from Dr R. Wyatt, National Institutes of Health, Bethesda, Md. U.S.A.

Virus propagation. Both avian rotaviruses were first propagated in PCEL cells, then in primary chick embryo (PEC) cells. Virus from PEC cells was passaged in MA104 cells, a continuous cell line derived from embryonic rhesus monkey kidney cells (Microbiological Associates, Walkersville, Md., U.S.A.). Prior to inoculation, MA104 cells were washed three times with Hanks' balanced salt solution on a roller apparatus for a minimum of 30 min per wash to remove residual trypsin and viral inhibitors in serum. The virus inoculum, consisting of virus-infected lysed cells, was prepared by mixing equal volumes of virus with trypsin (ICN Nutritional Biochemicals, Cleveland, Ohio, U.S.A.), 10 μg/ml in M199 without serum, and incubating for 1 h at 37 °C. Trypsin-treated virus was adsorbed onto cells at 1 to 2 particles/cell while rolling for 1 h at 37 °C. Cell culture medium M199 (Gibco), without serum but containing 5 μg/ml trypsin, was added. Cultures were incubated on a roller apparatus at 37 °C for up to 7 days or until 90% of the cells showed a degenerative cytopathic effect (c.p.e.). Viruses were also propagated the same way in PCEL and PEC cells.

Virus-infected cells were dislodged from the flask with a rubber scraper, and pelleted by centrifugation at 400 g for 15 min. The supernatant from this centrifugation was retained. The cell culture flasks were rinsed with sterile distilled water, 7 ml water per 490-cm^2 roller bottle (Corning), and the same water was used to lyse the cell pellet. A 1/10 vol. of 10 x M199 was added to the lysed cell suspension. Both the lysed cell and the supernatant preparations were examined for viruses by negative-contrast electron microscopy (EM) using the pseudoreplica technique (Smith, 1967) with 0.5% uranyl acetate. More virus was present in the lysed cell preparations than in the supernatants, so the former were used as inocula. The supernatants, however, also contained large numbers of particles. These were used as HA antigens because of their larger volume. Virus preparations were frozen at ~70 °C.

Virus purification. Ty 1 and Ch 2 were inoculated onto MA104 cells grown on 490-cm^2 roller bottles as described above. Cultures were incubated until at least 90% of the cells showed c.p.e. Any cells remaining on the roller bottles were dislodged into the media and then pelleted by centrifugation at 400 g for 30 min. Each roller bottle was rinsed with 1 to 2 ml TCN buffer (10 mM-Tris–HCl, 10 mM-CaCl_, 100 mM-NaCl, pH 7.2 to 7.4). The cell pellets were lysed in the TCN rinse buffer and M199 was added. The virus was precipitated from the culture media by adding 10% polyethylene glycol (PEG 6000, J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.) and 2.3% NaCl and stirring overnight at 4 °C. The precipitate was pelleted by centrifugation at 10000 g for 30 min. The pellets were resuspended in TCN buffer and a 1/10 vol. of 10 x M199 was added.

The lysed cell preparation was homogenized by 20 strokes of a Dounce homogenizer and then combined with the pellet virus preparation. Fifteen ml of Genesolv D (trichlorotrifluoroethane; Allied Chemical Corporation, Morristown, N.J., U.S.A.) was added to 15 ml of virus suspension in a 50 ml centrifuge tube, mixed on a vortex mixer for 4 min, and centrifuged at 1600 g for 20 min. The supernatant was removed and saved. Ten ml of TCN buffer was added to the cell interface and the mixing and centrifugation repeated. This step was repeated, and the supernatant was pooled with the two previously obtained. The pooled supernatants were extracted with Genesolv D; 20 ml supernatant plus 10 ml Genesolv D per 50 ml centrifuge tube. This supernatant was recovered and the virus in it pelleted by centrifugation at 70000 g for 2 h. Each pellet was resuspended in 1 ml TCN buffer. The resuspended pellets were pooled, and layered on top of a 30% glycerol-saturated potassium tartrate gradient in TCN buffer, then centrifuged to equilibrium at 120000 g. Light-scattering virus bands were collected and examined for purity by EM. Bands of purified virus were pooled and dialysed against distilled water at 4 °C, then frozen at ~70 °C.

Phenol extraction of RNA. RNA from purified virus was extracted with phenol as described by Pennica et al. (1980).

Polyacrylamide gel electrophoresis. Virus RNA was separated by discontinuous (disc) SDS-polyacrylamide gel electrophoresis (PAGE) as described by King & Laemmli (1971) using 7.5% resolving and 3% stacking gels. Slab gels that were 1.5 mm thick and measured 13 × 26 cm were subjected to electrophoresis at a constant current of 25 mA for 18.5 h. RNA was stained with ethidium bromide, which was included at 0.5 μg/ml in the upper electrode buffer solution (0.025 M-Tris base, 0.192 M-glycine, 0.1% SDS, pH 8.0).

Haemagglutination and haemagglutination-inhibition tests. HA and HI tests were performed in polystyrene 'U' well microtitre plates by the method of Martin et al. (1979). For HA tests with erythrocytes from different species, mammalian erythrocytes were diluted to 0.4%, and avian erythrocytes to 0.5%. Four HA units of antigen in 0.025 ml were used in the HI test. For all HI testing, except for tests using sera treated with heparin–MnCl_, phosphate-
buffered saline (PBS; 0.01 M-Na₂HPO₄/NaH₂PO₄, 0.15 M-NaCl, pH 7.2) was the diluent for serum, antigen, and erythrocytes. For heparin–MnCl₂-treated sera, the erythrocytes were washed in dextrose–gelatin–veronal buffer (DGV), pH 7.3, then suspended to 0.4% with HEPES–saline–albumin–gelatin buffer (HSAG), pH 6.2 (Palmer et al., 1980a) on the day of the test. HSAG was also used as the serum and antigen diluent. Tests were incubated at 4, 20, or 37 °C.

Enzyme immunoassay (EIA) tests were performed as described by Palmer et al. (1980b) except that goat anti-rabbit IgG (Cappel Laboratories, Cochranville, Pa., U.S.A.) was conjugated to alkaline phosphatase by the method of Voller et al. (1980) and used as the conjugate.

Serum treatments. Sera were heat-inactivated at 56 °C for 30 min. Sera were adsorbed with human group O erythrocytes and with kaolin and treated with receptor-destroying enzyme (RDE) as described by Hierholzer et al. (1969). Sera were treated with heat, trypsin and periodate as described by Dowdle et al. (1979).

For the treatment of sera with heparin–MnCl₂, 1 vol. of serum was added to 2 vol. of HSAG buffer. One vol. of heparin–MnCl₂ solution, prepared by combining equal volumes of heparin (5000 units/ml) and 1 M-MnCl₂, was added to the serum dilution and incubated for 30 min at 4 °C. Six vol. of HSAG buffer was added to bring the serum dilution to 1:10. The treated serum was recovered by centrifugation at 900 g for 15 min. Treated sera to be heat-inactivated were heated at 56 °C for 30 min then centrifuged again at 900 g for 15 min (Palmer et al., 1980a).

Sera treated with heparin–MnCl₂ and adsorbed with human group O erythrocytes were processed as follows. One vol. of serum was added to 1.5 vol. of HSAG buffer. One vol. of heparin–MnCl₂ solution was added to the serum dilution and incubated for 15 min at 4 °C. One vol. of 50% O erythrocytes (in HSAG buffer) was added and incubated for 1 h at 4 °C. Six vol. of HSAG buffer was added to bring the serum dilution to 1:10. The treated serum was recovered by centrifugation at 900 g for 20 min (Palmer et al., 1980a).

Treatment of human group O erythrocytes with RDE. The method of Lerner et al. (1963) was used.

Antisera. Antisera to Ty 1 and Ch 2 rotaviruses were prepared in rabbits. One-half ml of purified virus was mixed with 0.5 ml of Freund's complete adjuvant, and equal volumes were injected intradermally or subcutaneously in two sites on the back and intramuscularly in both hind legs. Rabbits immunized with Ty 1 were re-immunized on day 22 and rabbits immunized with Ch 2 were re-immunized on day 46. Those booster immunizations followed the initial immunization procedure, except that Freund's incomplete adjuvant was used.

RESULTS

Virus propagation

Ty 1 rotavirus was passed five times in PCEL cells. Virus particles were first observed by EM at the second passage and were numerous at the fourth passage. Cytopathic effect was not easily observed in these cells because trypsin in the cell culture medium disrupted the monolayer in both the inoculated and uninoculated flasks. After the first passage in PCEL cells, Ty 1 was passed in PEC cells eight times. Virus particles were first observed by EM at the first passage but were not numerous until the fourth passage; c.p.e. was first observed at the seventh passage. After the eighth passage, Ty 1 rotavirus was passed in MA104 cells. At the first passage, c.p.e. was observed and EM revealed numerous virus particles. Ch 2 was passed once in PCEL cells, three times in PEC cells, and then in MA104 cells. A few virus particles were observed by EM in the PCEL cells and in the first passage of PEC cells. At the second passage in PEC cells and the first passage in MA104 cells, c.p.e. and numerous virus particles were observed. Double-shelled, single-shelled, and empty virus particles were seen in all preparations examined by EM.

Passage of Ty 1 and Ch 2 rotaviruses without trypsin treatment of the virus and cells was attempted. The second passages of Ty 1 and Ch 2 in MA104 cells were inoculated into MA104 cells without trypsin treatment and passed three times. By the second passage, characteristic c.p.e. was not observed and EM revealed few virus particles. At the third passage, no particles were observed. Lysed cell preparations from the three trypsin-free passages were tested for their ability to haemagglutinate erythrocytes. HA titres of 512 and 64 for the Ty 1 and Ch 2 passages used as inocula, respectively, dropped to 4 at the first passage without trypsin and to 2 and < 2, respectively, at the third passage.

Polyacrylamide gel electrophoresis of rotavirus RNA

Ch 2 rotavirus RNA was separated into 10 bands. Like HRV, segments 7 and 8 were not resolved (Fig. 1). Ty 1 rotavirus RNA was separated into 11 bands (Fig. 1). The electrophoretic profiles for the avian rotaviruses are very similar to those previously reported (Todd et al., 1980).
Fig. 1. Polyacrylamide slab gel electrophoresis of Ch 2, Ty 1, HRV, and reovirus type 3 double-stranded RNA segments. Migration is from top to bottom, and the segments are indicated.

except for one important difference: in our system, segments 10 and 11 were well separated.

The most striking difference between the human and avian rotaviruses is the difference in the electrophoretic migration of RNA segment 5. Unlike HRV, segment 5 from Ch 2 and Ty 1 migrates with size class I, not with size class II. Avian rotavirus size class I contains segments 1, 2, 3, 4, and 5; size class II contains only segment 6.

Haemagglutination tests

Ty 1 and Ch 2 propagated in PEC and MA104 cells, haemagglutinated human O erythrocytes. The haemagglutinating ability of Ty 1, Ch 2, and SA-11 rotaviruses was further tested by using a variety of erythrocytes commonly used in HA and HI tests (Table 1). HA activity was found to
Characterization of two avian rotaviruses

Table 1. HA titres of Ty 1, Ch 2, and SA-11 rotaviruses with various erythrocytes at 4, 20, and 37 °C

<table>
<thead>
<tr>
<th>Virus</th>
<th>Temperature of incubation (°C)</th>
<th>Type of erythrocyte</th>
<th>Human</th>
<th>Guinea-pig</th>
<th>Dog</th>
<th>Rat</th>
<th>Gerbil</th>
<th>Mouse</th>
<th>Sheep</th>
<th>Rhesus monkey</th>
<th>Vervet monkey</th>
<th>Chick</th>
<th>Goose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ty 1</td>
<td>4</td>
<td></td>
<td>256</td>
<td>512</td>
<td>128</td>
<td>128</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>256</td>
<td>512</td>
<td>128</td>
<td>128</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
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<td>37</td>
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<td>256</td>
<td>512</td>
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<td>Ch 2</td>
<td>4</td>
<td></td>
<td>128</td>
<td>512</td>
<td>128</td>
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<td>64</td>
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<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>SA-11</td>
<td>4</td>
<td></td>
<td>256</td>
<td>1024</td>
<td>&lt;2</td>
<td>64</td>
<td>128</td>
<td>16</td>
<td>128</td>
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<td>512</td>
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<td>32</td>
<td>32</td>
<td>64</td>
<td>256</td>
<td>128</td>
<td>&lt;2</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. HA titres of Ty 1, Ch 2, and SA-11 rotaviruses with human group O erythrocytes treated with RDE

<table>
<thead>
<tr>
<th>Virus</th>
<th>RDE (units/ml) used to treat erythrocytes</th>
<th>Mock-treated*</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ty 1</td>
<td></td>
<td>1024</td>
<td>128</td>
<td>64</td>
<td>4</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Ch 2</td>
<td></td>
<td>256</td>
<td>64</td>
<td>32</td>
<td>8</td>
<td>&lt;2</td>
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<tr>
<td>SA-11</td>
<td></td>
<td>256</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

* Erythrocytes treated with calcium–saline solution

be temperature-sensitive with only 3 of the 33 virus–erythrocyte combinations tested. Marked differences were noted in the HA ability of each virus with the various erythrocytes. Highest HA titres with the avian rotaviruses were obtained when either human O or guinea-pig erythrocytes were used. Highest HA titres with SA-11 were obtained with human O, guinea-pig, rhesus monkey, and vervet monkey erythrocytes. On the basis of these results, all subsequent HA and HI testing was done with human erythrocytes at 37 °C.

Erythrocytes treated with 400 units RDE per ml were not agglutinated by Ty 1 or Ch 2 (Table 2). Haemagglutination by SA-11 virus was prevented when erythrocytes were pretreated with 50 units RDE per ml (Table 2).

Before testing heparin–MnCl2-treated sera, the HA ability of Ty 1, Ch 2, and SA-11 with human group O erythrocytes in HSAG buffer was assessed. All three viruses haemagglutinated to the same titre as that obtained when using PBS as the diluent.

Serum treatments

Rabbit and avian sera were treated in various ways prior to use in HI assays to determine the best method for removing non-specific inhibitors of HA. The efficacy of the various methods was evaluated as a reduction of the preimmune serum titres without a reduction in the immune serum titres. All preimmune sera were tested by EIA and found to be free of rotavirus antibody. Any preimmune HI titres greater than 10 were judged to be due to non-specific inhibitors of HA. Preliminary testing of rabbit sera showed no need for adsorption with human O cells. The method of choice for treatment of rabbit serum was shown to be heparin–MnCl2 followed by heat inactivation (Table 3). Heat–trypsin–periodate treatment was also effective. Increasing the concentration of RDE from 100 to 800 units/ml did not decrease the preimmune serum titres. Similar results were obtained when rabbit anti-Ch 2 sera were treated and tested for their ability to inhibit haemagglutination of Ch 2.
Table 3. HI titres of Ty 1 anti-rotavirus sera treated in different ways to remove non-specific inhibitors of HA

<table>
<thead>
<tr>
<th>HA antigen</th>
<th>Serum</th>
<th>Heat</th>
<th>Heat Trypsin</th>
<th>Heparin MnCl₂</th>
<th>Heparin MnCl₂ Heat</th>
<th>RDE (100 units/ml)</th>
<th>RDE (800 units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ty 1</td>
<td>Preimmune</td>
<td>320</td>
<td>10</td>
<td>20</td>
<td>160</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Ty 1</td>
<td>S₁</td>
<td>640</td>
<td>320</td>
<td>640</td>
<td>640</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td>Ty 1</td>
<td>S₂</td>
<td>≥2560</td>
<td>320</td>
<td>1280</td>
<td>≥2560</td>
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Table 4. HI and EIA of anti-rotavirus sera

<table>
<thead>
<tr>
<th>Serum</th>
<th>HI titre</th>
<th>EIA titre</th>
<th>Antigen</th>
<th>Antigen</th>
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<tr>
<td>Rabbit anti-Ty 1 Preimmune</td>
<td>Ty 1</td>
<td>Ty 1</td>
<td>Ch 2</td>
<td>SA-11</td>
<td>Ch 2</td>
</tr>
<tr>
<td>S₁</td>
<td>640</td>
<td>640</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>S₂</td>
<td>2560</td>
<td>≥65536</td>
<td>20</td>
<td>160</td>
<td>≥65536</td>
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<tr>
<td>Rabbit anti-Ch 2 Preimmune</td>
<td>Ty 1</td>
<td>Ty 1</td>
<td>Ch 2</td>
<td>HRV</td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>10</td>
<td>10</td>
<td>160</td>
<td>10</td>
<td>128</td>
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<tr>
<td>S₂</td>
<td>160</td>
<td>32768</td>
<td>80</td>
<td>≥65536</td>
<td>1024</td>
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</table>

Six avian sera, four chicken sera, one pigeon serum, and one turkey serum were tested by HI following various serum treatments (data not shown). Two of the sera were preimmune obtained from chickens from specific-pathogen-free flocks (Spafas Inc., Norwich, Conn., U.S.A.). The turkey serum, obtained from Dr R. W. Dellers (Virginia Polytechnic Institute, Blacksburg, Va., U.S.A.), was known to contain rotavirus antibody. Preliminary testing indicated that O cell adsorption was necessary to remove natural agglutinins except when the sera were treated with RDE or heat–trypsin–periodate. The method of choice for treating avian sera was shown to be heparin–MnCl₂ with O cell adsorption. Heat inactivation following heparin–MnCl₂ treatment was not necessary. Heat–trypsin–periodate and RDE serum treatments were no more effective than heat inactivation followed by O cell adsorption.

Haemagglutination-inhibition and enzyme immunoassay tests

Rabbit anti-avian rotavirus immune sera inhibited HA of the homologous virus and had negligible HI activity with the heterologous viruses (Table 4). An HI titre of 10 was considered to be a negative result, based on the observation that preimmune serum titres that were 10 by HI were < 8 by EIA, a test considered more sensitive than HI. When tested by EIA (Table 4), no difference was found between the homologous and heterologous titres of rabbit anti-avian rotavirus sera. The titre of the same sera to HRV was significantly lower.

DISCUSSION

Avian rotaviruses have been reported to replicate only in primary cell cultures (McNulty et al., 1979a, 1980; Todd et al., 1980). We successfully adapted two avian rotaviruses, Ty 1 and Ch 2, to replicate in MA104, a continuous cell line. Cells and viruses were trypsin-treated for adaptation and serial propagation of the viruses. Attempts to pass the adapted viruses in MA104 cells without trypsin treatment were unsuccessful by the third passage. Other investigators have documented the trypsin-dependent and trypsin-enhanced propagation of many rotavirus strains.
in cell culture (Almeida et al., 1978; Clark et al., 1979; McNulty et al., 1979a; Fukusho et al., 1981).

Todd et al. (1980) reported that avian rotavirus gene segments 10 and 11 had identical or closely similar electrophoretic mobilities and suggested that the co-migration of these segments is a feature unique to the avian rotavirus genome. However, we found that segments 10 and 11 were separable by disc SDS-PAGE using a 3% stacking and 7.5% resolving gel. We are suggesting that the feature unique to the avian rotavirus genome is the electrophoretic migration of gene segment 5 with size class I.

Among the rotavirus for which the presence of a haemagglutinin has been demonstrated are Nebraska calf diarrhoea virus (Spence et al., 1976; Inaba et al., 1977), SA-11 (Kalica et al., 1978), and human rotavirus purified from stools (Shinozaki et al., 1978). We have demonstrated that avian rotaviruses Ty 1 and Ch 2 agglutinate erythrocytes. Marked differences were noted in the HA ability of each virus with various erythrocytes. Highest HA titres for both viruses were obtained with human O and guinea-pig erythrocytes. For the most part, HA activity was found to be independent of the temperature of incubation.

HA of Ty 1 and Ch 2 is inhibited by homologous immune sera. Any inhibition by the heterologous immune sera is minimal. By EIA, no difference is detectable between Ty 1 and Ch 2. EIA does distinguish the avian rotaviruses from HRV. Thus, it appears that HI detects a type-specific antigen and EIA an avian group or subgroup antigen. Further testing with other avian rotavirus isolates is needed to confirm this observation.

HI testing may prove to be a useful method for typing avian rotavirus isolates and for seroepidemiological studies of avian sera. But for an HI assay to be useful, an effective serum treatment to remove non-specific inhibitors of HA must be available. Previous HI studies with anti-rotavirus sera have used kaolin treatment, RDE treatment, and adsorption with erythrocytes (Fauvel et al., 1978; Shinozaki et al., 1978; Spence et al., 1978). Though effective, kaolin has the disadvantage of removing approximately 60% of the total IgG and IgM and 40% of the IgA (Mann et al., 1967). We sought a method that reduced the preimmune serum titre without reducing the immune serum titre.

We were able to confirm the observation that pretreatment of human O erythrocytes with RDE prevents HA by SA-11 (Bastardo & Holmes, 1980). We also showed that RDE pretreatment of erythrocytes prevents HA by Ty 1 and Ch 2, although higher concentrations of RDE were required for the avian viruses than for SA-11. This observation suggests that these viruses interact with neuraminic acid-containing receptors on the erythrocytes, and that some non-specific inhibition of HA could be caused by neuraminic acid-containing molecules in sera. However, RDE treatment of rabbit sera was only partly effective in reducing the preimmune sera titres. Increasing the amount of RDE used to treat the sera did not further reduce the preimmune titres. Serum inhibitors other than, or in addition to, neuraminic acid-containing molecules which can function as substrates for RDE appear to be preventing HA. Heat–trypsin–periodate, which is often used to remove neuraminic acid-containing serum inhibitors not removed by RDE (Dowdle et al., 1979), was more effective than RDE treatment. Heat–trypsin–periodate treatment, however, slightly lowered the immune sera titres but has the disadvantage that precise timing is required during incubation. Similar results were obtained with avian sera, except that adsorption with O cells was necessary to remove natural agglutinins.

The best method for removing non-specific serum inhibitors of the HA was treatment with heparin–MnCl₂. Sulphated polysaccharides, such as heparin, in the presence of divalent cations specifically precipitate beta-lipoproteins (Cornwell & Kruger, 1961). Treatment with heparin–MnCl₂ has been successfully used to remove the non-specific serum inhibitors of reovirus type 1 (Mann et al., 1967). The non-specific inhibitor of avian rotavirus HA appears to be either a beta-lipoprotein or a substance associated with beta-lipoprotein that is removed along with the beta-lipoprotein in the precipitate. For the treatment of rabbit sera, heating at 56 °C for 30 min following heparin–MnCl₂ treatment was essential for the removal of non-specific inhibitors. Heating increases the precipitation of the residual reagent (Cremers, 1974), perhaps by converting some soluble lipoprotein–heparin complexes to insoluble complexes. Heating following heparin–MnCl₂ treatment of avian sera was not necessary.
Before elucidating the relationship of the avian rotaviruses to human and other mammalian rotaviruses, characterization of additional avian isolates must be undertaken. This task could be greatly simplified if adaptation of other avian rotaviruses to MA104 cells proves successful. It will be of interest to determine whether all avian rotaviruses haemagglutinate, because most mammalian rotaviruses do not.

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REFERENCES


Characterization of two avian rotaviruses


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