PURIFICATION AND MOLECULAR ANATOMY OF THE VARICELLA-ZOSTER VIRION

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SUMMARY Varicella-zoster virus (VZV) infected cell cultures were harvested and sonically disrupted when cytopathic effect was advanced. Infectious cell-free virus in the sonicates, as well as that in the culture medium, was further concentrated by precipitation with 8% (w/v) polyethylene glycol in the presence of high salinity (0.5 *M*). The virus-enriched pellet was layered onto 15–45% linear metrizamide gradients and sedimented for 18 h at 70,000 *g*. Of the three visible bands (designated upper, middle and lower), the middle band at a buoyant denisty of 1.156–7 g/cm^3 was enriched for enveloped virions. Electron microscopic enumeration of particles demonstrated a total of 10.04 $\log_{10}$ enveloped particles and 8.26 $\log_{10}$ unenveloped particles from middle bands representing the yield from a 150 cm^2 VZV-infected monolayer. Fractionation of radiolabeled virion preparations by SDS-PAGE revealed 30 polypeptides between 30 and 200 kilodaltons (K) with a total mol wt of 2,240,000. Prominent structural polypeptides included the major capsid protein (155K) and three glycoproteins—62K, 98K and 118K. Certain polypeptides better labeled by [^{14}C] amino acids than by [^{35}S] methionine included a higher mol wt polypeptide (174K) and 45K protein comigrating with actin. Immune precipitation of a Nonidet-extracted virion fraction again demonstrated the three major glycoproteins, as well as the 155K and 45K polypeptides. Comparison of structural polypeptides with the 16 constituents of the VZV-specific immunoprecipitation profile identified at least one polypeptide (145K) which was not represented in the virion and assumed, therefore, to be nonstructural.

INTRODUCTION

The purification of varicella-zoster virus (VZV) has been an elusive goal because this human herpesvirus remains predominantly cell-associated throughout the in vitro infectious cycle (Weller et al., 1958). Titration of culture medium overlying VZV-infected monolayers has

demonstrated little infectivity even when cytophathic effect is far advanced. Generally, therefore, cell-free virus has been recovered by freezing or sonic disruption of VZV-infected cells and slow speed centrifugation to remove gross debris; further concentration has been achieved by precipitation with nonionic hydrophilic polymers. Prior attempts at virus purification by sedimentation in several density-gradient media have been hampered by loss of infectivity and disenvelopment of individual virions (Grose et al., 1979).

To circumvent those obstacles, we first defined glycosylated and nonglycosylated infected cell-specific polypeptides by radioimmune precipitation with high titer VZV xenoantisera (Grose, 1980; Grose et al. 1981a; Grose and Friedrichs, 1982). In order to segregate the above immunogenic polypeptides into structural and nonstructural viral components, we further characterized the molecular anatomy of the VZ virion. First, a method was devised for purification of the enveloped virion by sedimentation in a nonionic density-gradient medium. Second, the polypeptide composition of the purified virion was resolved by electrophoresis in polyacrylamide gels under denaturing conditions, and finally immunogenic structural proteins were tentatively identified by comparison with VZV-specific immunoprecipitation profiles.

METHODS

1. *Cells and virus*

Human melanoma cell monolayers (HMC; Mewo strain, passages 28–45) (Grose et al., 1979) were grown in Eagle's minimum essential medium supplemented with 0.002 *M* glutamine, 1% nonessential amino acids, penicillin (100 U/ml), streptomycin (100 μg/ml) and 8% fetal bovine serum (MEM-FBS). The source of virus for all experiments was the " VZV-32 " strain, which was isolated from the vesicular fluid of a child with chickenpox (Grose et al., 1979) and serially propagated at 32 C no more than 20 times by passage of trypsin-dispersed infected HMC cultures.

2. *Infection of cells and isotopic labeling*

One day after subcultivation, nearly confluent HMC monolayers were inoculated with VZV-infected cells obtained from trypsinized monolayers which exhibited advanced cytopathic effect (CPE). Infected cells from one 75-cm^2 monolayer were equally distributed among six 75-cm^2 monolayers. CPE appeared within 24 h, at which time the culture medium was replaced with either MEM deficient in methionine but supplemented with [^{35}S] methionine (10 μCi/ml) or regular medium containing 0.5 μCi/ml of tritiated thymidine, 10 μCi/ml of a tritiated sugar (fucose or mannose), or 4 μCi/ml of [^{14}C] amino acid mixture. The cultures were harvested at the end of the second day when syncytial CPE covered nearly all of the monolayer.

3. *Cell-free virus*

Cell-free virus was released from VZV-infected cells by sonic disruption as described in detail in earlier publications (Brunell, 1967; Grose et al., 1979). After sedimentation at 400 *g* for 15 min to remove gross cellular debris, the cell-free virus sample was mixed with the culture medium which had also been clarified by low speed centrifugation. Sodium chloride and polyethylene glycol 6,000 (PEG; J. T. Baker Chemical Co., Pillipsburg, NJ) were added to final concentrations of 0.5 *M* and 8% (w/v), respectively, and the solution was incubated overnight at 4 C. Virus was recovered from the pellet after sedimentation for one h at 10,000 *g* at 4 C.

4. *Sedimentation in potassium tartrate-glycerol*

Positive density-negative viscosity gradients were prepared in a two-chambered gradient maker (Buchler Instrument Co., Fort Lee, NJ) by mixing 8 ml of 30% (w/w) glycerol in TE buffer (0.002 *M*-EDTA in 0.002 *M* Tris, pH 7.4) with 7 ml of 50% (w/w) potassium tartrate (KT) in the same buffer. After addition of one ml of cell-free virus, the gradient was centrifuged at 25,000 rpm for 17 h in a SW 27.1 (Beckman) rotor. A visible virus-enriched band near the center of the gradient was removed, dialyzed against Tris-buffered saline (pH 7.4) and layered onto a linear metrizamide gradient (see below).

5. *Sedimentation in metrizamide*

Metrizamide (Nyegaard, Oslo, Norway) is an iodinated derivative of 2-deoxyglucose (2-[3-aceta-

mido-5-N-methylacetamido-2, 4, 6, tri-iodobenzamido]-2-deoxy-D-glucose) which has become widely used as a density gradient medium because of its low osmotic and nonionic properties (Rickwood, 1978). Analytical grade metrizamide (purchased from Accurate Chemical and Scientific Corp., Westbury, NY) was preferred over the centrifugation grade because contaminants present in the latter preparation occasionally interacted with proteins in the virus preparations to form specious bands in the density gradients. Metrizamide was prepared in three different solutions: these included 0.001 *M* sodium phosphate (pH 7.0) containing either 0.2% (w/v) bovine serum albumin (BSA, Reheis Fine Chemicals, Scottsdale, AZ) or 0.01% (w/v) gelatin (Difco Laboratories, Detroit, MI) as a protein additive; the third solution consisted of 0.01% (w/v) gelatin and 0.001 *M* EDTA dissolved in 0.05 *M* Tris (hydroxylmethyl) aminomethane (pH 7.4)-buffered 0.15 *M* NaCl (TEN buffer) (Server, 1980). The linear density gradients were poured in a standard two-chambered gradient-maker by mixing 8 ml of a 15% (w/v) metrizamide solution with an equal amount of a 45% (w/v) solution.

Each 16 ml gradient was overlaid with one ml virus preparation (representing no more than the equivalent of cell-free virus recovered from one 25-cm^2 infected monolayer) and centrifuged for 18 h at 23,000 rpm in an SW 27.1 (Beckman) rotor. All sedimentations were carried out at 4 C. Individual 10-drop fractions were collected by puncture from below and analyzed for acid-precipitable radioactivity. Densities were calculated from the refractive indices of duplicate fractionated gradients; standard curves comparing refractive index, concentration and density of the three metrizamide solutions were established from our own data and those of Rickwood (1978; 1979).

6. *Electrophoresis and fluorography*

Polyacrylamide slab gels containing 0.1% (v/v) sodium dodecyl sulfate (SDS) were prepared from acrylamide cross-linked with bisacrylamide at either a 37.5: 1 or a 77: 1 ratio. The higher ratio was used to optimize conditions for fractionation of multiple polypeptides (Peluso et al., 1977). Electrophoresis of the solubilized samples was carried out by standard procedures in a Tris-glycine-SDS buffer system (pH 8.1) at ambient temperature (Maizel, 1971; Grose, 1980); protein standards, purchased from BioRad Laboratories (Richmond, CA) and labeled with carbon-14 by reductive alkylation (Rice and Means, 1971), were added to one lane of each gel. The slab gels were suffused with 2, 5-diphenyloxazole dissolved in dimethylsulfoxide prior to drying onto chromatography paper (Bonner and Laskey, 1974), and the dried gels were exposed to Kodak XR-5 or XRP-5 film from 4 to 21 days.

7. *Immunoprecipitation*

High titer VZV antisera were produced in inbred guinea pigs inoculated with VZV infected syngeneic cells (Edmond et al., 1981; Grose, 1981). Viral antigen was prepared from VZV-infected HMC cultures solubilized in modified Schwyzer's buffer containing final concentrations of 1% (v/v) Nonidet P40 and 1% (w/v) deoxycholate (Grose and Friedrichs, 1982). Antisera were added to 100 μl aliquots of radioactive antigen and the mixtures were incubated overnight at 4 C. On the following day 110 μl of pre-swollen protein A-sepharose CL-4B beads (Pharmacia Fine Chemicals, Piscataway, NJ) were added to each reaction mixture. The immunoprecipitated samples were processed and analyzed by SDS-PAGE as described (Kessler, 1975; Grose and Friedrichs, 1982).

8. *Radioisotopes*

L-[5, 6-^{3}H] fucose (40–60 Ci/mmol) and [^{14}C] formaldehyde (10 mCi/mmol) were obtained from New England Nuclear Corp. (Boston, MA); [methyl-^{3}H] thymidine (40–60 Ci/mM) was purchased from Schwarz / Mann Bioresearch (Orange, NJ). Amersham (Arlington Heights, IL) supplied L-[^{35}S] methionine (>600 Ci/mmol), D-[2-^{3}H] mannose (10–20 Ci/mmol), and L-amino acids labeled with carbon-14 (25 mCi/milligramatom of carbon labeled).

RESULTS

1. *Purification of VZ virions by sedimentation in metrizamide*

Cell-free virus was recovered from sonically disrupted VZV-infected cells, as well as from culture medium overlying virus-infected monolayers, by PEG precipitation as described (Grose et al., 1979). The virus enriched pellet was disrupted by a 5-second sonic burst and layered onto 15–45% (w/v) linear density gra-

dients. After isopycnic centrifugation, the gradients were fractionated and analyzed for radioactivity and optical density. Sedimentation profiles of [^{14}C] amino acid labeled cell-free virus in each of three metrizamide density gradient media are represented in Fig. 1. In each instance three bands were visualized and designated upper, middle and lower relative to their location in the gradient; in the metrizamide / phosphate / gelatin gradient, the lower two visible bands occupied adjacent fractions. Similar sedimentation profiles were recorded after centrifugation of cell-free virus isotopically labeled with either tritiated mannose or thymidine (Fig. 2).

The PEG pellet and the three bands from a metrizamide / phosphate / BSA gradient were examined by electron microscopy to document morphology (Fig. 3) and enumerate viral particles (Table I). The pellet revealed a diversity of cellular organelles, cell debris and herpesviruses. After separation in a metrizamide gradient, the upper band consisted mainly of cellular membranes with relatively few viral particles. Those viral particles that were found were frequently closely associated with membranes and occasionally possessed distorted envelopes. The lower band included large numbers of predominantly unenveloped viral particles interspersed among cellular organelles, especially mitochondria. The middle band, which was always the least prominent visually,was enriched for enveloped viral particles in both absolute number (titer $\geq 10 \log_{10}$) and in relative numbers compared with nucleocapsids (10: 1 ratio). This band also contained the smallest amount of contamination by cellular elements.

The middle bands were collected and subsequently sedimented in a second metrizamide density gradient prepared in the same buffer (Fig. 1). The isodense position of the middle band was determined from the optical density of the corresponding fractions. A value of 1.156 g/cm^3 was calculated for the virion-enriched band in metrizamide dissolved in 0.001 *M* phosphate / BSA, while the equivalent

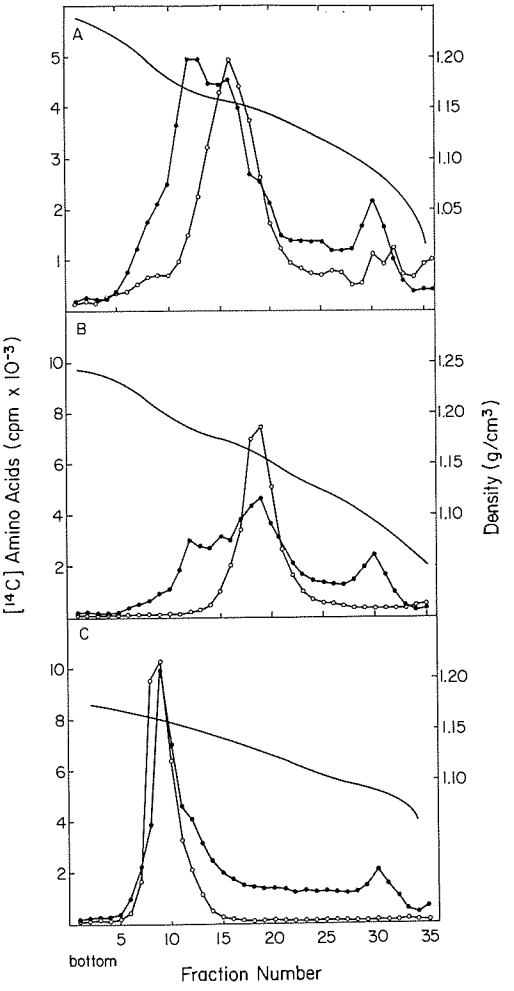

FIGURE 1. Sedimentation profiles of cell-free virus labeled with [^{14}C] amino acids in 15–45% linear metrizamide gradients prepared in three different buffers. The solutions included (A) 0.001 *M* sodium phosphate (pH 7.0), 0.2% BSA; (B) 0.05 Tris (pH 7.4), 0.15 *M* NaCl, 0.001*M* EDTA, 0.01% gelatin; and (C) 0.001 *M* phosphate (pH 7.0), 0.01% gelatin. The first sedimentation profile (–•–) consisted of upper, middle and lower bands. The virion-enriched middle band was subjected to a second sedimentation (–◦–) in metrizamide dissolved in the same buffer. Density as calculated from the refractive index of alternate fractions is represented by the continuous line.

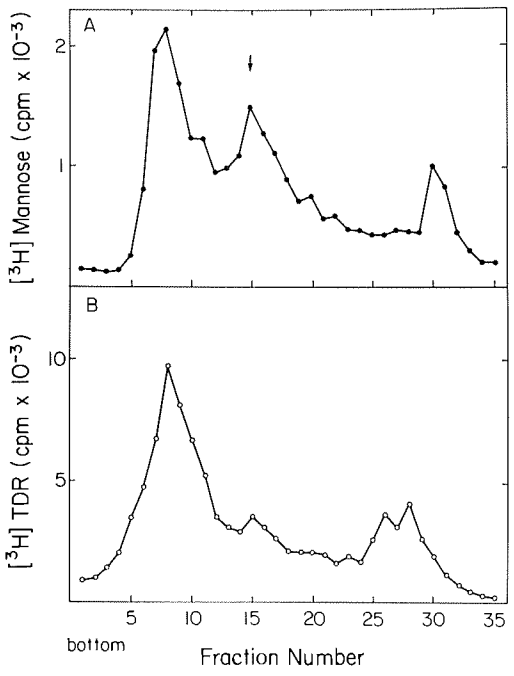

FIGURE 2. Sedimentation profiles in metrizamide. Cell-free VZV isotopically labeled with either [^{3}H] mannose (A) or [^{3}H] thymidine (B) was sedimented in 15–45% linear gradients of metrizamide dissolved in 0.001 *M* phosphate (pH 7.0), 0.2% BSA. The location of virion-enriched middle band is indicated by an arrow (↓).

peak of radioactivity in the other two metrizamide-buffer systems had a buoyant density of 1.157 g/cm^3. The respective values for the upper and lower bands from the first sedimentation were 1.118 g/cm^3 and 1.184 g/cm^3.

In an earlier paper (Grose et al., 1979), we reported that low titers of cell-free virus could be recovered from the medium overlying VZV-infected HMC cultures which displayed advanced cytopathic effect. When we concentrated virus from culture media by PEG precipitation and subjected the pellet to metrizamide density gradient sedimentation, three bands corresponding in location and density to the previously described upper, middle, and lower bands were identified. Enumeration of virus particles demonstrated a similar profile to that observed in earlier experiments (Table II). However, the absolute number of viral particles was approximately one log fewer (titer ~9 $\log_{10}$) than that recovered from 150 cm^2 VZV-infected monolayers and media together. Therefore, the titer of virus from culture medium alone represented roughly one-tenth of the total yield.

To further verify the results of our centrifugation procedures, we subjected cell-free virus first to lyophilization in phosphate-buffered saline prior to sedimentation in metrizamide. We have previously shown that freeze drying in suspension medium devoid of sugars or protein effects disenvelopment of all virus particles (Grose et al., 1981b). As anticipated, subsequent metrizamide gradients contained only two major bands corresponding to the isodense positions of the membranes and the nucleocapsids. No band was detected in the region of 1.156–7 g/cm^3.

Metrizamide gradients also were assayed for infectivity after fractionation into one ml aliquots. Each fraction was adsorbed onto 2–2.5 cm^2 HMC monolayers. No cytopathic effect appeared in monolayers inoculated with fractions corresponding to the middle and lower bands, although an occasional focus was seen in the cells inoculated with the upper (membrane) band. In a reconstitution experiment, virus from a middle band was mixed with uninfected cell membranes recovered in an upped band; however, infectivity was not restored. Additional titrations of unsedimented cell-free VZV in the presence of 5% 10% and 30% metrizamide demonstrated reductions in titer of 89%, 97% and 100%, respectively. Therefore, metrizamide alone appeared to inhibit infectivity while preserving the integrity of the virion.

2. *Sedimentation in potassium tartrate and glycerol*

We demonstrated previously that infectious virus could be recovered after sedimentation of clarified VZV sonicate through density-viscosity gradients composed of potassium tartrate

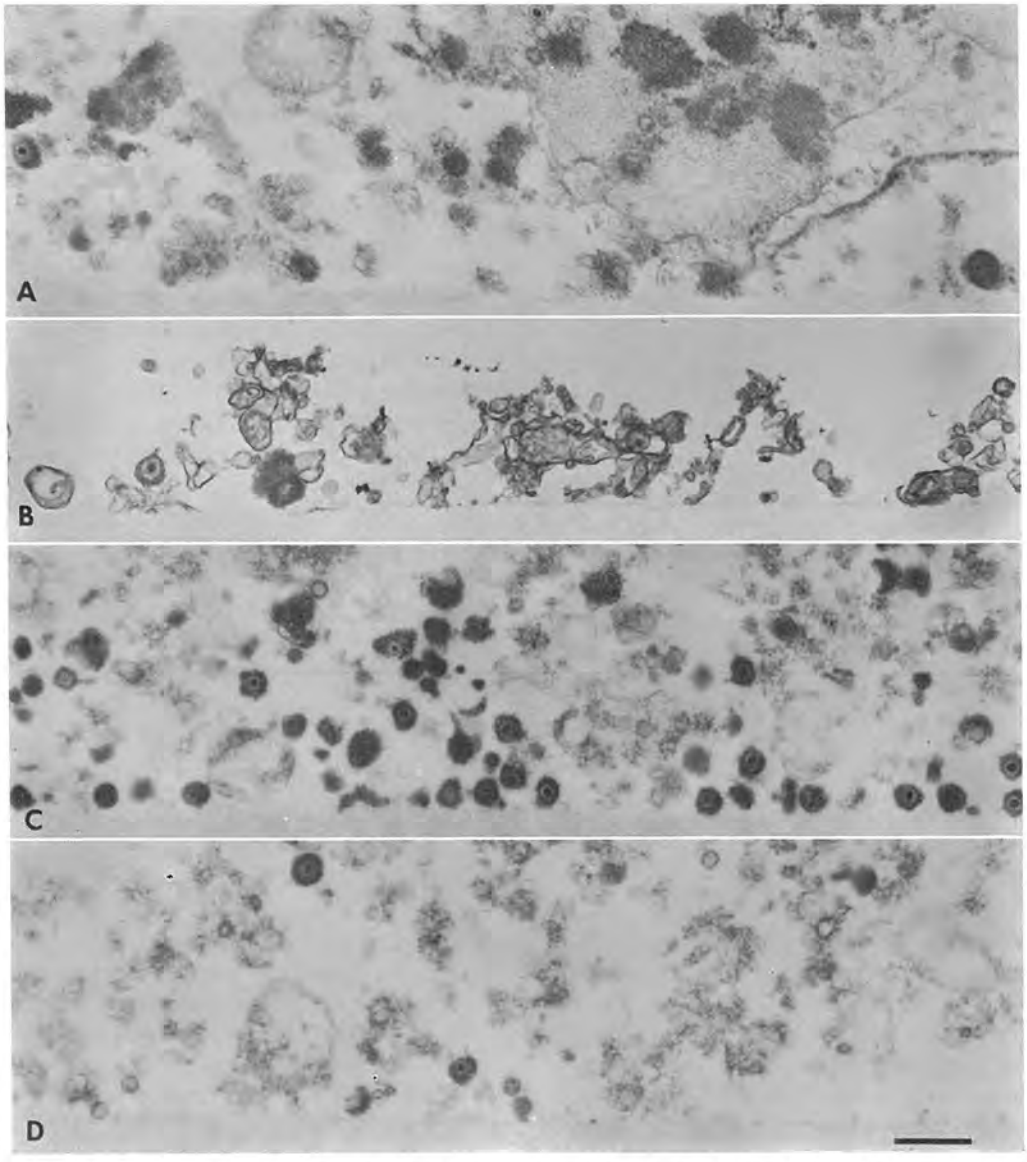

FIGURE 3. Electron microscopy. Quantitative electron microscopy was based on a thin section method which involves the sedimentation of virus-containing specimens onto a Millipore filter (Miller et al., 1973). The four panels represent specimens from the pellet after PEG precipitation of medium and sonicate (A), and from the upper (B), middle (C), and lower (D) bands of a metrizamide density gradient after centrifugation of the PEG-precipitated pellet; a Millipore filter in cross section is visible near the bottom of each panel. As described in greater detail in a recent publication (Weiss et al., 1981), the specimens on the filters were post-fixed in chrome-osmium, dehydrated in graded ethanols, and embedded in Epon. Filters with sedimented virus were cut perpendicularly to the plane of the membrane, and pale gold sections were mounted on 300 mesh copper grids. Sections were photographed at 3000× and enlarged to 9,000×, prior to enumeration of viral particles with the aid of a dissecting microscope. Total numbers of viral particles sedimented onto a filter were calculated according to previously described quantitation formulas (Miller et al., 1973; Weiss et al., 1981). Magnification of the electron micrographs was determined using a carbon grating replica. Scale: 0.5 μm.

TABLE 1. *Sedimentation of viral particles present in VZV-infected monolayers and media*[a]

Location in metrizamide *Density gradient*	Enumeration of viral particles ($\times \log_{10}$)		
	Enveloped	*Unenveloped*	*Total*
Upper band	8.72	8.88	9.11
Middle band	10.04	8.26	10.11
Lower band	9.82	10.49	10.57

[a] Cell-free virus from 150 cm^2 VZV-infected monolayers consisted of 10.92 and 10.51 $\log_{10}$ enveloped and unenveloped particles, respectively, after precipitation with 8% PEG in 0.5 *M* NaCl. The pellet was distributed among six metrizamide density gradients. After sedimentation, the three major bands from each gradient were removed and pooled prior to enumeration by electron microscopy, as described in the legend to Fig. 3.

TABLE 2. *Sedimentation of viral particles present in culture medium*[a]

Location in metrizamide *Density gradient*	Enumeration of viral particles ($\times \log_{10}$)		
	Enveloped	*Unenveloped*	*Total*
Upper band	7.77	8.23	8.36
Middle band	8.91	8.04	8.97
Lower band	8.28	8.88	8.98

[a] Cell-free virus in culture medium overlying 150 cm^2 VZV-infected monolayer was precipitated by the addition of PEG and NaCl to final concentration of 8% and 0.5 *M*, respectively. The pellet was fractionated by sedimentation in a metrizamide density gradient, and the viral particles in the three major bands were counted as described in the legend to Fig. 3.

(KT) and glycerol (Grose et al., 1979). As part of the present study, we subjected the band of infectivity from a KT-glycerol gradient to sedimentation in metrizamide (Fig. 4). Fractionation of the latter gradient demonstrated an accumulation of radioactivity near the top with trailing into the regions of higher density. The metrizamide sedimentation profile of radioactive cell-free virus from the same

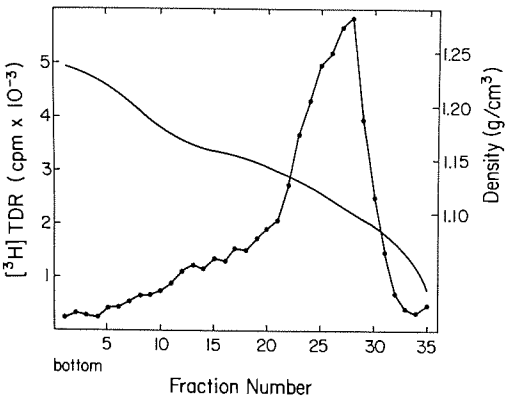

FIGURE 4. Effect of KT-glycerol on sedimentation profile in metrizamide. A band of infectious cell-free virus was recovered from a combination density-viscosity (KT-glycerol) gradient and subjected to a second sedimentation in a 15–45% linear metrizamide gradient.

experiment which had never been placed into KT-glycerol resembled that described in Fig. 1. Addition of KT-glycerol to sonically disrupted VZV-infected cells, therefore, effected aggregation between virus and cellular membranes in the preparation and thereby inhibited further purification of enveloped virions.

3. *Structural polypeptides of virion and nucleocapsid*

After the second sedimentation in metrizamide, aliquots of the middle bands were collected. The viral particles were sedimented by ultracentrifugation, resuspended in sample buffer and analyzed in polyacrylamide gels prepared at a 77:1 ratio. Thirty polypeptides were detected in the electrophoretic profile of the twice-sedimented virion-enriched band and were designated according to their apparent molecular weights as determined by plotting their migration relative to marker proteins (Fig. 5). The most prominent of the higher mol wt polypeptides labeled with [^{35}S] methionine had an estimated weight of 155 kilodaltons (K). A closely migrating band at 160K was observed in this gel, but not in gels

mixed at a lower ratio (fluorogram not shown). A lower mol wt polypeptide which was always identifiable in the virions comigrated with radioactive actin marker (45,000). The cumulative mol wt of the 30 polypeptides between 30 and 200K was 2,240,000.

When virions labeled with [^{14}C] amino acid mixture were similarly purified and analyzed, a structural polypeptide at 174K which incorporated little [^{35}S] methionine was better visualized (Fig. 6, lane G). In addition, analysis of virion fractions labeled with tritiated sugars exhibited three major glycoproteins which corresponded in mobility with polypeptides designated 62K, 98K and 118K (Fig. 6, lane D), Glycoproteins present in the nucleocapsid enriched fraction presumably represented con-

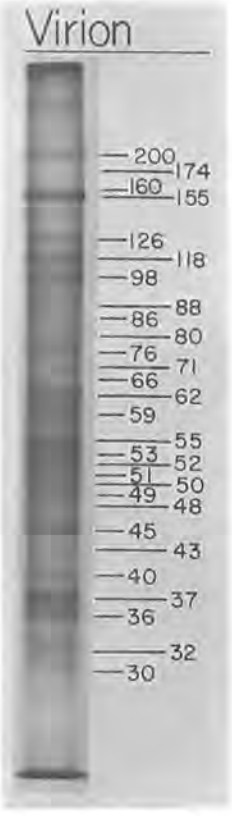

FIGURE 5. Structural polypeptides of the virion. Cell-free virus labeled with [^{35}S] methionine was recovered from the medium and infected cells and concentrated by PEG precipitation. After two sedimentations in 15–45% linear metrizamide gradients, the virion-enriched middle bands were collected and solubilized. A sample containing ~40,000 cpm was subjected to electrophoresis in a 10% acrylamide / MBA slab gel (77: 1 ratio). The molecular weights of the structural polypeptides between 30 and 200K, as estimated by the method of Shapiro et al. (1967), are enumerated in the right margin.

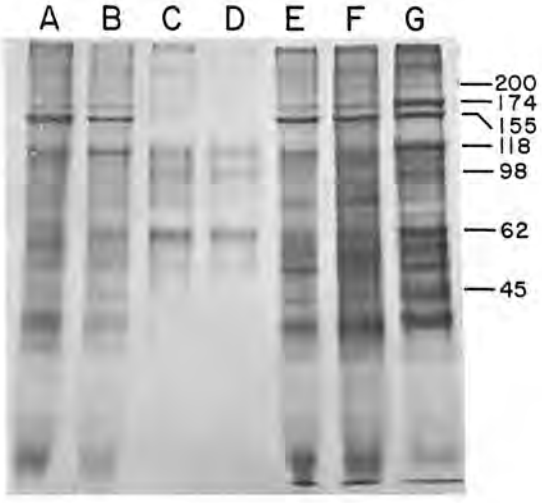

FIGURE 6. Electrophoretic analyses of virion- and nucleocapsid-enriched fractions. Individual VZV-infected cultures were isotopically labeled with [^{35}S] methionine or [^{14}C] amino acid mixture. The cultures were harvested and cell-free virus was further purified by two sedimentations through linear metrizamide gradients. The middle bands (virion-enriched) and lower bands (nucleocapsid-enriched) were collected and analyzed by electrophoresis in a 6–12% gradient acrylamide / MBA gel (37.5: 1 ratio). The seven lanes from left to right contain the following samples: A, [^{35}S] methionine labeled nucleocapsids (45,400 cpm); B, [^{35}S] methionine labeled virions (34,400 cpm); C, [^{3}H] fucose labeled nucleocapsids (23,400 cpm); D, [^{3}H] fucose labeled virions (17,000 cpm); E, [^{35}S] methionine labeled nucleocapsids (46,300 cpm); F, [^{14}C] amino acid labeled nucleocapsids (11,400 cpm); and G, [^{14}C] amino acid labeled virions (14,500 cpm). The molecular weights of seven prominent polypeptides are designated in the margin on the far right side; the 45K protein comigrates with [^{14}C] actin.

tamination by enveloped virions as well as cellular elements (Fig. 6, lane C).

The structural polypeptides of the [^{35}S] methionine and [^{14}C] amino acid labeled virion fractions also were compared with those of similarly labeled nucleocapsid enriched preparations (lower bands in metrizamide). The 155K polypeptide was the single most distinctive band in all samples (Fig. 6). The 174K polypeptide was less prominent in both nucleocapsid preparations regardless of radioisotope. A second difference noted between the two bands involved actin. Actin, which is relatively deficient in methionine, incorporated radioactive amino acids and was easily visualized in the virion fraction (Fig. 7). However, in the nucleocapsid band the amount of this cellular protein was considerably reduced.

4. *Solubilization of virions.*

Detergent solubilized extracts of virion-enriched fractions were centrifuged to remove particulate matter and subjected to immunoprecipitation with high titer VZV antiserum in order to identify prominent soluble proteins on or near the surface of the virion. The two

FIGURE 7. Actin in virion- and nucleocapsid-enriched fractions. Virions labeled with [^{35}S] methionine and nucleocapsids labeled with [^{14}C] amino acids were collected from the middle and lower bands, respectively, of two different 15–45% linear metrizamide gradients. After solubilization, an aliquot from the virion fraction (35,000 cpm) and a second from the nucleocapsid fraction (25,000 cpm) were added to lanes A and B, respectively, and subjected to electrophoresis in an 8% acrylamide / MBA gel (77 : 1 ratio). Radioactive actin [^{14}C] was added to lane C as a marker protein.

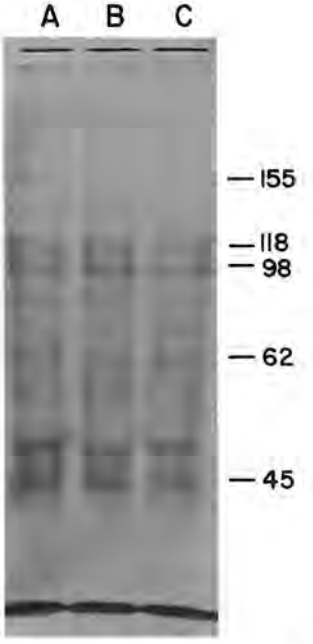

FIGURE 8. Nonidet solubilization of virions. Three aliquots (40 μl) of an [^{35}S] methionine labeled and gradient-purified virion fraction were solubilized by the addition of 1% NP40 and 1% DOC in phosphate buffer (pH 6.5) or modified Schwyzer's buffer (pH 9.0) with or without 0.1 *mM* EDTA. Insoluble materials was removed by sedimentation at 100,000 *g* for 60 min in a Beckman airfuge. Each of the three supernatants (containing 250,000 cpm in 100 μl) was incubated with 25 μl of high titer VZV antiserum; 10,000 cpm from each of the three immunoprecipitates (A, phosphate buffer; B, Schwyzer's buffer; and C, Schwyzer's buffer with EDTA) were subjected to SDS-PAGE in an 8% acrylamide / DATD gel.

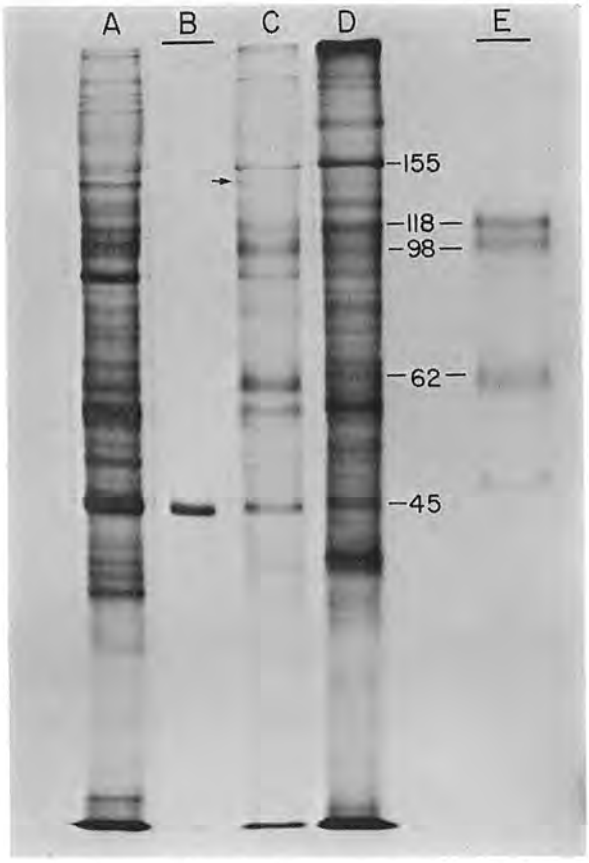

FIGURE 9. Structural and immunoprecipitable VZV-specific polypeptides. Detergent-solubilize [^{35}S] methionine and [^{3}H] fucose labeled VZV antigen stocks were prepared from infected cell cultures, and immunoprecipitation was carried out with high titer caviid VZV antiserum (5 μl) in the presence of Staphylococcal protein A-antibody adsorbent. Aliquots of the initial [^{35}S] methionine labeled antigen sample (40,000 cpm) and the corresponding immunoprecipitate (10,000 cpm), as well as the [^{3}H] fucose labeled immunoprecipitate (25,000 cpm), were added to lanes A, C, and E, respectively, of a 10% acrylamide / MBA gel (77 : 1 ratio). A [^{35}S] methionine labeled virion fraction collected from the middle band of a linear metrizamide gradient (40,000 cpm) was added to lane D; [^{14}C] actin was added to lane B. An immunoprecipitable polypeptide (lane C) with an estimated mol wt of 145K is designated by an arrow (→). In the column between lanes D and E the most prominent virion and immunoprecipitable polypeptides are enumerated according to their previously defined molecular weights.

detergents NP 40 and DOC were dissolved in three different buffer solutions because of previous data about variable solubilization of actin (Tilney, 1976). Analysis of the immunoprecipitates in three buffer systems were essentially the same; all three electrophoretic profiles demonstrated the three major glycoproteins (62K, 98K and 118K), the major capsid protein (155K) and a 45K polypeptide (Fig. 8).

5. *Comparison of structural and immunoprecipitable polypeptides.*

In an earlier report (Grose and Friedrichs, 1982), we described 16 polypeptides in the immunoprecipitation profile of highly specific caviid VZV antisera; these polypeptides ranged in mol wt from 32 to $\gg$200K. In this study, we subjected similarly prepared immunoprecipitates to electrophoresis along side of aliquots of purified virions, in order to identify which immunogenic polypeptides comigrated with VZV-specific structural proteins (Fig. 9). Counterparts of the most prominent components in the immunoprecipitates—the nonglycosylated 155K protein and the three major glycoproteins (62K, 98K and 118K)—were easily identified among the more numerous structural polypeptides. The glycoproteins, however, were less conspicuous when fractionated in polyacrylamide gels containing the higher ratio (77: 1) of monomer to crosslinker. Actin was easily distinguished in both the virion fraction (as previously observed in Figs. 5–7) and the immunoprecipitates. Of the 10 immunogenic polypeptides $\geq$62K, only a 145K constituent was not present in the virion profile and presumed therefore to be nonstructural. Because of numerous closely migrating structural polypeptides below 62,000 daltons, we were unable to consistently distinguish other putative VZV-specified nonstructural polypeptides by their differential electrophoretic mobilities.

DISCUSSION

VZ virions have been successfully recovered from virus infected cells and culture medium, concentrated by precipitation with nonionic polymers, and isolated from cellular organelles and membranes by equilibrium sedimentation in linear metrizamide density gradients. The buoyant density of the enveloped virion in metrizamide was 1.156–7 g/cm^3; this value corresponded closely with an estimate of 1.153 g/cm^3 for the isodense position of channel catfish herpesvirus in the same density-gradient medium (Robin and Rodrique, 1978). However, both of the above buoyant densities differed sharply from a value of 1.12–1.14 g/cm^3 published in an earlier report describing the purification of herpes simplex virus (Blomberg et al., 1976). Since the band of infectivity in the latter report was rather wide, it may have represented an incomplete separation of enveloped virus from infected-cell membranes which sediment at a lower density in metrizamide ($<$1.14 g/cm^3) (Rickwood, 1978). We observed a distinct upper band ($\sim$1.12 g/cm^3) in our preliminary metrizamide centrifugations which represented a mixture of virus and cellular membranes; these membranes have been preliminarily characterized and manifested several of the proteins specific to the infected cell (unpublished data). A band of higher density ($\sim$1.18 g/cm^3) consisted of mainly naked particles; however, cell organelles, especially mitochondria, sedimented to a very similar isodense position (cf. Rickwood, 1978). Cosedimentation of enveloped virions in the latter band may also be a reflection of reversible protein-metrizamide interactions (Rickwood et al., 1974; Huttermann and Wendlberger-Schieweg, 1976) which would increase the density of the particles.

Although high numbers of intact enveloped VZ virions were present in the middle band of the metrizamide gradient, an unexpected discrepancy was the low infectivity titers. This result differs from that of Robin and Rodrique (1978), who recovered infectious channel catfish herpesvirions from metrizamide. However, a similar loss in infectivity was observed when attempts were made to purify measles

virus in metrizamide (electron micrographs were not presented); the author speculated that this effect was secondary to binding between metrizamide and the measles glycoprotein (Vanden Berghe, 1976). Other possible explanations include inhibition of virus-specified glycoprotein synthesis secondary to release of the sugar analog 2-deoxyglucose from the parent compound metrizamide or, alternatively, a direct inhibitory effect of metrizamide on certain galactosyl transferases (Wattiaux et al., 1978). When we sedimented VZV cell-free virus in KT-glycerol rather than metrizamide gradients, the enveloped virions adhered tenactiously to contaminating cellular membranes. Previously Bellini et al., (1979) had observed that measles virions aggregated with cellular material when applied directly to KT gradients. De Villiers (1979) working with a strongly cell associated bovid herpesvirus (*H. ovis*) also cited his lack of success with either KT or KT-glycerol density gradient media. (Metrizamide was not examined.) Instead, De Villiers added heparin ($\geq$150 units/ml) to all buffer solutions to inhibit re-aggregation of *H. ovis* to cellular membranes during ultracentrifugation in sucrose. We found, however, that an equal concentration of heparin destroyed all VZV infectivity (unpublished data).

In contrast with our experience, Shemer et al. (1980) were able to purify VZV by successive sedimentations in glycerol and KT-glycerol gradients. When they characterized the molecular composition of the virion, they found a total of 33 polypeptides ranging in mol wt from 16 to 244K after analysis of [^{35}S] methionine labeled VZV preparations in either 11 or 20% acrylamide gels. Five of the polypeptides incorporated [^{14}C] glucosamine; the three most easily visualized in their autoradiograms corresponded in mol wt to those described both in this paper and by Grose (1980) in VZV-infected cultured cells. Recently Shiraki et al. (1982) purified VZ virions by rate zonal centrifugation in sucrose followed by sedimentation to equilibrium in CsCl. They enumerated 32 [^{35}S] methionine labeled structural polypeptides which ranged in mol wt from 21.5 to 280K, among which were six species which also incorporated [^{14}C] glucosamine. The major capsid polypeptide was designated 145K and the six glycoproteins were assigned molecular weights of 115K, 80–100K, 64K, 59K, 55K and 45K (Shiraki et al., 1982; Shiraki and Takahashi, 1982).

Upon comparison of the fluorograms in this paper with those of Shemer et al. (1980) and Shiraki et al. (1982), it is readily apparent that the basic electrophoretic pattern is quite similar although there are some minor variations in designations of molecular weights for the prominent viral structural proteins and glycoproteins. Perhaps of most interest is the major capsid polypeptide. Shemer et al. (1980) designated this protein as 180K, which corresponded to the 174K polypeptide in our profile. As previously mentioned, we found polypeptide 174K to be present mainly in the enveloped virion while noticeably diminished to absent in the nucleocapsid. In our analyses in this paper and in an earlier publication (Grose et al., 1981a), the major capsid protein was a somewhat faster migrating polypeptide (155K), which corresponded to the 153K polypeptide in the classification by Shemer et al. (1980). A value of 155,000 was also assigned to this protein by Zweerink and Neff (1981) who fractionated gradient-purified VZV nucleocapsids in 10% acrylamide gels. Asano et al. (1979, 1980) and Shiraki et al. (1982), in their extensive analyses of both infected cell-specific and virus-specific polypeptides, have calculated the mol wt of the major capsid protein to be 145,000 daltons. A major capsid polypeptide around 150K has been a common feature of all herpes group viruses studied to date (Killington et al., 1977).

The cumulative molecular weights of the 30 designated virion polypeptides totals 2,240,000, an amount encodable by the VZV genome whose size is estimated to be 80×10^6 (Dumas et al., 1980; Ecker and Hyman, 1981). The total complement of viral gene products and their sequence of formation has not yet been

elucidated because of the asynchronous conditions of in vitro VZV infection following inoculation with infected cells. Studies with the prototype herpes simplex virus by one-dimensinal SDS-PAGE have demonstrated some 50 infected cell-specific proteins, which have been temporally segregated into a cascade beginning with immediate early proteins as prerequisites for the formation of early nonstructural (e.g., viral-induced enzymes) and late structural polypeptides (Roizman et al., 1975). In this paper we identify a 145K constituent of the immunoprecipitation profile of high titer VZV xenoantisera and also of all human post-zoster sera tested to date (Grose, 1983) which is not included among the virion polypeptides and presumably represents a nonstructural protein. On the basis of electrophoretic mobility, this protein is similar to HSV DNA polymerase which has a mol wt of approximately 150K and is also highly immunogenic (Powell and Purifoy, 1977). Thus, these delineations by ourselves and others of the electrophoretic profiles representing structural and nonstructural virally encoded polypeptides will facilitate further characterization and categorization of individual VZV-specific polypeptides present in wild and laboratory VZV strains, as well as in the live attenuated vaccine strain (Takahashi et al., 1975).

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REFERENCES

Asano, Y., Takahashi, M. 1979. Studies on the polypeptides of varicella-zoster (V-Z) virus. I. Detection of varicella-zoster virus polypeptides in infected cells. Biken J. 22: 81–89.

Asano, Y., Takahashi, M. 1980. Studies on the polypeptides of varicella-zoster (V-Z) virus. II. Synthesis of viral polypeptides in infected cells. Biken J. 23: 95–106.

Bellini, W. J., Trudgett, A., McFarlin, D. E. 1979. Purification of measles virus with preservation of infectivity and antigenicity. J. Gen. Virol. 43: 633–639.

Blomberg, J., Bjorck, E., Olofsson, S., Berg, G., Lycke, E. 1976. Purification of virions and nucleocapsids of herpes simplex virus by means of metrizamide and sodium metrizoate gradients. Arch. Virol. 50; 271–278.

Bonner, W. M., Laskey, R. A. 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 43: 83–88.

Brunell, P. A. 1967. Separation of infectious varicella-zoster virus from human embryonic lung fibroblasts. Virology 31: 732–734.

De Villiers, E. M. 1979. Purification of the JS-3 isolate of *Herpesvirus ovis* (bovid herpesvirus 4) and some properties of its DNA. J. Virol. 32: 705–709.

Dumas, A. M., Geelen, J.L.M.C., Maris, W., Van Der Noorda, J. 1980. Infectivity and molecular weight of varicella-zoster DNA. J. Gen. Virol. 47: 233–235.

Ecker, J. R., Hyman, R. W. 1982. Varicella zoster virus DNA exists as two isomers. Proc. Natl. Acad. Sci. USA 79: 156–160.

Edmond, B. J., Grose, C., Brunell, P. A. 1981. Varicella-zoster virus infection of diploid and chemically transformed guinea-pig embryo cells: Factors influencing virus replication. J. Gen. Virol. 54: 403–407.

Grose, C. 1980. The synthesis of glycoproteins in human melanoma cells infected with varicella-zoster virus. Virology 101: 1–9.

Grose, C. 1981. Immunization of inbred guinea pigs with varicella-zoster virus grown in a syngeneic transformed embryo cell line. J. Clin. Microbiol. 14: 229–231.

Grose, C. 1983. Zoster in children with cancer: Radioimmune precipitation profiles of pre- and post-illness sera. J. Infect. Dis. 147: 47–56.

Grose, C., Edmond, B. J., Friedrichs, W. E. 1981a. Immunogenic glycoproteins of laboratory and vaccine strains of varicella-zoster virus. Infect. Immun. 31: 1044–1053.

Grose, C., Friedrichs, W. E. 1982. Immunoprecipitable polypeptides specified by varicella-zoster virus. Virology 118: 86–95.

Grose, C., Friedrichs, W. E., Smith, K. O. 1981b. Cryopreservation of varicella-zoster virions without loss of structural integrity or infectivity. Intervirology 15: 154–160.

Grose, C., Perrotta, D. M., Brunell, P. A., Smith, G. C. 1979. Cell-free varicella-zoster virus in cultured human melanoma cells. J. Gen. Virol. 43: 15–27.

Huttermann, A., Wendlberger-Schieweg, G. 1976. Studies on the metrizamide-protein interactions. Biochim. Biophys. Acta 453: 176–184.

Kessler, S. W. 1975. Rapid isolation of antigens from cells with a Staphylococcal protein A-antibody adsorbent: Parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. 115: 1617–1624.

Killington, R. A., Yeo, L., Honess, R. W., Watson, D. H., Duncan, B. E., Halliburton, I. W., Mumford, J. 1977. Comparative analyses of the proteins and antigens of five herpesviruses. J. Gen. Virol. 37: 297–310.

Maizel, J. V. 1971. Polyacrylamide gel electrophoresis of viral proteins. p. 179–246. *In* Maramorosch K., Koprowski, H. [ed.] Methods Virol., Vol. 5, Academic Press, New York.

Miller, M. G., Allen, P. T., Dmochowski, L. 1973. Quantitative studies of oncornaviruses in thin sections. J. Gen. Virol. 21: 57–68.

Peluso, W., Lamh, R. A., Choppin, P. W. 1971. Polypeptide synthesis in simian virus 5-infected cells. J. Virol. 23: 177–187.

Powell, K. L., Purifoy, D.J.M. 1977. Nonstructural proteins of herpes simplex virus. I. Purification of the induced DNA polymerase. J. Virol. 24: 618–626.

Rice, R. H., Means, G. E. 1971. Radioactive labeling of proteins *in vitro*. J. Biol. Chem. 246: 831–832.

Rickwood, D. 1978. Isopycnic centrifugation in nonionic media. p. 219–250. *In* Birnie, G. D., Rickwood, D. Centrifugal Separations in Molecular and Cell Biology. Butterworths, Boston.

Rickwood, D. 1979. A Gradient Medium for Centrifugation Studies, p. 11. *In* Rickwood, D. [ed.] Metrizamide. Nyegaard & Co., Oslo.

Rickwood, D., Hell, A., Birnie, G. D., Gilhuus-Moe, C. C. 1974. Reversible interaction of metrizamide with protein. Biochim. Biophys. Acta 342: 367–371.

Robin, J., Rodrique, A. 1978. Purification of channel catfish virus, a fish herpesvirus. Can. J. Microbiol. 24: 1335–1338.

Roizman, B., Kozak, M., Honess, R. W., Hayward, G. 1975. Regulation of virus macromolecular synthesis; evidence for multilevel regulation of herpes simplex 1 RNA and protein synthesis. Cold Spring Harbor Symp. Quant. Biol. 39: 687–701.

Server, P. 1980. A metrizamide-impermeable capsid in the DNA packaging pathway of bacteriophage T7. J. Mol. Biol. 138: 65–91.

Shapiro, A. L., Vinuela, E., Maizel, J. V. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28: 815–820.

Shemer, Y., Leventon-Kriss, A., Sarov, I. 1980. Isolation and polypeptide characterization of varicella-zoster virus. Virology 106: 133–140.

Shiraki K., Okuno, T., Yamanishi, K., Takahashi, M. 1982. Polypeptides of varicella-zoster virus (VZV) and immunological relationship of VZV and herpes simplex virus (HSV). J. Gen. Virol. 61: 255–269.

Shiraki, K., Takahashi, M. 1982. Virus particles and glycoproteins excreted from cultured cells infected with varicella-zoster virus (VZV). J. Gen. Virol. 61: 271–275.

Takahashi, M., Okuno, Y., Otsuka, T., Osame, J., Takamizawa, A., Sasada, T., Kubo, T. 1975. Development of a live attenuated varicella vaccine. Biken J. 18: 25–33.

Tilney, L. G. 1979. Nonfilamentous aggregates of actin and their association with membranes. p. 513–528. *In* Goldman, R., Pollard T., Rosenbaum, J. [ed.] Cell Motility, Book B. Cold Spring Harbor Laboratory, New York.

Vanden Berghe, D.A.R. 1976. The influence of a tri-iodinated benzamido-derivative of glucose on measles virus. Arch. Int. Physiol. Biochim. 84: 415–416.

Wattiaux, R., Wattiaux-De Coninck, S., Ronveaux-Dupal, M.-F., Dubois, F. 1978. Isolation of rat liver lysosomes by isopycnic centrifugation in a metrizamide gradient. J. Cell Biol. 78: 349–368.

Weiss, S. A., Smith, G. C., Kalter, S. S., Vaughn, J. L., Dougherty, E. 1981. Improved replication of *Autographa californica* nuclear polyhedrosis virus in roller bottles: Characterization of the

progency virus. Intervirology 15: 213–222.
Weller, T. H., Witton, H. M., Bell, E. J. 1958. The etiologic agents of varicella and herpes zoster. Isolation, propagation, and cultural characteristics *in vitro*. J. Exp. Med. 108: 843–868.
Zweerink, H. J., Neff, B. J. 1981. Immune response after exposure to varicella-zoster virus: Characterization of virus-specific antibodies and their corresponding antigens. Infect. Immun. 31: 436–444.