Biochemical Characterization of Infantile Gastroenteritis Virus (IGV)

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SUMMARY

Enzymic and biophysical studies with purified infantile gastroenteritis virus (IGV) nucleic acid indicated that the virion contained a double-stranded RNA genome of approx. $14 \times 10^6$ daltons which could be separated by gel electrophoresis into eight bands of RNA which were comprised of 15 RNA species. Two major virus proteins, VP2 (mol. wt. = 135,000) and VP8 (mol. wt. = 40,000), which composed about 85% of the total virion protein, were detected in IGV particles by polyacrylamide gel electrophoresis. Eight additional minor proteins were also resolved.

INTRODUCTION

The Reoviridae family of viruses currently consists of two recognized genera, Reovirus and Orbivirus. Since the family was established, several other viruses with physicochemical characteristics similar to those of the Reoviridae have been described. These viruses cause enteritis in the young of several species of mammals and are antigenically (Flewett et al. 1974; Kapikian et al. 1974, 1975) and morphologically distinct (Flewett, Bryden & Davies, 1973; Martin, Palmer & Middleton, 1975) from either of the current genera. It has been proposed that these viruses – Nebraska calf scour virus, epidemic diarrhoea virus of infant mice, simian virus SA-11, and the virus causing enteritis in young children which we term infantile gastroenteritis virus (IGV) – be placed into a separate genus, either Rotavirus (Flewett et al. 1974) or Duovirus (Davidson et al. 1975; Holmes et al. 1975) within the Reoviridae virus family. However, only one of these viruses, a calf rotavirus, has been adequately characterized by biochemical and biophysical methods (Newman et al. 1975; Rodger, Schnagl & Holmes, 1975; Schnagl & Holmes, 1976).

Infantile gastroenteritis virus, which was first detected in late 1973 (Bishop et al. 1973), is now known to be a major cause of enteritis in young children in many parts of the world (Editorial, Lancet, 1975). It has been shown to be an isometric virus with complex capsid symmetry of a $T = 9$ icosadeltahedron (Martin et al. 1975), which is unique among the known isometric viruses. The capsid is, in turn, surrounded by an outer layer which gives the virus a precise margin when viewed by negative contrast electron microscopy (Flewett et al. 1973). By thin section, the virus resembles the orbiruviruses (Murphy et al. 1971) in all aspects of morphology and morphogenesis (Bishop et al. 1973; Holmes et al. 1975). Recent work has shown that IGV isolated in Canada has a density of 1.36 g/ml in caesium chloride and that the genome is RNA (Petrie, Szymanski & Middleton, 1975). A morphologically identical virus isolated in Australia also had a density of 1.36 g/ml in caesium chloride, was
composed of eight or nine polypeptides and had an RNA genome of 11 segments (Rodger et al. 1975; Schnagl & Holmes, 1976). Another similar virus, designated as a human reo-virus-like agent was passed in calves; the virus purified from the stools of these infected animals also contained a segmented, double-stranded genome of 11 segments (Kalica et al. 1976).

We have investigated the biochemical and biophysical characteristics of IGV isolated in the United States. Data on the polypeptide and nucleic acid composition of the virus are presented in this communication.

METHODS

Chemicals and reagents. Specially purified reagents for preparing polyacrylamide gels were purchased from Bio-Rad Laboratories, Richmond, California. Other reagents used were optically pure caesium sulphate (Cs₂SO₄) and absolute grade urea from Research Plus Labs., Inc., Denville, N.J.; sodium dodecyl sulphate (SDS), glycerol, potassium tartrate from Gallard-Schlesinger Chemical Corp., Long Island, N.Y.; cytochrome c, myoglobin, chymotrypsinogen, ovalbumin, bovine serum albumin from Mann Research Labs., New York, N.Y.; and Genesolv-D from Allied Chemicals, Morristown, N.J. Worthington Biochemical Corp., Freehold, N.J., supplied β-galactosidase, phosphorylase A, DNase and pancreatic ribonuclease (RNase).

Virus. Infantile gastroenteritis virus was obtained from faecal extracts of children hospitalized in Grady Memorial Hospital, Atlanta, Georgia with acute enteritis. These specimens were generously supplied by Dr Robert Ryder, Bureau of Epidemiology, Center for Disease Control (CDC), Atlanta, Georgia. The Dearing strain of type 3 reovirus was from a stock collection at CDC and was propagated in mouse L cells.

Virus purification. The procedure for the purification of IGV from stool specimens consisted of a combination of fluorocarbon extraction and equilibrium density gradient centrifugation. A 10% (w/v) suspension of pooled faecal material, which was shown previously by electron microscopy to contain many IGV particles, was prepared by homogenizing in distilled water. After the large particulate debris was removed by low-speed centrifugation the supernatant fluid was combined with an equal volume of Genesolv-D and was mixed vigorously for 2 min with a Vortex mixer. The phases were separated by centrifuging at 2000 g for 10 min and the upper aqueous phase was collected. An equal volume of distilled water was added to the interphase and Genesolv phase, and the extraction procedure was repeated twice more. After each extraction the aqueous phase was collected and pooled. Bacteria and other debris were removed at this point by centrifuging at 8000 g for 15 min.

The virus was concentrated by centrifuging for 1 h at 35000 rev/min at 4 °C in a Beckman SW 40 rotor. The virus pellet was suspended in TS buffer (0.05 M-tris buffer, pH 7.8, and 0.15 M-NaCl) and layered on to pre-formed equilibrium:viscosity gradients of saturated potassium tartrate: 30% (w/w) glycerol (KT:GLY) prepared in TS buffer (Martin et al. 1975). After centrifuging at 4 °C for 3 h at 39000 rev/min in a SW41 Beckman rotor, three bands were visible in the gradients. A large, diffuse band was seen at the top of the gradient; when it was examined by electron microscopy, a few virus particles were found trapped in a large amount of debris. The two remaining bands located slightly below the centre of the gradient, contained most of the virus particles as well as some debris. The two bands were collected and dialysed against several changes of TS buffer to remove the glycerol and potassium tartrate. After dialysis this material was treated with 1% (v/v) Nonidet P-40 (NP 40) for 15 min at 37 °C and was centrifuged to equilibrium in KT:GLY gradients for 18 h at 39000 rev/min at 4 °C. Purified virus was obtained from these gradients as a single,
homogeneous, visible band at a density of 1.34 g/ml. The virus was collected with a pipette, examined by electron microscopy to monitor purity, and dialysed against TSE buffer (0.02 M-tris buffer, pH 7.8, 0.15 M-NaCl, 0.002 M-EDTA). Purified virus preparations were stored at -70°C until used.

Reovirus was purified from infected mouse L cells by a procedure already described (Palmer & Martin, 1976).

Isolation and analysis of IGV nucleic acids and proteins. Purified preparations of IGV were suspended in TSE buffer at a concentration of 1 mg/ml and were dissociated with 2-mercaptoethanol (1%, v/v) and SDS (2%, v/v). The virus nucleic acids and proteins were extracted three times with an equal volume of phenol mixture (100 g phenol, 14 g m-cresol, 0.1 g 8-hydroxyquinoline). The nucleic acids were precipitated from the aqueous phase (1 ml) with 2 vol. of ethyl alcohol in the presence of 0.2 M-LiCl. After a second alcohol precipitation the virus nucleic acid was suspended in TS buffer and stored at -20°C. The virus proteins were retrieved from the combined phenol phases (3 ml) by adding 7 vol. of cold ethanol (-20°C) and 10 ml of 5 M-NaCl and allowing the proteins to flocculate at -20°C for 4 h. The precipitate was collected by centrifuging at 10000 rev/min for 30 min at 4°C, washed twice with acetone, and dissolved in 0.5 ml of phosphate dissociation buffer (PDB; 0.02 M-sodium phosphate buffer, pH 7.0, 2.5%, w/v, SDS, 5%, v/v, 2-mercaptoethanol and 8 M-urea). Reovirus RNA and proteins were extracted from purified virus by the same method. Tobacco mosaic virus labelled with 3H-uridine (sp. act. = 16152 ct/min/µg RNA) was supplied by Dr G. A. Tannock, CDC, and 14C-thymidine labelled vaccinia DNA (sp. act. = 14620 ct/min/µg DNA) was supplied by Dr J. J. Esposito, CDC.

The separation of virus proteins by SDS polyacrylamide gel electrophoresis (PAGE) with either an 8% (w/v) polyacrylamide gel and a continuous phosphate buffer system (pH 7.0) with 8 M-urea (CONT-SDS) or a discontinuous gel system with a 3% (w/v) stacking gel and 10% (w/v) resolving gel (DISC-SDS) has already been described (Obijeski et al. 1974). After electrophoresis the gels were stained for total protein with Coomassie brilliant blue in methanol:acetic acid:water as described earlier (Obijeski et al. 1974). Purified IGV and reovirus RNA were analysed in 7.5% (w/v) polyacrylamide gels containing 6 M-urea (Schuerch, Mitchell & Joklik, 1975). Each gel was electrophoresed at a constant current of 2.5 mA/gel for 48 h to obtain the best resolution of the RNA species. After electrophoresis the gels were washed for 24 h in several changes of 7% (v/v) acetic acid to remove the SDS and urea. The gels were stained for 2 h in 0.2% methylene blue prepared in 0.4 M-sodium acetate buffer (pH 5.0) and decolourized with distilled water (Shatkin, Sipe & Loh, 1968).

Enzyme treatment of virus nucleic acids. Purified unlabelled IGV nucleic acid (~ 25 µg), 3H-labelled TMV-RNA (~ 16000 ct/min), and 14C-labelled vaccinia DNA (~ 14000 ct/min) in 0.2 ml TS buffer were digested with pancreatic RNase (15 µg/ml) for 20 min at 30°C. After incubation the mixture was loaded on top of a Cs2SO4 solution (starting density = 1.55 g/ml), prepared in TSE buffer containing 0.4 M-NaCl and was centrifuged to equilibrium in a Beckman SW 50.1 rotor at 35000 rev/min for 60 h at 15°C. After centrifuging, fractions were collected from the bottom of the tube, and the density was measured by weighing 0.05 ml samples. The distribution of radioactivity in the gradient was determined by counting a 0.05 ml portion of each fraction in a toluene-Triton scintillation cocktail.

In some experiments, the same mixture of the above nucleic acids in TS buffer containing 10 mM-MgCl2 was treated with DNase (15 µg/ml) for 20 min at 30°C and then analysed in Cs2SO4 equilibrium density gradients as previously described.

Electron microscopy. IGV prepared by a pseudoreplica technique (Palmer, Martin &
Gary, 1975) and stained with 0.5% aqueous uranyl acetate was examined by electron microscopy. The relative number of virus particles present in 0.4 ml gradient fractions collected by bottom puncture of the tubes was determined by counting the number of particles present in 10 squares of a 300-mesh copper grid. Virus counting was facilitated by preparing 11 x 14 in prints of grid squares from electron image plates.

**Complement fixation (CF) tests.** Complement fixation tests were performed by the standardized LBCF method (Casey, 1965). Sera used in these tests were the acute and convalescent phase sera from an infant who had experienced gastroenteritis. The acute phase serum had no detectable CF titre with purified virus as the test antigen, whereas the convalescent phase serum had a titre of 1:128 with the same antigen.

**Analytical methods.** The concentration of protein in purified virus preparations was determined by the Folin-biuret method with BSA used as a standard (Lowry et al. 1951).

The proteins and nucleic acids in stained gel electropherograms were recorded at 620 nm and 610 nm, respectively, in a Gilford spectrophotometer fitted with a linear gel transport accessory. The percentage of each virion protein species in purified virus particles was computed by determining the area under each protein peak in densitometer tracings and calculating the fraction of the total stained protein each species represented.

To determine the molar ratios of the RNA genome segments of IGV and reovirus, ten copies of the scanned electropherograms were photographically reproduced and the areas under each peak were cut out and weighed. From this information, the molar ratios were calculated by dividing the weight in each RNA peak (mean of ten determinations) by the apparent mol. wt. of each RNA species.

**RESULTS**

**Gradient centrifugation and electron microscopy of IGV**

When a purified preparation of IGV was centrifuged to equilibrium in a KT:GLY gradient, a single coincident peak of CF activity and virus particles was found at a density of 1.34 g/ml (Fig. 1). No other material was observed in the gradient.

Fig. 2 shows the morphological appearance of negatively stained IGV particles obtained from the 1.34 g/ml region of the KT:GLY gradient. They measured approx. 65 nm in diam. and were intact. The sharply defined outer surface, which is characteristic of complete IGV, was clearly evident and only an occasional particle was seen to be penetrated by the negative stain. Disrupted virus particles were not observed in the preparations after final purification in KT:GLY gradients. Apparently, this procedure for purifying IGV by fluorocarbon extraction and sedimentation in combination equilibrium:viscosity gradients produced no degraded virus particles devoid of nucleic acid with a corresponding lower density. By electron microscopy, we were unable to detect any contaminating structures in our purified virus preparations.

**Nucleic acid composition of purified IGV**

Since we were unable to grow IGV in cell culture, and since only limited quantities of virus were available for study, we chose the following indirect methods to determine the type of genome nucleic acid present in IGV particles. A purified preparation of IGV nucleic acid (≈ 20 μg), was mixed with 3H-labelled TMV-RNA and 14C-labelled vaccinia DNA, and the densities of each nucleic acid species were determined after equilibrium centrifugation in Cs2SO4. The nucleic acid of IGV had a density of 1.59 g/ml, whereas single-stranded TMV-RNA displayed a characteristic density of 1.65 g/ml (Szbalski, 1968) in these gradients (Fig. 3a). Double-stranded, vaccinia-virus DNA was recovered at a density of 1.41 g/ml. When
IGV nucleic acids and proteins

Fig. 1. Centrifugation of IGV in an equilibrium viscosity gradient of potassium tartrate and glycerol. A sample of IGV purified from faecal extracts by fluorocarbon treatment and gradient centrifugation was loaded onto a KT : GLY gradient and centrifuged for 18 h at 39000 rev/min at 4 °C in a Spinco SW41 rotor. Fractions (0.4 ml) were collected from the bottom of the tube, and the tartrate and glycerol were removed by dialysis. The CF activity (○) and virus particle count (△) of each fraction were determined as described in the text. The density (□) was measured by weighing 0.10 ml samples of each fraction at room temperature.

the same mixture of nucleic acids was incubated with pancreatic RNase before equilibrium centrifugation, we observed that the TMV-RNA was totally sensitive to the degrading action of RNase and that all the $^{3}$H-label was recovered at the top of the gradient (Fig. 3b). However, IGV and vaccinia nucleic acids were still recovered at their same positions in the gradient (Fig. 3b). When treated with DNase, both the TMV-RNA and the IGV nucleic acid maintained their same relative densities (i.e., 1.65 g/ml and 1.59 g/ml) in the gradient, whereas approx. 95% of $^{14}$C-vaccinia radioactivity was solubilized by this treatment (Fig. 3c). From this information, we concluded that IGV nucleic acid was resistant to enzymic digestion by either pancreatic RNase or DNase and its apparent density (1.59 g/ml) in Cs$_{2}$SO$_{4}$ was similar to the value of 1.61 g/ml reported for the segmented, double-stranded genome of reovirus (Iglewski & Franklin, 1967; Shatkin, 1965). Taken together, these results indicate that IGV, like other members of the Reoviridae family (Joklik, 1974), contains a double-stranded RNA genome.
Fig. 2. Electron photomicrograph of purified IGV particles. A sample of IGV, which banded at a density of 1.34 g/ml in a KT:GLY gradient, was processed for electron microscopy by pseudoreplication and was stained with 0.5% uranyl acetate.

Fig. 3. Caesium sulphate equilibrium density gradient centrifugation of IGV-RNA, TMV-RNA, and vaccinia DNA. (a) A mixture of IGV-RNA (~25 μg), 3H-TMV-RNA, and 14C-vaccinia DNA in TS buffer was layered on top of a Cs₂SO₄ solution. After centrifugation for 60 h at 35,000 rev/min, fractions were collected from the bottom of the tube and the density, extinction at 260 nm, and radioactivity of each fraction were determined. (b) same mixture as in (a), but incubated with 15 μg/ml pancreatic RNase before centrifugation. (c) same mixture as in (a), but incubated with 15 μg/ml DNase before centrifugation. ●—●, TMV-RNA; ○—○, IGV-RNA; □—□, vaccinia DNA.
PAGE analyses of IGV and reovirus RNAs

The double-stranded RNA isolated from purified IGV was resolved into at least eight distinct bands by electrophoresis in 7.5% polyacrylamide gels (Fig. 4 and Fig. 5b). The ten double-stranded RNA species of the reovirus genome were also resolved in the gel system (Fig. 4 and Fig. 5a). The numbering of the IGV genome segments (Fig. 4) refers to the total number of RNA species present in each of the bands recorded by the densitometer (Fig. 5) and was determined by a calculation of their molar ratios (Table I). Similarly, the reovirus RNA species were lettered as recommended by Joklik (1974). The same number of visible bands and the same relative amounts of each RNA species were found when either IGV
or reovirus RNA was incubated with RNase, DNase, or a combination of both, before PAGE analysis (data not shown).

The mol. wt. of the IGV-RNA segments were calculated by comparing their mobilities to those of the RNA species of reovirus which were electrophoresed in parallel gels. By this method, the eight visible segments of IGV-RNA were found to have a mol. wt. range of $2.3 \times 10^6$ to $0.24 \times 10^6$ (Table 1). From ten densitometer tracings (Fig. 5a and b) the molar ratios of the RNA genome segments for both IGV and reovirus were calculated as described in the Methods section. Apparently, each of the ten reovirus RNA segments was present in a proportion of one copy per virus particle (Table 1). However, when an identical computation was made for IGV-RNA, we noticed that band 2 from the tracing contained three species of RNA, while band 6 contained at least six molar equivalents of RNA (Table 1 and Fig. 5b).

**Protein composition of IGV**

The purpose of these experiments was to determine the number of proteins present in purified IGV particles, their electrophoretic mobilities and, hence, their mol. wt. (Weber & Osborn, 1969). In all of the experiments, IGV was purified from stool specimens, and the proteins were extracted with SDS and phenol. The virus proteins (VP) were recovered from the phenol phase with ethanol, solubilized with SDS, mercaptoethanol and urea, and resolved by SDS-gel electrophoresis.
**Table 1. Molecular weights and molar ratios of the RNA genome species of IGV and reovirus**

<table>
<thead>
<tr>
<th>Visible* band</th>
<th>Number of RNA genome species†</th>
<th>Mol. wt. (× 10^6)$</th>
<th>Molar ratio$</th>
<th>Visible band</th>
<th>Number of RNA genome species</th>
<th>Mol. wt. (× 10^6)$</th>
<th>Molar ratio$</th>
</tr>
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<tr>
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<td>0.98</td>
<td>1</td>
<td>L₁</td>
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<td>1.19</td>
</tr>
<tr>
<td>2</td>
<td>2, 3, 4</td>
<td>1.66</td>
<td>3.3</td>
<td>2</td>
<td>L₂</td>
<td>2.71</td>
<td>1.21</td>
</tr>
<tr>
<td>3</td>
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<td>4</td>
<td>6</td>
<td>0.85</td>
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<td>4</td>
<td>M₁, M₂</td>
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</tr>
<tr>
<td>5</td>
<td>7</td>
<td>0.76</td>
<td>0.91</td>
<td>5</td>
<td>M₃</td>
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</tr>
<tr>
<td>6</td>
<td>8, 9, 10, 11, 12, 13</td>
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<td>S₁</td>
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<td>0.79</td>
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<td>S₂</td>
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<tr>
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<td>15</td>
<td>0.24</td>
<td>0.89</td>
<td>8</td>
<td>S₂, S₄</td>
<td>0.63**</td>
<td>2.12</td>
</tr>
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</table>

* Refers to the numbering of the RNA peaks found in the scanned gel electropherograms (see Fig. 5 and Fig. 6).
† Indicates the number of genome segments found in each RNA peak as determined by the molar ratios calculation.
‡ Mol. wt. of IGV-RNA species were determined by comparing their rates of electrophoretic migration to those of reovirus run in parallel polyacrylamide gel.
§ Molar ratios were determined by using the densitometer tracings of stained gels shown in Fig. 5 and the calculation described in the text.
¶ Mol. wt. for reovirus type 3 (Dearing strain) were those reported by Martin & Zweerink (1972), and the nomenclature for the three size classes of reovirus RNA (large, L; medium, M; small, S) was adopted from Joklik (1974).
†† Average mol. wt. of M₁ (1.62 × 10⁶) and M₂ (1.55 × 10⁶) was used to calculate the molar ratio.
** Average mol. wt. of S₂ (0.65 × 10⁶) and S₄ (0.61 × 10⁶) was used to calculate the molar ratio.

**Table 2. Proteins of infantile gastroenteritis virus (IGV)**

<table>
<thead>
<tr>
<th>Virus protein</th>
<th>Percentage of total protein*</th>
<th>Estimated mol. wt.$</th>
<th>CONT-SDS gel system</th>
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<tr>
<td>1</td>
<td>2.4</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>12.6</td>
<td>135000</td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td></td>
</tr>
<tr>
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<td>102000</td>
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</tr>
<tr>
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<td>70000</td>
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</tr>
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<td>42000</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.4</td>
<td>31000</td>
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</tr>
<tr>
<td>10</td>
<td>0.8</td>
<td>20000</td>
<td></td>
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<table>
<thead>
<tr>
<th>Virus protein</th>
<th>Percentage of total protein*</th>
<th>Estimated mol. wt.$</th>
<th>DISC-SDS gel system</th>
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<tr>
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<td>118000</td>
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<td>2.7</td>
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<td>74.9</td>
<td>40000</td>
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</tr>
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<td>9</td>
<td>3.2</td>
<td>32000</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.8</td>
<td>24000</td>
<td></td>
</tr>
</tbody>
</table>

* The percentage of each protein species in IGV virions is based on a computation of the amount of Coomassie brilliant blue stain bound by each protein species as described in the text (mean of three determinations).
† The mol. wt. of IGV proteins were established by comparing their electrophoretic mobility with that of proteins of known mol. wt. (β-galactosidase, phosphorylase A, bovine serum albumin, ovalbumin, chymotrypsinogen, myoglobin, and cytochrome c). Each value is the mean from three separate determinations in each gel system.
Fig. 6. Polyacrylamide gel electrophoresis of IGV proteins. IGV proteins were solubilized with SDS, mercaptoethanol, and urea, and were separated by electrophoresis in either (a) the DISC-SDS or (b) CONT-SDS polyacrylamide gels. After being stained with Coomassie brilliant blue for 2 h, the gels were destained and photographed.

Fig. 6 represents a typical stained electropherogram of IGV proteins separated in either CONT-SDS or DISC-SDS polyacrylamide gels. In both gel systems, two major virus proteins—VP2 and VP8—were observed, which accounted for about 85% of the total virion protein (Table 2). Several other minor proteins were also resolved in both gel systems. The approximate mol. wt. obtained for IGV proteins with the two different electrophoretic systems are given in Table 2. Although not shown here, reovirus proteins extracted from purified virus by the same procedure used for the IGV were resolved into seven distinct bands in both gel systems (Joklik, 1974; Martin & Zweerink, 1972).

The electropherogram patterns reported here for IGV proteins were strikingly similar to those reported for human enteritis virus isolated in Australia (Rodger et al. 1975); however, our mol. wt. estimates were somewhat higher, particularly for the major structural proteins.
We also identified two minor proteins, VP5 and VP6, in our preparations of IGV which were not seen by Rodger et al. (1975). This observation could reflect differences in the electrophoretic system used to resolve the virus-proteins.

**DISCUSSION**

We have demonstrated that purified IGV particles contain a double-stranded RNA genome which can be resolved by gel electrophoresis into eight bands which comprised 15 RNA species. Other investigators (Newman et al. 1975; Rodger et al. 1975; Kalica et al. 1976; Schnagl & Holmes, 1976), using different criteria, have also suggested that the genomes of human mammalian enteritis and calf diarrhoea viruses are double-stranded RNA. Our mol. wt. estimates of the RNA segments of IGV ranged between $2.3 \times 10^6$ for the largest species to $0.24 \times 10^6$ for the smallest (Table 1). These values differ only slightly from the values reported by others (Kalica et al. 1976; Schnagl & Holmes, 1976). The size of the IGV-RNA genome reported in this study is about $14 \times 10^6$ daltons, which is similar to the value of $12$ to $15 \times 10^6$ daltons reported for other members of the Reoviridae family (Joklik, 1974).

By gel-electrophoretic analyses, we found the same eight major, visible RNA segments for IGV-RNA that others reported for calf and human enteritis virus. The occurrence of multiple RNA species in band 2 and band 6 from the densitometer tracing in Fig. 5b was determined by a molar ratio calculation (Table 1). We consistently found three RNA species in band 2 and six RNA segments in band 6. At present we have no information that would suggest whether each of the multiple RNA species actually contributes distinct genetic information with different coding functions or, particularly in the case of band 6, whether these RNA species are multiple copies of the same genetic information which are enclosed in the mature virion particle. Schnagl and co-workers (Rodger et al. 1975; Schnagl & Holmes, 1976), who studied the genome of human and calf diarrhoea viruses, reported two RNA species in band 2 and three RNA species in band 6. Perhaps the variation in the number of RNA segments in band 2 and band 6 is due to differences in experimental technique. Alternatively, these differences in the molar ratios of the RNA species for human enteritis reported by us and by Rodger et al. (1975) and Schnagl & Holmes (1976) reflect fundamental, yet indeterminate morphogenetic differences between the viruses isolated in different hemispheres. A more accurate assessment of the complete genetic complement of these viruses will be forthcoming when radiolabelled virus preparation grown in tissue culture can be analysed.

Two major structural proteins, VP2 (mol. wt. = 135,000) and VP8 (mol. wt. = 40,000), were found in purified IGV particles and accounted for approx. 85% of the virion protein mass. Also, eight minor proteins were always observed in our preparations (Table 2). No large discrepancies in the mol. wt. values of IGV proteins in either gel electrophoresis system were noticed. The same total number of virus proteins (10) was resolved in each gel system; however, some of the virus proteins, particularly VP9 and VP10, were more distinct in the DISC-SDS system than in the CONT-SDS gel system (Fig. 6). The total mol. wt. of the ten IGV proteins was about 850,000, or 8500 amino acid residues. Assuming an RNA to protein coding ratio of 18:1 (Martin & Zweerink, 1972) for double-stranded RNA, we concluded that the coding capacity of the $14 \times 10^6$ dalton genome of IGV (approx. 7700 amino acid residues) was slightly less than would be needed to code for all the virion structural proteins.

We were unable to determine which of the virion structural proteins was associated with a
core structure since chymotrypsin treatment, which has been used successfully to produce core particles of reovirus (Joklik, 1974), had no effect on intact IGV particles. However, trypsin treatment at various concentrations completely digested IGV particles into soluble components (unpublished observation).

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