Characterization of Three Antigenic Particles of Swine Vesicular Disease Virus

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SUMMARY

Three distinct particles were isolated from cell culture harvests of swine vesicular disease virus (SVDV) by sucrose and CsCl gradient centrifugation. Virions (148S), RNA-free empty capsids (81S), and a third particle (49S) also free of RNA showed immune reactivity with SVDV antiserum. The 81S and 49S particles had polypeptides typical of naturally occurring empty capsids. Injection of purified antigens into guinea pigs produced antisera which distinguished empty capsids from virions on immunodiffusion; the 49S antigen appeared similar to virions. Antisera produced to freshly prepared virus antigen grown in brains of baby mice distinguished SVDV from the serologically related Coxsackie B-5 virus but did not distinguish the individual S particle antigens. Partly purified virus preparations degraded to empty capsids when incubated in guinea pig serum. The possible origin of empty capsids and 49S particles and their relationship to antigenicity of virus preparations are discussed.

INTRODUCTION

Swine vesicular disease (SVD) was first recognized in Italy in 1966 (Nardelli et al. 1968; Nardelli, 1973) and has since appeared elsewhere in Europe and in Asia (Brooksby, 1972; Mowat, Derbyshire & Huntley, 1972; Dawe, Forman & Smale, 1973; Larenaudie et al. 1973). The disease is clinically indistinguishable from foot-and-mouth disease (FMD); but steers fail to develop clinical signs, and tissue extracts of lesions from diseased swine fail to show FMD virus (FMDV) complement-fixing antigen with antisera of the seven serotypes of FMDV (Nardelli et al. 1968; Mowat et al. 1972). The causal agent, identified as a porcine enterovirus, was distinguished from FMD as well as from vesicular exanthema and vesicular stomatitis viruses by its biological and physical properties (Nardelli et al. 1968; Brooksby, 1972; Mowat et al. 1972). The virus is spherical, with a diam. of 28 to 32 nm and has a sedimentation coefficient of 150S and a buoyant density of 1.34 g/ml. The virus is resistant to acid conditions or ether, and infectivity is stabilized at 50°C by 1 M-MgCl₂.

A serological relationship between SVD virus (SVDV) and Coxsackie virus type B-5 was shown by neutralization (Graves, 1973), complement-fixation (P. D. McKercher, unpublished observations) and immunodiffusion tests (Brown, Talbot & Burrows, 1973). However, Coxsackie B-5 virus was clearly distinguished from isolates of SVDV by specific spur lines in immunodiffusion. Differences in antigenicity between SVDV isolates have also been noted (Brown et al. 1973).

During purification of radiolabelled virus harvests, three fast-sedimenting particles were
observed on sucrose gradient centrifugation. Also, Ouchterlony double diffusion of SVDV concentrates versus certain SVDV antisera formed double precipitin lines. These results indicated that multiple antigenic forms of the virus might be present. This paper describes the isolation of three distinct antigenic particles that appear in SVDV-infected tissue culture fluids. Some biological and physical properties of the individual antigens are described.

**METHODS**

**Viruses.** The British isolate, UKG 27/72 of SVDV (SVDV-UKG) principally used in this study, and the Hong Kong 36/71 isolate (SVDV-HK) were supplied by the Animal Virus Research Institute, Pirbright, Surrey, England, as the second IB-RS-2 passage of virus isolated from vesicular lesions of swine. Coxsackie B-5 virus was the Faulkner strain obtained from the American Type Culture Collection, Rockville, Maryland.

The viruses were passed twice in a swine kidney cell line (MVPK), and samples in Eagle’s minimal essential medium (MEM) plus 10% foetal bovine serum were stored at -90°C as virus stocks. Virus was passed once from frozen storage and used as inoculum to produce virus for the experiments as the fourth MVPK passage.

**Cell culture, virus growth and concentration.** The MVPK cell line was obtained from Dr William Mengeling of the National Animal Disease Center, Ames, Iowa. The cell line is of epithelioid morphology and was developed for the propagation of porcine enteroviruses. Cells were grown in 2 l roller bottles for virus production or in stationary flasks for maintenance of the cell line. Cells were grown in MEM containing non-essential amino acids, 10% foetal bovine serum, penicillin (100 units/ml), streptomycin (50 µg/ml) and sodium pyruvate (1 mM).

Viruses were grown in MEM without serum and inoculated at a multiplicity of approx. 1 TCD₅₀ per 100 to 500 cells. Tissue culture fluids were collected 16 h post-infection and cleared of debris by centrifuging at 5500 g for 15 min. Radiolabelled virus was produced by adsorption of virus at 10 TCD₅₀/cell for 30 min and incubated in Hanks’ balanced salt solution with 0.02 M-tris-HCl at pH 7.5 (HBSS); penicillin, streptomycin, and pyruvate were added as for MEM. At 3 h post-inoculation, ¹⁴C-amino acid mixture (0.2 µCi/ml, 278 mCi/mM, average) or ¹⁴C-amino acids plus ³H-uridine (2 µCi/ml, 7.83 Ci/mmol) were added in 25 ml HBSS as appropriate. Cultures were incubated for 15 h when fluids were collected and processed as above for unlabelled virus. When desired, radioactivity in the cytoplasm of collected cells was extracted by treatment with 1% (v/v) Triton X-100 detergent.

Virus harvests were concentrated by polyethylene glycol (PEG) precipitation as previously described (Wagner, Card & Cowan, 1970) except that 8% (w/v) PEG 6000 was used for a single- or two-step precipitation. Precipitates were resuspended in tris-buffered saline (TBS) containing 0.15 M-NaCl and 0.02 M-tris-HCl at pH 7.5. Sodium azide, 0.02% (w/v) was added to virus harvests or concentrates and stored at 4°C if not used immediately. Fractions from gradients were concentrated by vacuum dialysis versus appropriate buffer.

**Preparation of antisera.** Antiserum to crude virus antigen (SVDV-UKG) was produced in guinea pigs by the subcutaneous injection of 0.5 ml of a 10% suspension of infected brain of newborn mice emulsified in incomplete Freund’s adjuvant. Animals were re-inoculated at 30 days, and sera were collected 10 days later.

Antisera were also produced against individual virus antigens which were purified by CsCl banding and sucrose gradient sedimentation. These were prepared from virus harvests treated with 0.05% (v/v) acetylenimine at 25°C for 48 h to prevent possible virus
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replication in inoculated animals. Initially, a 25 μg dose of virus or other protein antigen emulsified in complete Freund’s adjuvant was given subcutaneously to each guinea pig. Additional injections of 10, 10 and 5 μg of protein in phosphate buffered saline (PBS), pH 7.4 were given at 3, 5 and 7 weeks, respectively; the inoculum was divided between the original subcutaneous site and the peritoneal cavity. Sera were collected 10 days after the final injection.

Infectivity and neutralization tests. Virus infectivity was estimated by the method of Spearman–Kärber (Finney, 1964) in a tenfold virus dilution, four-well replicate microtitre system. Portions (0.1 ml) of virus dilution were incubated with 0.1 ml cell suspension containing 20000 MVPK cells. Plates were incubated for 72 h in a humidified atmosphere of 5% CO₂ in air, and wells containing 50% or greater c.p.e. were scored as infected.

The neutralizing activities of antisera were measured in a plaque reduction assay adapted from McVicar, Sutmoller & Andersen (1974). The reciprocal of the serum dilution, calculated to neutralize 70% of a test dose of 50 to 100 p.f.u. in 1 h at 37°C, was taken as the neutralizing titre. Plaques were developed in 4 oz prescription bottle MVPK cell cultures overlaid with MEM as used for cell growth plus 0.01 M-tris base and 0.6% (w/v) gum tragacanth (Mirchamsy & Rapp, 1968).

Complement-fixation tests. A tube method was used for complement fixation assays of virus antigens taken from sucrose density gradients (Cowan & Trautman, 1967). Appropriate antisera dilution (to crude virus antigen) was determined by block titration with PEG concentrated SVDV-UKG antigen. Complement fixation titres are expressed as the reciprocal of antigen dilution fixing 4 or 5 C₅₀ units of complement at optimum serum dilution.

Immunodiffusion and acridine orange staining. Antigens and antisera were diffused through 0.7% (w/v) agarose in a glycine-barbital buffer, pH 7.9, containing sodium azide, 0.05%, w/v (Cowan & Graves, 1966). Precipitin lines containing RNA were identified by acridine orange staining (Cowan & Graves, 1968).

Gradient and analytical centrifugation. For isodensity centrifugation, virus preparations were layered on to pre-formed 1.25 to 1.38 g/ml CsCl gradients in TBS and sedimented into the gradients at 150000 g for 3 h at 13°C in a SW 50.1 rotor (Spinco, Beckman Instruments, Inc., Palo Alto, California). In some isodensity runs, the sample was mixed with saturated CsCl in TBS to a density of 1.31 g/ml, and density gradients were formed by centrifuging to equilibrium for 16 h at 190000 g at 13°C. The density of certain fractions was determined by weighing 50 μl and correcting to g/ml at 20°C. Sucrose gradient sedimentation was performed in 20 to 45% (w/v) sucrose gradients in TBS at 13°C for 2.5 h at 190000 g in a SW 50.1 rotor unless otherwise specified. Drop fractions were collected from the bottom or top of the gradients as appropriate.

Moving boundary analytical ultracentrifugation analysis was done in a Spinco Model E analytical ultracentrifuge (Beckman Instruments) equipped with Schlieren optics according to standard procedures (Trautman & Hamilton, 1972).

Protein and RNA determinations. Protein concentration was estimated by the method of Lowry et al. (1951); bovine serum albumin was used as a protein standard. The concentration of purified virus antigen was roughly estimated for immunodiffusion and ultracentrifugation analysis by E₂₅⁹ measurements; the extinction coefficient for purified FMDV was used (Bachrach, Trautman & Breese, 1964). The amount of RNA contaminating purified virus protein antigen was estimated from E₂₅₀:E₂₆₀ ratios (Layne, 1957).

SDS-polyacrylamide gel electrophoresis. Samples in TBS were precipitated, resuspended in disruption buffer, and electrophoresed essentially as described by Harris & Brown.
Cylindrical 0.6 cm diam. gels were used and urea was omitted from stacking and resolving gels. Inclusion of urea from 0.5 to 4.0 M caused co-migration of VP0 and VP1 in procapsids and VP2 and VP3 in virions as shown elsewhere (Delagneau, Bernard & Lenoir, 1975; Rowlands et al. 1975). Polypeptides VP0 and VP2 were only resolved similar to patterns reported by Harris & Brown (1975) in 8 M-urea or if urea was omitted. Gels were fixed for 1 h in 50% (w/v) trichloroacetic acid, rinsed for 18 h in 25% (v/v) isopropanol and 10% (v/v) acetic acid, and stained with 0.2% (w/v) Coomassie brilliant blue in 50% (v/v) methanol and 7.5% acetic acid. Gels were diffusion destained in 5% methanol and 7.5% acetic acid. For determination of radioactivity in gels, 1 mm fractions were collected directly from gel tubes into 0.1 M-NH4OH using an automatic gel dividing device (Gilson Medical Electronics, Inc., Middleton, Wisconsin).

**Determination of radioactivity.** Radioactivity in aqueous samples of 3H- and 14C-labelled virus antigens was counted in a liquid scintillation counter (Beckman LS 335) using a toluene-Triton X-100 liquid scintillation mixture. Trichloroacetic acid-insoluble radioactivity was determined by precipitation with 10% trichloroacetic acid at 4°C for 2 h. Precipitates were collected on membrane filters (0.45 μm, 25 mm, Gelman GS-6), washed with 5% trichloroacetic acid at 4°C, dissolved in 1 ml 0.1 M-ammonium hydroxide, and counted in 10 ml Triton-toluene scintillator. Relative amounts of 3H and 14C in each sample were determined by appropriate correction for background and spillover.

**Materials.** Triton X-100 was obtained from Rohm and Haas Co., Philadelphia in 5 gallon batches that were tested for background and quench before use. Tritiated uridine was obtained from New England Nuclear, Inc., Boston, Massachusetts, and 14C-labelled amino acids from Schwartz BioResearch, Orangeburg, New York.

**RESULTS**

**Identification of three antigens of SVDV**

Tissue culture fluids collected from SVDV-UKG infected and uninfected MVPK cells incubated with 14C-amino acids were PEG-concentrated and centrifuged in sucrose gradients. Three sedimenting peaks of radioactivity were observed with SVDV-infected cell harvests (Fig. 1a). The largest peak sedimented first, followed by two smaller, poorly separated peaks. Results were identical with 14C-labelled preparations of the Hong Kong isolate of SVDV (not shown). No fast-sedimenting radioactivity was seen with concentrated culture fluids of uninfected cells (Fig. 1b) despite large amounts of incorporation of 14C-amino acids by these cells (Table 1). A small amount (less than 4%) of the total radioactive protein collected from the cytoplasm of uninfected cells sedimented, but at a slightly slower rate than the two small peaks from infected cells. Figures 1(c) and (d) show similar results of 14C labelled protein obtained from SVDV infected and uninfected primary pig kidney cultures. Three peaks of fast sedimenting radioactive material were also seen in fresh, unconcentrated harvest fluid or if harvest fluids were processed and sedimented in PBS instead of TBS (not shown).

The reactivity of the three particles with SVDV antiserum was investigated by comparing profiles of immunodiffusion reactions, complement fixation, and infectivity from a preparative sucrose gradient of concentrated harvest to the distribution of radioactivity of a labelled virus harvest in a duplicate tube (Fig. 2). Infectivity was associated with the fastest sedimenting peak and both the infectious particles and the slower sedimenting particles fixed complement and produced precipitin lines with antiserum to SVDV-UKG. Both zones also fixed complement and gave precipitin lines in agarose with an antiserum to
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Fig. 1. Sedimentation through a sucrose gradient of 14C-amino acid-labelled PEG-concentrated culture fluid of (a) infected MVPK; (b) control MVPK; (c) infected primary pig kidney cells; (d) control primary pig kidney cells.

Table 1. TCA-insoluble 14C-amino acids from SVDV-infected and control MVPK cell cultures

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Fraction</th>
<th>TCA-insoluble ct/min/culture</th>
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<tbody>
<tr>
<td>SVDV-infected</td>
<td>Culture fluid</td>
<td>38,400</td>
</tr>
<tr>
<td></td>
<td>Cells</td>
<td>42,000</td>
</tr>
<tr>
<td>Control</td>
<td>Culture fluid</td>
<td>388,500</td>
</tr>
<tr>
<td></td>
<td>Cells</td>
<td>343,800</td>
</tr>
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SVDV-HK mouse brain antigen. The immune reactivity of both slower sedimenting particles was demonstrated by re-sedimentation with labelled virus harvest until separated at the middle of the gradient. Complement fixation and precipitin lines associated with both peaks. When immunodiffusion patterns were stained with acridine orange, precipitin lines from fractions of the infectious zone showed fluorescence typical of single stranded nucleic acid (Cowan & Graves, 1968) whereas the slower sedimenting component precipitin lines were not stained.

Precipitation of all three components was confirmed in a liquid precipitation system (Fig. 3) in which antibody bound antigens were precipitated by the addition of rabbit anti-guinea pig immunoglobulin serum at equivalence. In addition, no reduction in
Fig. 2. Sedimentation through a sucrose gradient of concentrated SVDV harvest comparing (a) immunodiffusion; (b) complement fixation; (c) infectivity; (d) radioactive profile of $^{14}$C-amino acid-labelled SVDV harvest.

Radioactivity at the top of the gradient (Fig. 3) and no immunoprecipitation in agarose from the top fractions (Fig. 2) indicated that SVDV antiserum did not react with any significant part of the non-sedimenting material.

Physical characteristics of SVDV antigens

Sedimentation rate

Efforts to obtain a sedimentation rate of virus in PEG concentrates directly were unsuccessful because of viscosity interference caused by contamination with PEG. Pools of fractions taken from sucrose gradients and dialysed against TBS were used for sedimentation analysis. The pool of infectious virus particles sedimented at 148S (average of 3 runs), and the pool of slower sedimenting particles (taken as a pair) resolved two peaks of 81S and 49S. Estimation of mass by refractive increment indicated a relative proportion of 40% 148S, 20% 81S and 40% 49S particles recovered from the preparative sucrose gradient.
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Fig. 3. Supernatant radioactivity on a sucrose gradient after double antibody precipitation. ○—●, foot-and-mouth disease virus antiserum as a control; ▲—▲, SVDV antiserum.

Fig. 4. Sedimentation of $^{14}$C-amino acid (○—○) and $^3$H-uridine (▲—▲) labelled SVDV on a sucrose gradient.
Fig. 5. Polypeptide profiles of 14C-amino acid-labelled SVDV antigens on SDS-polyacrylamide gels. (a) 148S; (b) 81S; (c) 49S.

Density

A sample of PEG-concentrated SVDV-UKG was centrifuged to equilibrium in CsCl. The lowest sharp light-scattering band in the gradient was 1.35 g/ml and above this were two closely spaced light-scattering bands at 1.28 g/ml. Both fractions gave strong immunoprecipitin lines. The presence of both the 49S and 81S particles in the 1.28 g/ml band was demonstrated by sucrose gradient sedimentation of light-scattering zones taken from a pre-formed CsCl gradient run. Material at 1.34 g/ml sedimented, as does virus, to the bottom of the gradient and the 1.28 g/ml pool resolved into two components corresponding to the 81S and 49S antigens.

Nucleic acid content of 81S and 49S particles

The nucleic acid content of preparations of 81S and 49S particles was estimated by $E_{280}:E_{260}$ ratios of purified components that were prepared by CsCl banding and sucrose gradient sedimentation. The 81S-49S preparation contained 0.5% or less nucleic acid. The lack of RNA in the slower sedimenting particles was confirmed in a sucrose sedimentation run of concentrated SVDV-UKG grown in MVPK cells labelled with 3H-uridine and 14C-amino acids (Fig. 4). Clearly, 3H-uridine (RNA) was associated only with virus and the top of the gradient.
Polypeptide content of SVDV antigens

Two successive sucrose gradient sedimentations were used to isolate \(^{14}\)C-labelled SVDV-UKG virion, 81S, and 49S antigens. The distribution of \(^{14}\)C-labelled polypeptides on SDS-polyacrylamide gels is shown in Fig. 5. Purified virus resolved into three polypeptides corresponding to VP\(_1\), VP\(_2\) and VP\(_3\); VP\(_4\) was not clearly defined. The 81S antigen lacked VP\(_3\) and VP\(_9\) migrated just behind VP\(_1\); the 49S antigen contained the same proportion of VP\(_6\), VP\(_1\), and VP\(_3\) as the 81S antigen.

Immunodiffusion of three SVDV antigens and antigens of Coxsackie B-5

Guinea pig antisera to SVDV preparations or sera from SVDV-infected swine formed a common precipitin line with purified SVDV isolates and Coxsackie B-5 virus, but SVDV precipitin lines formed specific spurs over adjacent Coxsackie B-5 lines (Brown et al. 1973). Fig. 6(a) shows a similar test in which concentrated SVDV antigen as well as the purified antigens 148S, 81S and a 81S-49S mixture were precipitated adjacent to concentrated Coxsackie B-5 virus. Guinea pig antiserum to crude mouse brain SVDV-UKG antigen was used. As shown in Fig. 2 and 3, all three SVDV particles were precipitated. In adjacent wells, the individual SVDV antigens showed a common line of identity without spur formation (Fig. 6b); this finding indicated that the antiserum to mouse brain SVDV did not recognize any antigenic differences among the isolated SVDV antigens. Coxsackie B-5 also precipitated with a common precipitin line, but a strong spur line formed over the Coxsackie B-5 lines from each of the purified SVDV components. When the HK and UKG isolates were compared with this antiserum no specific spur appeared (not shown). More recently, similar guinea pig antisera to crude SVDV-UKG antigen have shown slight specific spurring over the other SVDV isolate, but SVDV-HK sera have not (S. Lai, personal communication).

Possible antigenic differences in the three SVDV particles were investigated in tests of the specificity of antisera from guinea pigs inoculated repeatedly with purified virus or a mixture of the 81S and 49S particles (see Methods for inoculation protocol). Antisera prepared to purified virus and the 81S-49S pair precipitated virus at a twofold or fourfold higher serum dilution than antiserum produced against crude SVDV antigen; plaque reduction neutralization titres were high for both 148S and 81S-49S antisera, log\(_{10}\) 4·58 and 5·01, respectively (c.f. 4·35 for guinea pig mouse brain SVDV-UKG antiserum).

Antiserum to 148S antigen precipitated all SVDV antigens (Fig. 6c). Two distinct precipitation lines formed against PEG-concentrated harvest: the inner line identified with the 148S particle, and the outer with the 81S particle. The 49S particle line appeared to coalesce with the 148S line and in another pattern (not shown) aligned with the inner PEG concentrate line. The 81S precipitin line formed a spur over the 49S antigen. Precipitin patterns formed with the 81S-49S antiserum were identical to those shown for 148S antiserum. No 148S precipitin line spurred over a 81S or 49S line with any of the antisera tested.

Specific spurring of 81S over 148S precipitin lines was also found when 1·28 g/ml and 1·34 g/ml CsCl fractions from SVDV-HK were tested (Fig. 6d) with antiserum to 148S antigen. When the UKG and HK isolates of SVDV were compared, precipitin lines of the 1·28 g/ml and the 1·34 g/ml CsCl fractions of each isolate coalesced (Fig. 6e). Figure 6(f) shows a similar pattern in which Coxsackie B-5 empty capsids and virions isolated by CsCl gradients were compared to SVDV-UKG with 148S SVDV-UKG antiserum. Both the 81S precipitin lines of each virus and the 148S lines of each coalesced separately (cf. the spurring seen with serum of guinea pigs injected with mouse brain antigen, Fig. 6a). In fact, the
Fig. 6. Immunodiffusion patterns demonstrating the specificity of guinea pig SVDV antisera produced against (a, b) crude; and (c to f) purified SVDV antigens. (a) Antiserum to mouse brain SVDV antigen distinguished SVDV antigens from Coxsackie B-5 virus, but (b) SVDV antigens were not distinguished from each other. (c) Identification of SVDV antigens with antiserum to purified SVDV; (d, e) full and empty capsids of SVDV-UKG and SVDV-HK; (f) purified SVDV antigens versus full and empty capsids of Coxsackie B-5 virus. Purified antigens of SVDV-UKG are indicated as 148S, 81S, 49S; PEG-concentrated virus by PEG-SVDV; Coxsackie B-5 virus by CB5; and CsCl fractions by 1.34 (virions) and 1.28 (empty capsids).
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Conversion of virus particles to empty capsids

When precipitation of all three SVDV antigens was shown in a liquid precipitation system (Fig. 3), a part of the 148S class radioactivity was apparently precipitated in the control serum tube. However, when sucrose gradient profiles of serum-incubated and unincubated PEG-concentrated preparations were compared, it was obvious that a conversion of 148S to 81S rate particles had occurred. Incubation at 37°C for 2 h in normal heated guinea pig serum (1:1, v/v, with TBS) caused a 40 to 60% loss of radioactivity in the virus peak and an equivalent increase in the 81S radioactive peak (Fig. 7c); no change in profile was observed when preparations were incubated in TBS (Fig. 7a and b). A reduction in the 49S empty capsid Coxsackie B-5 line spurred over the SVDV 148S line. Thus, the sera produced against purified components apparently were antigen class specific, whereas the sera developed against crude mouse brain SVDV antigen were virus type specific (SVDV versus Coxsackie virus, and occasionally SVDV isolate versus SVDV isolate).

Fig. 7. The effect of incubation on PEG-concentrated SVDV-UKG for 2 h (a) at 4°C in TBS; (b) at 37°C in TBS; (c) at 37°C in heated (56°C for 30 min) 50% normal guinea pig serum. (d) Lack of RNA in newly formed 81S antigen: • - •, 14C-amino acids; △ -- △, 3H-uridine. (e) Inhibition of serum-induced conversion to 81S by 0.2 M-MgCl₂. (f) Stabilization of virions at 50°C by 1 M-MgCl₂: • - •, 1 M-MgCl₂ in TBS; △ -- △, TBS alone. Sedimentation was in sucrose gradients.
peak was also noted with incubation in serum (Fig. 7c). Immunodiffusion analysis of sucrose gradients of purified 148S antigen showed a shift of immunoprecipitation to the middle of the gradient with serum incubation; virus incubated in TBS continued to sediment as virus. The newly formed antigen showed spurring over 148S and coalesced with 81S antigens on immunodiffusion with 81S-49S specific antiserum characteristic of empty capsids isolated from concentrated virus harvest. The serum-induced, 81S-like antigen contained little RNA (Fig. 7d).

The serum-induced conversion of virus occurred whether the serum was fresh, previously heated at 56°C for 30 min or dialysed against TBS. However, addition of 0.2 M MgCl₂ inhibited the serum-induced degradation (Fig. 7e), and 1 M MgCl₂ retarded the conversion to 81S in buffer at 50°C for 1 h (Fig. 7f). Incubation at 50°C also caused a loss of the 49S peak whether Mg was present or not. A peak of slowly sedimenting material appeared in this test, but insufficient material was obtained for immunodiffusion or centrifugation analysis.

**DISCUSSION**

The physical properties of full and empty capsids of SVDV are comparable with those reported elsewhere for SVDV (Nardelli et al. 1968; Mowat et al. 1972; Delagneau et al. 1974; Harris & Brown, 1975). Coxsackie virus preparations contain both full and empty particles (Philipson, Beatrice & Crowell, 1973) and artificial empty capsids can be formed in vitro (Schmidt et al. 1963). The 81S antigen isolated here from freshly purified stocks would appear by its polypeptide content to be naturally occurring empty capsids. Artificially produced empty capsids are distinguished by the loss of VP₄ and RNA, but contain the amounts of VP₁, VP₂ and VP₃ seen in virions (Maizel, Phillips & Summers, 1967; Korant et al. 1972; Philipson et al. 1973). These results are similar to those found for several isolates of SVDV (Harris & Brown, 1975), and unlike those of Delagneau et al. (1975), where poorer resolution prevented differentiation of full and empty capsid polypeptides.

Radioactive virus harvests always contained an 81S component, but empty capsids were not seen in electron micrographs of infected cells (unpublished data). The lower concentration or failure to associate with virion in crystalline arrays may have prevented observation of empty capsids. Virus degraded quickly on incubation in serum, purified virus preparations contain a significant number of empty capsids (Delagneau et al. 1974), and PEG concentrates of SVDV stored at 4°C eventually contain only empty capsid antigen (unpublished data). This indicates that older preparations of virus antigens may contain a significant quantity of artificial empty capsids as well.

No 49S structure has been reported for SVDV in sucrose gradient centrifugation (Delagneau et al. 1974; Delagneau et al. 1975; Harris & Brown, 1975). The conditions of centrifugation may have been such that 49S particles were not resolved from the 81S zone of empty capsids. The appearance of 49S particles found in this study did not depend on a particular cell type to grow virus, on the buffering system used, or on a concentration step before sedimentation analysis, but they were lost when incubated in serum or at high temperatures. Early reports on the purification and crystallization of Coxsackie virus (Briefs et al. 1952; Mattern, 1958) showed the presence of material sedimenting at about 80S and 40S, but such material was regarded as contaminants. The 80S particles were certainly empty capsids to be described later, and the 40S material termed ‘normal component’ remained unidentified. Recently, a particle was isolated from bovine enterovirus lysates; it sedimented at about 45S and contained VP₀, VP₁ and VP₃ (Su & Taylor, 1976). However, the particle was unstable at high ionic strength when it sedimented at about 80S.
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The loss of the 49S antigen on incubation in serum or at high temperature may reflect a conversion similar to that described by Su & Taylor (1976). Philipson et al. (1973) produced a number of subcapsid components of Coxsackie B-3 virus by incubation in urea solutions. A 40S component arose from natural empty capsids and contained VP0 and smaller quantities of VP1 and VP3. However, the immune reactivity of degraded components with antiviral antisera was not investigated. The 49S particle reported here contains similar quantities of VP0, VP1 and VP3 and probably represents an antigenically active subcapsid component of provirions (natural empty capsids).

The specificity of immunodiffusion reactions varied, depending on the method used to prepare antiserum. Virus antigen was obtained from homogenates of infected mouse brain in an effort to avoid swine tissue antigens that were present in harvests of infected pig kidney cell cultures. All three isolated antigens of SVDV precipitated with antiserum to mouse brain antigen but were not distinguished from each other. The antiserum distinguished all three SVDV antigens from Coxsackie B-5 virus by specific spurring on immunodiffusion. However, the HK and UKG isolates of SVDV usually produced reactions of identity. Brown et al. (1973) reported that specific spur lines formed between several SVDV isolates as well as between SVDV and Coxsackie B-5 virus when convalescent swine or guinea pig hyperimmune antiserum was used. Results were different when antisera were produced to purified acetylhexylamine-treated antigens. Although empty capsids could be distinguished from intact virions and 49S particles, the antiserum produced reactions of identity between SVDV and Coxsackie B-5 virions as well as between their empty capsids.

The difference in reactivity of the sera is similar to the 'group' (C type) and 'specific' (D type) reactivity seen with sera to other enteroviruses (Schmidt, Lennette & Dennis, 1962; Connant, Barron & Milgram, 1966). Serum from laboratory animals injected with purified antigens or serum taken during acute stages of infection showed group specificity and failed to distinguish types within an enterovirus group. Serum taken during convalescence became type specific. The guinea pig serum to purified SVDV antigens appears to be group specific even though 81S antigen could be distinguished from virus. The antiserum to crude SVDV antigen was type specific. The relative amount of virus and its persistence after injection may be important because the specific antigen of most enteroviruses is associated with intact (dense) virus and the group antigen with empty capsids (Hummeler & Tumilowicz, 1960; Schmidt et al. 1963).

Inoculation of purified virus or the 81S-49S antigens produced antisera with the same immunodiffusion specificity. The 148S antiserum did not produce virus-specific spur lines over 81S or 49S antigen indicating that either virus and 81S-49S antigens share virus-specific determinants, as was shown for rhinovirus (Lonberg-Holm & Yin, 1973) and FMDV (Rowlands, Sangar & Brown, 1975), or that the virus inoculum degraded rapidly and provided only empty capsid determinants.

The high neutralization titre of antiserum to the 81S-49S antigen pool suggests that it contains immunogenic determinants. However, enterovirus empty capsids are considered to be poor immunogens (Hummeler & Tumilowicz, 1960; Ghendon & Yakobson, 1971). Because multiple injections of antigen were given here, the high titres obtained may not represent immunogenicity comparable with that of virions. However, the coalescing lines of 49S and 148S on immunodiffusion indicates that they are similar antigenically and that 49S might contain an immunogenic site.
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