Characterization of the *Drosophila C* Virus

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**SUMMARY**

Some properties of *Drosophila* C virus (DCV), a non-occluded isometric virus, have been studied. The virus particles were 30 nm in diam., their sedimentation coefficient was 153S and their buoyant density was 1.34 g/ml in caesium chloride in the pH range 7 to 9. These particles contained about 31% ribonucleic acid (RNA) and 69% protein. The reaction of formaldehyde with DCV particles suggested that the RNA *in situ* is single-stranded. The infectivity of DCV was stable at pH 3. The virus capsid contained three major polypeptides with mol. wt. of 31000, 30000 and 28000, and two minor components of mol. wt. 37000 and 8500.

Virus preparations also contained a small number of infective particles 24 nm in diam. banding at a density of 1.44 g/ml. Preliminary results indicated that these heavy particles probably correspond to the dense particles recently reported in several vertebrate picornaviruses.

RNA extracted from DCV was single-stranded and infectious. Its mol. wt. was calculated to be approx. 3 x 10^6. It is proposed that this virus be included in the enterovirus group. The cryptogram of the virus is R/I:3.0/31:S/S :I/O.

**INTRODUCTION**

During the last five years several viruses with small isometric particles have been reported in natural and laboratory populations of *Drosophila* (Plus & Duthoit, 1969; Jousset, 1972; Jousset *et al.* 1972; Plus *et al.* 1975a, b). In naturally infected flies these viruses do not produce marked pathogenic effects; however, when injected they become lethal after one or several passages.

The virus particles are paraspherical and measure approx. 25 to 30 nm in diam. Some of these viruses have been shown to multiply in the cytoplasm of different cell types of their hosts and to give a positive reaction when stained for RNA by the method of Bernhard (1969). These properties, along with the apparent resistance to acid pH and organic solvents of these viruses in crude extracts of *Drosophila*, suggested their relation to the picornaviruses (Jousset *et al.* 1972; Teninges & Plus, 1972). However, in the absence of precise data concerning the physicochemical properties of their particles, it was not possible to include

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them with certainty in this group. In order to further characterize these viruses we have studied the *Drosophila* C virus (DCV) isolated in 1972 from the ‘Charolles’ strain of *Drosophila melanogaster* Meigen (Jousset *et al.* 1972). This virus kills the flies in 3 to 4 days at the first passage and the yield of virions is greater than that obtained from other *Drosophila* virus isolates. We report here the results of our studies of the biophysical and biochemical properties of purified DCV particles as well as those of the RNA extracted from the virion and discuss them in comparison with those of the insect and mammalian picornaviruses.

**METHODS**

*Insect culture.* The ‘Champetières’ strain of *Drosophila melanogaster*, free of known viruses of this species, was used to produce the virus and for infectivity tests. The flies were reared at 25°C in bottles and fed on an axenic diet (David, 1959).

*Virus strain.* The virus used for this investigation was the *Drosophila* C virus (DCV) isolated from the ‘Charolles’ strain of *D. melanogaster* by Jousset *et al.* (1972).

*Virus production.* Adult flies were anaesthetized with ether and each was injected with approx. 0.2 μl of virus suspension containing 10^5 LD_{50}, using micro-needles (L’Heritier, 1952). The flies died within 3 to 4 days. They were harvested and stored at -30°C.

*Virus assays.* Infectivity assays of virus suspensions were made by injecting tenfold dilutions to groups of 50 flies. Daily mortality was recorded for 10 days and the LD_{50} estimated according to Reed & Muench (1938).

*Purification of the virus.* The flies were crushed in approx. thirty times their weight of cold 0.05 M-phosphate buffer (PB), pH 7.4, in a conical glass grinder. About 1000 flies were used for each virus preparation. The extract was clarified at 2000 g for 15 min at 4°C, the supernatant fluid kept and the pellet re-extracted twice by sonication (20 kHz in an MSE disintegrator) in the same volume of PB. The resulting supernatants were mixed and the virus was pelleted by centrifuging at 42000 rev/min for 1 h at 4°C in the 42.1 Beckman rotor. The pellets were allowed to re-suspend overnight in small volumes of PB. After sonication as above, the virus suspension was layered on to pre-formed gradients of sucrose (15 to 45%, w/w) in PB. The gradients were centrifuged for 2 h at 25000 rev/min in the SW 27 Beckman rotor and analysed through an ISCO gradient fractionator. The fractions containing the virus bands were diluted in PB, sedimented and resuspended as indicated above. Two additional cycles of sedimentation in sucrose gradients were carried out, the last one in the SW 50.1 Beckman rotor at 35000 rev/min for 1 h. The virus bands were mixed and dialysed against at least four changes (at 12 h intervals) of PB (200 volumes).

*Electron microscopy.* Purified virus preparations were negatively stained with 2% (w/v) sodium phosphotungstate, pH 7.0, or uranyl acetate, and the grids examined in a Hitachi HU 11C electron microscope at an accelerating voltage of 75 kV.

*Determination of the chemical composition of the virion.* The chemical composition of DCV was calculated assuming that the virion contained only protein and RNA. RNA concentration was determined by the orcinol reaction of Mejbaum (1939). The presence of DNA was tested by the diphenylamine reaction as modified by Burton (1956). Protein concentration was measured by the Folin method of Lowry *et al.* (1951) as modified by Hartree (1972). Since only very small amounts of virus RNA and protein were available it is necessary to use bovine serum albumin and yeast RNA as standards.

*Buoyant density in caesium chloride.* The buoyant density of the virus was determined by analytical ultracentrifugation (see below) and by a method similar to that described by Rowlands, Sangar & Brown (1971). Pre-formed 30 to 45% (w/w) CsCl gradients were
prepared in 0.1 M-phosphate at pH 7.0, 8.0 and 9.0 in 13 ml tubes. A sample (0.5 ml) of the virus was then layered on top. The tubes were centrifuged at 20°C in the SW 41 Beckman rotor at 35000 rev/min for 16 h. Fractions of 0.3 ml were collected using an ISCO gradient fractionator and their density was determined from measurements of their refractive index at 20°C.

**Gel electrophoresis of virus polypeptides.** Virus particles in PB were dissociated by heating for 2 min at 100°C in 1% SDS, 0.1% β-mercaptoethanol and 8 M-urea. The preparations were electrophoresed in 7.5 to 15% polyacrylamide gels in the SDS-Phos or SDS-Disc system of Maizel (1971). The gels were stained overnight in a 0.25% solution of Coomassie brilliant blue in methanol:acetic acid:water (50:7:43) and then destained in several changes of methanol:acetic acid:water (50:7:43) over a period of 2 to 3 days. The stained gels were scanned with a Vernon microdensitometer. The mol. wt. of the virus polypeptides were estimated from their migration rates relative to those of catalase, ovalbumin, aldolase, chymotrypsinogen, parvalbumin and cytochrome c.

**Extraction of the RNA.** Virus pellets were resuspended in 0.01 M-tris, 0.004 M-EDTA, 0.1 M-NaCl, pH 8.0. The virus suspensions had an E260 of about 2. Proteinase K pre-incubated for 1 h at 37°C was added to a final concentration of 50 μg/ml. The solution was then made 1% in Sarkosyl (Geigy). After leaving at room temperature for 5 min, the solution was incubated at 37°C for 15 min, cooled and extracted with an equal volume of buffer-saturated phenol. The aqueous and phenol layers were separated by centrifuging at 3000 g for 15 min and the upper phase containing the RNA was dialysed against 0.01 M-tris, 0.01 M-EDTA, 0.1 M-NaCl (TEN), pH 8.0.

**Infectivity of RNA preparations.** The infectivity of extracted RNA preparations was estimated by injecting groups of 100 flies with RNA solutions treated, or not treated, with pancreatic ribonuclease (RNase). Mortality was recorded daily and flies were checked individually for the presence of infective virus.

**Spectrophotometric measurements.** U.v. extinctions of purified virus or RNA preparations were made with Gilford model 240, DK 2A or Acta C III Beckman recording spectrophotometers.

**Thermal denaturation.** A sample of RNA in TEN buffer with an extinction at 260 nm of about 0.4 was placed in a microcell and sealed with a Teflon stopper to prevent evaporation. The temperature of the cells was increased 1°C/2 min from 30°C to 100°C and extinction at 260 nm was recorded. The cell was cooled rapidly and the extinction at 260 nm measured again.

**Formaldehyde reactions.** DCV particles and RNA extracted from the virus were each tested for reactivity at 30°C with neutralized formaldehyde. The samples were first scanned in u.v. light then formaldehyde was added to give a final concentration of 1.8% (Fraenkel-Conrat, 1954; Sinsheimer, 1959). The extinction changes at 260 nm were recorded over a period of 24 h for the intact virion and 7 h for extracted RNA.

**Analytical ultracentrifugation.** Sedimentation velocities of virions and RNA were measured in a Spinco model E analytical ultracentrifuge equipped with u.v. absorption optics. Virus preparations in PB with an extinction between 0.3 and 0.8 at 260 mm were centrifuged at 20°C and 20,410 rev/min using an AnD rotor and 12 mm standard cells. U.v. photographs were taken at 4 min intervals. Sedimentation velocities of RNA were measured in the same rotor and same conditions at 44770, 40000 or 34000 rev/min. The RNA was in TEN buffer at concentrations of 20 to 25 μg/ml. In some experiments the RNA was also centrifuged after treating with 3.3% formaldehyde (Boedtker, 1968) to overcome the effects of secondary structure on the rate of sedimentation. In each case photographs
were taken every 4 min. Photographic negatives were analysed with a Joyce Loebl micro-
densitometer. Sedimentation coefficients were corrected to standard conditions in water.
Equilibrium centrifugation in CsCl was performed with virus preparations diluted in PB
to an extinction of 0.004 at 260 nm. CsCl (Merck Suprapur) was added to bring the density
to about 1.34 g/ml. The actual densities of the CsCl solutions were determined by measuring
their refractive index in an Abbe refractometer and correcting for the refractive index of PB.
Gradients were centrifuged for 24 h at 44,770 rev/min at 25°C. After this time, absorption
photographs were taken. The buoyant densities were calculated using the method described
by Meselson, Stahl & Vinograd (1957).

RESULTS
Studies on the virion

Virus purification

DCV replicates in most tissues of adult Drosophila melanogaster and causes marked
pathogenic effects (Jousset et al. 1972). The virus was released by crushing dead flies and
concentrated by pelleting the particles from the clarified crude extract. Fractionation of a
virus preparation by centrifuging in a sucrose gradient yielded three bands (revealed by
analysis at 254 nm): the upper band, when examined under the electron microscope, was
found to contain mostly disrupted cellular debris, the second band (located near the middle
of the gradient) consisted mainly of intact virus particles with a few impurities, and the third
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Fig. 2. Extinction spectrum of DCV before (+—+) and after (○—○) treatment for 24 h with formaldehyde. The DCV was originally in 0.05 M-phosphate buffer, pH 7.4. Neutralized formaldehyde was added to give a final concentration of 1.8 % HCHO and the virus suspension was incubated for 24 h at 30°C. Corrections were made for virus dilution.

band (near the bottom of the tube) was found to contain large aggregates of virus particles trapped in amorphous material. The upper band was discarded whereas bands two and three were sonicated and refractionated separately in sucrose gradients. The major band of intact virions was found in both gradients and the gradients loaded with band three still contained a band of aggregated particles. The virus bands were pooled and loaded on to a third sucrose gradient. The single homogeneous band resulting from this centrifugation was used for all further virus particle and RNA studies. This band proved to be almost pure virus particles as judged by u.v. spectrophotometry, electron microscopy and analytical ultracentrifugation.

Criteria of purity of virus preparations

Examination of virion suspensions under the electron microscope showed large numbers of intact 30 nm particles (Fig. 1). There was no evidence of cell debris or 'empty' particles. Our preparations were therefore considered to be essentially free from contaminants.

The u.v. extinction spectra of virus preparations also served as a criterion of their purity. The spectra were typical of a nucleoprotein (Fig. 2), with a maximum at 258 nm and a minimum at 240 nm. The average ratio of extinction at 260 nm to that at 280 nm was 1.80, suggesting a large nucleic acid content.

Chemical composition

The purified virus preparations gave positive orcinol but negative diphenylamine reactions. Assuming that the virus contained only RNA and protein, the analysis of five different preparations gave an average of 31 % of RNA and 69 % of protein in the virion.
Fig. 3. The effect of formaldehyde (1.8 %) at 30°C on DCV virions (○—○) and DCV-RNA (●—●). Extinction at 260 nm was recorded over a period of 7 h (DCV-RNA) and 24 h (DCV virions). The virion was in 0.05 M-phosphate buffer, pH 7.4; the RNA was in 0.01 M-tris, 0.001 M-EDTA, 0.1 M-NaCl buffer, pH 8.0.

Fig. 4. Microdensitometer tracings of u.v. extinction patterns of purified DCV in 0.05 M-phosphate buffer, pH 7.4, during a sedimentation velocity run at 20410 rev/min. Successive boundaries were from photographs taken at 4 min intervals. Direction of sedimentation was from left to right.
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Fig. 5. Caesium chloride equilibrium centrifugation of DCV. Pre-formed 30 to 45 % (w/w) CsCl gradients were prepared in 0.1 M-phosphate buffer at pH 7.0. Centrifugation was at 35000 rev/min for 16 h at 20°C in a Beckman SW41 rotor. U.v. extinction at 254 nm was monitored and 0.3 ml fractions collected, using an ISCO Model UA-5 extinction monitor and Model 640 fraction collector. The density of each fraction was calculated from the refractive index at 20°C, measured in an Abbe refractometer: ○-○, E_{254}; ○-○, density, g/ml.

Strandedness of the nucleic acid in the particles

Examination under the light microscope of histological sections of infected flies stained with acridine orange revealed a red cytoplasmic fluorescence in the infected cells (F.-X. Jousset, unpublished data). Control preparations did not exhibit such fluorescence. This suggested that the virus particles contained single-stranded nucleic acid. In order to confirm these observations, purified virus preparations were allowed to react for 24 h at 30°C with formaldehyde at 1.8 % final concentration. This resulted in an increase in extinction and a shift in the maximum extinction to a longer wavelength (Fig. 2). The original extinction maximum at 258 nm shifted to 261 nm after 24 h formaldehyde treatment. Four preparations treated for 24 h gave an average hyperchromicity of 19.8 % with variations of less than 4 % (Fig. 3). No additional significant changes in extinction were caused by heating the preparations for 10 min at 100°C. These results suggested that the genome is single-stranded in the intact virion.

Sedimentation coefficient

Ultracentrifugal analysis of purified DCV preparations showed that the material absorbing at 260 nm gave a single sharp boundary (Fig. 4). These results indicated that the preparations were homogeneous. Sedimentation coefficients of five different virus preparations, corrected for solvent viscosity and density, gave an average value of 153 ± 2S.

Buoyant density in caesium chloride

When subjected to isopycnic gradient centrifugation at pH 7.4 in the analytical ultracentrifuge, DCV banded as a single symmetrical peak of density 1.337 g/ml (average of 3 determinations). Similarly, the purified virus gave a single peak at a density 1.34 g/ml after centrifugation for 16 h at 35000 rev/min in pre-formed caesium chloride gradients at pH 7.0 in the SW 41 rotor (Fig. 5). The buoyant density of the virus was not modified by centrifuging.
Fig. 6. Densitometer tracing of DCV polypeptide patterns after electrophoresis on a 12% polyacrylamide-SDS gel containing 6 M-urea. Dissociation conditions of virus particles and staining of gel are described in Methods. The relative positions of protein standards are included in the figure for comparison with those of DCV capsid proteins (arrows).

in CsCl gradients prepared in 0.1 M-phosphate buffer at pH 8.0 or 9.0. These results indicated that DCV had the same density characteristics as mammalian enteroviruses and cardioviruses (Rowlands et al. 1971; Newman, Rowlands & Brown, 1973b).

Another discrete peak at a density 1.44 g/ml was also observed in these gradients. We discuss below the similarity between this peak and the 'dense particles' recently described in several vertebrate picornaviruses (Rowlands et al. 1975; Yamaguchi-Koll, Wiegers & Drzeniek, 1975).

Effect of pH on the infectivity of DCV particles

In order to study the stability of the DCV particles at different pH values, one vol. of virus suspension in PB was mixed with 9 vol. of 0.1 M-citrate buffer solutions at pH 3.0, 5.0 and 7.0. The suspensions were incubated at 20°C for 15 min before diluting in PB for titration. An untreated virus suspension in PB was also titrated. The virus suspensions subjected to pH 3.0, 5.0 and 7.0 contained between $4 \times 10^8$ and $6 \times 10^8$ LD$_{50}$/ml as compared to $5 \times 10^9$ LD$_{50}$/ml for the control preparation. These results clearly show that DCV particles are stable in acidic conditions and confirmed the preliminary results obtained with crude extracts (Jousset et al. 1972).

Polyacrylamide gel electrophoresis of virus proteins

The proteins prepared from purified virus particles were analysed by electrophoresis on 7.5 to 15% gels. Three major bands close to one another were repeatedly observed, the middle one sometimes hardly distinguishable from the slowest one. This pattern was the same whatever the gel concentration but the resolution was improved at high polyacrylamide concentration and in the presence of 6 M-urea (Fig. 6). The three polypeptides were roughly in equimolar proportions. The average mol. wt. values obtained from 7 determinations were about 31000, 30000 and 28000. These polypeptides corresponded in their mol. wt. to the
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Fig. 7. Extinction spectrum of RNA extracted from DCV in 0.01 M-tris, 0.001 M-EDTA, 0.1 M- NaCl buffer, pH 8 (+--+), and after treatment at 30°C for 7 h with 1.8% neutralized formaldehyde (●—●).

VP₁, VP₂ and VP₃ reported for most of the picornaviruses. In addition to these major polypeptides, two minor bands with mol. wt. of 37000 and 8500 were also detected in the gels.

**Partial characterization of dense particles**

When observed under the electron microscope the band with a density of 1.44 g/ml in CsCl equilibrium density gradients appeared to be a pure suspension of isometric particles 24 nm in diam. Only particles not penetrated by the stain were observed. The u.v. extinction spectrum of this suspension was almost identical to that of the main component thus indicating a similar RNA:protein ratio.

The infectivity of the dense particles was assayed by injecting tenfold dilutions of a sample into flies. The preparation was found to have the same infectivity titre as a suspension of light particles with the same $E_{260}$. This result clearly demonstrated that the particles observed under the electron microscope were highly infectious.

Polyacrylamide gel electrophoresis of dissociated dense particles revealed the presence of three polypeptides with the same electrophoretic mobility as the major polypeptides of the light particles. However, the two minor polypeptides could not be detected in dense particles probably because of the small virus content of the sample used for this analysis.

A crude extract of flies inoculated with dense particles was clarified, the virus particles sedimented and resuspended prior to centrifuging in a sucrose gradient. The profile described above for normal DCV preparations was recorded through the ISCO gradient analyser. The middle band (virus particles) was sedimented and then centrifuged to equilibrium in a
caesium chloride gradient. Two peaks were recovered from this gradient: a major peak at a density of 1.34 g/ml containing 30 nm particles and a minor one at a density of 1.44 g/ml consisting of 24 nm particles. This result strongly suggested that the two types of particles represent different configurations of the same virus rather than a contamination of DCV by another picornavirus.

Studies on the virus RNA

Ultraviolet extinction spectrum

The u.v. extinction spectrum of RNA extracted from DCV particles was typical of a nucleic acid: it had a maximum at 258 nm and a minimum at 231 nm (Fig. 7). The $E_{260}:E_{280}$ and $E_{260}:E_{230}$ ratios were 2.24 and 2.51, respectively.

Reactivity with formaldehyde

DCV-RNA reacted rapidly with formaldehyde, and the increase in extinction was virtually complete within 1 h (Fig. 3). Analysis of 4 different lots of RNA gave an average of 24% hyperchromicity after 4 h with a variation of less than 2%.

Heating at 100°C for 15 min then rapid cooling at 37°C did not produce a significant change in extinction. The extinction spectrum of RNA after reaction with formaldehyde showed a shift of $E_{\text{max}}$ from 258 nm to 261 nm (Fig. 7). These results are closely similar to those obtained when formaldehyde reacted with intact virions, thus providing further evidence that the RNA in the particles is single-stranded.
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Fig. 9. Microdensitometer tracings of u.v. absorption patterns of DCV-RNA in 0.01 M-tris, 0.001 M-EDTA, 0.1 M-NaCl buffer, pH 8.0, during a sedimentation velocity run at 41,000 rev/min. Successive boundaries were from photographs taken at 4 min intervals. Direction of sedimentation was from left to right.

Reaction with ribonuclease

When the RNA was incubated at 37°C with pancreatic ribonuclease (0.4 µg/ml), extinction at 260 nm increased to a maximum of 24% hyperchromicity within a few minutes.

Thermal denaturation

The single-strandedness of DCV-RNA was confirmed by thermal denaturation. The melting curve of the RNA showed a gradual increase in extinction at 260 nm with no marked discontinuity (Fig. 8). The increase reached a maximum of 25.7% at 73°C. When the RNA was allowed to cool slowly at room temperature or was quenched in an ice bath, a low residual hyperchromicity (< 3%) was observed.

Infectivity of virus RNA

Injection into Drosophila of four different preparations of RNA extracted from DCV clearly demonstrated that they were infectious. The infectivity titre of the RNA was approx. 6 logs lower than that of the virus from which it was extracted. Fourteen to 50% of the flies injected with the RNA preparations containing 15 to 46 µg of nucleic acid/ml died within 3 to 5 days. An extract from each dead fly was examined in the electron microscope and found to contain a large number of virus particles. The percentage of infected flies was not strictly proportional to the amount of RNA injected but was more probably related to the quality of the preparation. After incubation for 20 min with pancreatic ribonuclease (2.5 µg/ml) at 25°C, a solution containing 32 µg/ml of virus RNA was no longer infectious.
**Sedimentation coefficient**

Ultracentrifugal analysis showed that the extracted DCV-RNA was homogeneous (Fig. 9). The average of 8 determinations gave a sedimentation coefficient value of $37 \pm 2S$, as compared to $31S$ for the TMV-RNA used as a standard. This sedimentation coefficient corresponded to a mol. wt. of $3 \times 10^6$ for the DCV-RNA using the formula of Spirin (1963). When the RNA was first treated with formaldehyde at low ionic strength according to the method of Boedtker (1968), values of $12S$ for DCV-RNA and $16S$ for TMV-RNA were obtained. This S value for DCV-RNA after treatment with formaldehyde was significantly lower than those reported for several mammalian picornaviruses.

**DISCUSSION**

Some of the physical and chemical properties of highly purified *Drosophila* C virus (DCV) and its extracted RNA are reported here. The present studies show that this virus meets all the criteria for inclusion in the Picornaviridae family (Wildy, 1971; Brown & Hull, 1973): (1) the virus particles are 30 nm in diam. in negatively stained preparations, (2) they have a sedimentation coefficient of 153S, (3) their buoyant density in CsCl is $1.34 \text{ g/ml}$, (4) they contain single-stranded RNA, and (5) they mature within a cytoplasmic matrix and form crystalline arrays in infected cells (Jousset et al. 1972).

Newman et al. (1973b) have recently proposed a subgrouping of the mammalian picornaviruses based on their stability at pH 3 to 7, buoyant density in CsCl, and the base composition of the virus RNA. When these criteria were applied to both the intact virion and the extracted RNA of DCV, it appeared that DCV shared many properties of the members of the enterovirus subgroup. Indeed, infectivity of DCV preparations was unaffected by treatment at pH 3.0 or 5.0 and its morphology was maintained (Jousset et al. 1972). Similarly, the buoyant density of $1.34 \text{ g/ml}$ in CsCl of the virus was not modified by altering the pH from 7.0 to 9.0. Analysis of the base composition of the DCV-RNA will provide a further criterion for this comparison, though Newman et al. (1973b) quoted a rather large variation in RNA composition among the enteroviruses.

Density gradient fractionation of enterovirus preparations always yields two types of virus particles: empty particles (or 'procapsids'), forming a band near the top of the gradients, and full virions, sedimenting faster in the gradient column (Brown & Hull, 1973). However, only full particles were observed in DCV preparations, and in this respect, this virus resembled mouse Elberfeld virus (Rueckert & Schäfer, 1965).

Furthermore, a minor component consisting of particles with a high density ($1.44 \text{ g/ml}$) has recently been isolated from preparations of several vertebrate enteroviruses (Rowlands et al. 1975; Yamaguchi-Koll et al. 1975). These particles contained the same amount of RNA and had a similar polypeptide composition to the particles of density $1.34 \text{ g/ml}$ but were less infectious and less stable, and possibly differed in the structural arrangement of the capsid proteins. Analysis of profiles of DCV equilibrium density gradients repeatedly revealed a minor peak located precisely at a density of $1.44 \text{ g/ml}$ (Fig. 5). Preliminary results have shown that it consisted of dense particles with the same specific infectivity as the major component but with a smaller diam. The fact that both types of particles were recovered from flies inoculated with the dense component strongly supports the idea that the two types of particles are different virion forms of the same virus. Further experiments are, however, necessary to eliminate the possibility of a contamination by a second picornavirus and to test whether or not the two components are interconvertible forms resulting from our purification procedure.
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The reactivity of the whole virion with formaldehyde indicated the accessibility of the amino groups in the DCV-RNA, thus suggesting that the virus genome was mostly single-stranded in situ. However, when the RNA was extracted from the particles, the formaldehyde reacted more rapidly and more extensively with it. These results were in accordance with those reported for other single-stranded RNA viruses (Fraenkel-Conrat, 1954; Staehelin, 1958; Longworth, Payne & MacLeod, 1973) and also with DNA viruses (Sinsheimer, 1959; Crawford, 1966; Robinson & Hetrick, 1969).

Polyacrylamide gel electrophoresis of DCV revealed 3 major polypeptides of mol. wt. 31,000, 30,000 and 28,000, respectively and two minor proteins of mol. wt. 37,000 and 8,500. The major proteins and the low mol. wt. minor polypeptide would correspond to VP1, VP2, VP3 and VP4 of enteroviruses (Brown & Hull, 1973; Rowlands et al., 1975). The minor component of mol. wt. 37,000 could correspond to the VP6 protein of 'procapsids' or 'provirions', although these structures have never been observed in DCV preparations. The three major polypeptides were common to light and dense particles.

Characterization of RNA extracted from DCV showed that the preparations were homogeneous and tests proved that it was infective. The average value of 37S found for the sedimentation coefficient of the RNA was slightly higher than that reported for mammalian enteroviruses (Brown & Hull, 1973). As a consequence, the mol. wt. of $3.0 \times 10^8$, calculated from the $s_{20, w}$ value by using the relationship of Spirin (1963) for small single-stranded RNA, was also higher. This difference, if significant, might reflect a peculiarity of this insect virus. Estimation of the mol. wt. by other analytical techniques is underway. The abnormally low figure of 12S for the sedimentation coefficient after formaldehyde treatment is difficult to explain. As suggested by Newman et al. (1973b) for the human rhinoviruses, differences in secondary structure of these virus RNA species and in their base composition could account for some of the variation in their response to formaldehyde. In this respect, the DCV-RNA is expected to have a low GC content.

Comparisons of the properties of the DCV particle and of its genome with those of the vertebrate picornaviruses leads us to propose that it is classified in the enterovirus subgroup as defined by the International Committee for Nomenclature of Viruses (Wildy, 1971).

Compared to the considerable body of data available for the vertebrate picornaviruses, our knowledge of the small isometric RNA viruses of invertebrates is still scarce. A few of them have recently been partially characterized. These include the five viruses of the honey bee (Newman et al. 1973a; Bailey & Woods, 1974) for Hymenoptera, the Gonometra virus and β Nudaurelia virus for Lepidoptera (Longworth et al. 1973; Struthers & Hendry, 1974), the cricket paralysis virus for Orthoptera (Reinganum, O'Loughlin & Hogan, 1970; Reinganum, 1973), and the Nodamura virus and kelp fly virus for Diptera (Murphy et al. 1970; Newman & Brown, 1973, 1976; Scotti, Gibbs & Wrigley, 1976).

DCV shares several physicochemical properties with sacbrood virus and acute paralysis virus of the honey bee, although the first is unstable below pH 5 and the second exhibits an increase in buoyant density above pH 7 (Newman et al. 1973a). Furthermore, DCV and the two viruses of the honey bee are serologically unrelated (Plus et al. 1975b; Reinganum & Scotti, 1976). In addition, DCV has been found to be serologically indistinguishable from cricket paralysis virus (CrPV) using an antiserum prepared against DCV and CrPV as antigen (Reinganum & Scotti, 1976). In spite of this resemblance DCV differs markedly from CrPV in its host range, in the constancy of its buoyant density in CsCl above pH 7 and in polypeptide composition (Reinganum, 1973, 1975 and personal communication; Jousset, 1976). These results clearly point out the necessity for a more complete characterization of these small RNA viruses of invertebrates in order to compare them more easily with
one another and with the picornaviruses of vertebrates. This applies particularly to the many isometric cytoplasmic viruses reported recently in laboratory and wild populations of *D. melanogaster* (Plus et al. 1975b). So far, these isolates have been compared only serologically. Biophysical and biochemical studies similar to those reported in this paper will certainly permit us to assess the relatedness of these different virus isolates to one another and to the mammalian picornaviruses.

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