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Cytomegalovirus Proteins

I. Polypeptides of Virions and Dense Bodies

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Cytomegalovirus virions and dense bodies were purified by sucrose velocity and equilibrium centrifugation from the medium of fibroblasts infected with the strain AD169. The final virus preparations were purified more than 228-fold with respect to cellular proteins as determined by double-isotopic labeling and at least 1,600-fold on the basis of changes in the ratio of total protein to virus particles. The protein content of purified particles approximated that found for purified preparations of other herpesviruses. Twenty polypeptides ranging from 22,000 to >230,000 molecular weight were detected in purified virus preparations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Polypeptides of virions and dense bodies were allocated on the basis of analyses of preparations containing differing percentages of virions and dense bodies. Six polypeptides were represented predominantly or exclusively in virions, and four polypeptides were represented predominantly or exclusively in dense bodies, whereas the remainder appeared to be shared by both types of particles. Four polypeptides were glycosylated, and at least three of these appeared to be shared by both particle types. The protein composition of cytomegalovirus differs profoundly from that of herpes simplex virus.

Cytomegalovirus (CMV) infection is common throughout the world. Twenty to 90% of adults possess complement-fixing antibody varying with the population under study (15). The virus contributes to fetal and infant morbidity (26), is an important cause of heterophil-negative mononucleosis (14), and is a common pathogen in subjects with impaired immune defenses (7, 11). Although at least three antigenic subtypes have been recognized by cross-neutralization studies (25), there is little biochemical information on virus-specific proteins. Knowledge of protein composition and antigenic activity of CMV particles is a prerequisite for studies on the synthesis of CMV proteins in the infected cell and the role of different CMV subtypes in human diseases. In addition to the virions, the cytoplasm of CMV-infected cells also contains numerous membrane-bounded, electron-dense inclusions or dense bodies (1). In this paper, information is presented concerning the purification of virions and dense bodies from the medium of infected cells as well as on the molecular weights and glycoprotein nature of the constituent polypeptides.

MATERIALS AND METHODS

Cell cultures. Fetal tonsil fibroblasts (FTE strain) were kindly provided by R. Alexander and B. Wentworth. The cells were grown in Eagle minimum essential medium (MEM) supplemented with 10% fetal calf serum.

Virus strains. CMV strain AD169, passage 80 (21), was kindly donated by Thomas Weller. The virus was propagated in FTE cells, using as inoculum infected cells removed from glass by trypsin.

Infection of cells. FTE cells in four roller-drum bottles (each with a surface area of 690 cm^2) were infected with cells and medium from two 32-oz (900 ml) bottles of FTE cells with advanced virus cytopathic effects (approximately 2×10^8 focus-forming units). The virus inoculum was allowed to adsorb for 2 h and then was replaced with MEM containing 2% fetal calf serum (MEM-FCa2).

Microfocus assay of infectious virus. The previously described assay of infectious virus (6) was modified by the use of agarose in the overlay. Samples (0.05 ml) of 10-fold dilutions of virus in MEM-FCa2 were added to drained FTE monolayers in wells of a cell culture tray (FB-24-TC, Linbro Co.). After 2 h, 1.5 ml of an overlay containing MEM-FCa2 and 0.4% agarose (Bausch and Lomb) was added, and the plates were incubated at 37 C for 7 to

10 days at which time they were stained by the Giemsa technique. The foci of enlarged cells with intranuclear inclusions were counted at ×40 and ×100 magnifications. Virus titer was expressed in focus-forming units. The number of foci was linearly related to virus dose.

Radiolabeling of virus. Infected cells were incubated at 36 C for 24 to 48 h after inoculation until they became uniformly refractile. The medium was then replaced with MEM-FCa2 containing either 20% of the normal concentration of amino acids or the full complement of amino acids recommended by Eagle (3). In each case, [^{14}C]valine (280 mCi/mmol), [^{14}C]isoleucine (348 mCi/mmol), and [^{14}C]leucine (342 mCi/mmol), purchased from Amersham/Searle, were added to a final concentration of 0.1 μCi/ml. The cells were then incubated an additional 72 to 96 h before the supernatant medium was harvested.

Purification of virions and dense bodies. Preliminary experience showed that on day 5 after infection 75% of the infectious virus was located in the cytoplasmic fraction (prepared by hypotonic lysis of infected cells and homogenization in a glass tissue grinder), with only 3% in the nuclear fraction of infected cells and 22% in the supernatant medium. Attempts to purify virus from the cytoplasmic fraction were not successful. Large amounts of cell debris and dense bodies remained in the fractions comprising the infectious virus peak after sedimentation on sucrose or dextran 10 gradients. In the experiment to be described, the supernatant medium was used as starting material.

The supernatant medium was centrifuged at 5,000 rpm for 20 min in an SS-34 rotor of a Sorvall RC-2B centrifuge at 4 C to remove cell debris and then at 28,000 rpm for 60 min in a type 30 rotor of a Beckman model L ultracentrifuge at 4 C to pellet the virus. Virus pellets were suspended in a 0.5-ml total volume of 0.1 M Tris-hydrochloride (pH 7.4)-0.001 M EDTA buffer (0.1 M TE) and subjected to sonic oscillation with a probe (Sonifier cell disruptor, Ultrasonics Systems) for 5 s. 0.1 M TE was used in all subsequent steps. The virus suspension was layered on a 4.5-ml linear gradient of 10 to 30% (wt/wt) sucrose with a 0.2 ml cushion of 60% sucrose and centrifuged at 18,000 rpm for 30 min in an SW50 rotor of a Beckman model L ultracentrifuge. Two opalescent bands were visible after sedimentation of the supernatant of infected cells maintained in amino acid-deficient medium: one at 24% sucrose (A) and a second at 21% sucrose (B). When virus was propagated in cells maintained in complete Eagle medium, only one band was found at 21% sucrose (B). The bands were collected from the side of the tube and adjusted to 45% sucrose. A discontinuous sucrose gradient was then formed by layering the sample (2.0 ml in 45% sucrose) upon a 0.5-ml cushion of 60% sucrose and overlaying with 1.0 ml of 40% sucrose and 1.0 ml of 30% sucrose. This gradient was centrifuged at 25,000 rpm for 18 h in an SW50 rotor at 4 C. Band A resolved on the equilibrium gradient into three bands: AC in 60/45% sucrose interphase, AD in 45/40% interphase, and AE in 40/30% interphase. Band B resolved either into one band, BD, corresponding to band AD or into two bands, BD and BE, at sucrose concentrations corresponding to bands AD and AE.

Each band was collected and, after twofold dilution, was pelleted at 25,000 rpm for 60 min in an SW50 rotor. The pellets were suspended in 0.05 to 0.1 ml of 0.1 M TE to obtain a protein concentration of approximately 1 mg/ml, as determined by the method of Lowry et al. (17), and stored at −70 C.

Polyacrylamide gel electrophoresis. The apparatus used for polyacrylamide gel electrophoresis was designed according to Reid and Bieleski (20), using a discontinuous buffer system (2, 19), modified by the inclusion of sodium dodecyl sulfate (16). Up to 10 samples were run simultaneously on 10-cm long 7.5 or 9.5% polyacrylamide gel slabs. Samples containing approximately 0.025 mg of protein in 0.025 ml were solubilized by the addition of an equal volume of Tris buffer containing 3% sodium dodecyl sulfate and 5% mercaptoethanol with boiling for 2 min. A constant current of 10 mA was used in the polyacrylamide gel electrophoresis until the marker bromophenol blue dye had migrated to the end of the slab (ca. 4 h). Gels were fixed by immersion in 50% trichloroacetic acid for 30 min and then stained in 50% trichloroacetic acid with 0.2% Coomassie brilliant blue for 30 min. The gels were destained in a solution of 10% methanol and 10% acetic acid in water. The gel was dried onto filter paper and then exposed to X-ray film for 7 to 10 days (4). The absorbance measurement of the autoradiographic image was made in a Gilford recording spectrophotometer with a scanning attachment. The contribution of individual polypeptides to total protein content was determined by computer-aided planimetric analysis of absorbance tracings as described previously (12). Glycoproteins were visualized by staining with periodic acid fuchsin-sulfate (18) modified by fixation with trichloroacetic acid (27). Markers of known molecular weight, bovine serum albumin (fraction V, Miles Laboratories), ovalbumin (Sigma Chemical Co.), α-chymotrypsinogen (Sigma Chemical Co.), and radiolabeled herpes simplex virus type 1 virion polypeptides (9, 24), were run simultaneously with the unknown samples. Molecular weights of CMV proteins were determined by graphic interpolation with respect to the above-mentioned proteins.

Electron microscopy. Carbon-stabilized Formvar films on 300- or 400-mesh copper grids were floated on 5-μl drops of samples placed on Parafilm. The grids were then drained on a filter paper, rinsed three times by transfer to drops of bovine serum albumin in water (5 μg/μl), stained with 2% sodium or potasium phosphotungstate (pH 7.4), and examined at ×20,000 and ×30,000 magnifications in an AE1EM6B or a Phillips 300 electron microscope. The concentration of virions and dense bodies was estimated by mixing the virus preparation with an equal volume of a latex suspension containing 2.7 × 10^{10} particles per ml and determining the ratio of virions and latex particles in several microscope fields containing at least 100 virions. Virions were differentiated from dense bodies by the presence of a nucleocapsid structure, and only those particles in which a nucleocapsid was visible were designated as

virions. Dense bodies are membrane bounded and variable in diameter and, when they are penetrated by negative stain, appear to contain a fibrous matrix with no recognizable symmetry. This material is similar in this respect to that seen between the capsid and envelope of the herpes simplex virion, a structure that has been termed the "tegument" (21).

Assay of radioactivity. Liquid samples were dried onto 2.3-cm filter paper disks (Whatman), rinsed once in ice-cold 10% trichloroacetic acid, twice in 5% trichloroacetic acid at room temperature, and once in 95% ethanol, and finally dried for 1 h at 37 C. Radioactivity was measured in a Nuclear-Chicago liquid scintillation counter with a toluene-based scintillation fluid. Radioactivity in acrylamide gels was assayed by autoradiography as described above or the gel was sliced into 1-mm sections that were incubated in 0.4 ml of NCS solubilizer (Amersham/Searle) for 3 h at 60 C. After addition of 10 ml of Omnifluor solution (New England Nuclear Corp.) and incubation at 4 C overnight, radioactivity was assayed by liquid scintillation counting.

RESULTS

Purification of virions and dense bodies and criteria for purity of final products. Comparison of virus production in cells maintained in normal culture medium and amino acid-depleted medium showed that the concentration of amino acids was critical for the optimal release of virions into the medium of infected cells. Fractions containing a high proportion of virions (70 to 85%) and a low proportion of dense bodies (15 to 30%) were only obtained when infected cells were maintained in complete Eagle medium. Cells maintained in medium with 20% of the specified concentration of amino acids after infection yielded a fraction enriched for dense bodies (up to 70%) and another fraction rich in virions (60% virions) (Fig. 1). Complete Eagle medium was, therefore, used in all experiments except those dealing with the differential polypeptide composition of dense bodies and virions.

Data on the initial and final stages of a typical purification are summarized in Table 1. Although the recovery of infectivity in the final product was very low, ranging from 0.003 to 1.7%, the recovery of physical particles (virions and dense bodies) was much better (16%). The protein content per particle (virions and dense bodies) in the final product, calculated by dividing the protein content by the virus particle count in band BD, was 3×10^{-15} g. This represents a 1,600-fold purification compared with the starting material (4.8×10^{-12} g/particle) and approximates that obtained in purified preparations of herpes simplex virus (9).

Although this reduction in protein content per particle constitutes one criterion for the absence of large amounts of impurity, the degree of purification was also estimated by three additional methods. In the first, virus was purified from the medium of cells differentially labeled before and after infection. The ratio of counts in viral as opposed to host cell proteins increased 8.1-fold during the purification procedure (Table 1). In the second experiment, an artificial mixture of differentially labeled infected and uninfected cells was subjected to the purification procedure (Table 2). In the first part of this experiment, approximately eightfold purification was achieved by centrifugation of supernatant media of infected and uninfected cells at 28,000 rpm. Radiolabeled virus was pelleted from infected-cell medium, but little labeled material was recovered from uninfected-cell medium. In the second part, to stimulate substantial contamination with host cell materials, pelleted virus was mixed with a cytoplasmic extract of uninfected cells, and a simulated purification was carried out on the mixture by velocity and equilibrium centrifugation. This resulted in a further 30-fold purification. The overall degree of purification achieved, as indicated by this experiment, was therefore 228-fold. A comparison of electropherograms of partially purified (Fig. 2A) and purified (Fig. 2B) virus did not show significant ^{14}C-labeled host proteins in the final preparation.

In the third experiment the purity of the final product was estimated from a comparison of polypeptide profiles at each stage of purification (Fig. 3). The major polypeptides of virions and dense bodies (no. 1, 2, 3, 7, 8, and 9) could be seen in the polypeptide profile of the medium pellet from infected cells (slot 2, Fig. 3) but not from that of uninfected cells (slot 3).

The purification procedure clearly discriminated effectively against contaminating proteins of uninfected cells and components of the medium such as bovine serum albumin (most prominent polypeptide in slots 1 and 3). In addition all polypeptides in the final product appeared to have been newly synthesized since there was a complete concordance of total protein staining by Coomassie brilliant blue and the autoradiographic polypeptide profile (Fig. 4). This latter observation effectively eliminated significant contamination with serum proteins or with cellular proteins present before infection.

Polypeptide composition of CMV virions and dense bodies. We noted earlier that the medium from infected cells contains both dense bodies and virions. Although the majority of dense bodies can be separated from virions on the basis of their larger average size, a fraction

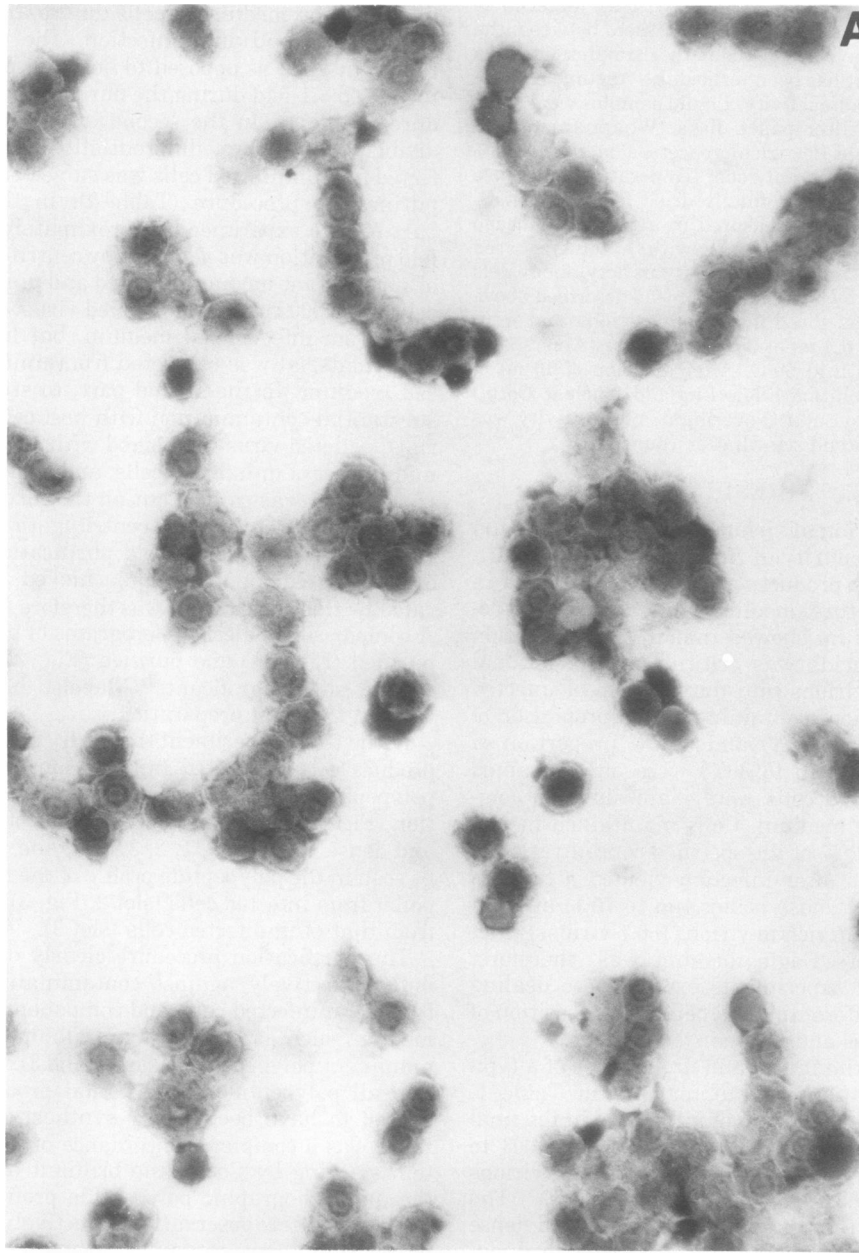

FIG. 1. *Electron micrographs of preparations of virions and dense bodies purified from infected-cell medium deficient in amino acid content (relative to that specified by Eagle[3]). (A) Fraction AC containing 60% virions and 40% dense bodies. (B) Fraction AD containing 30% virions and 70% dense bodies.*

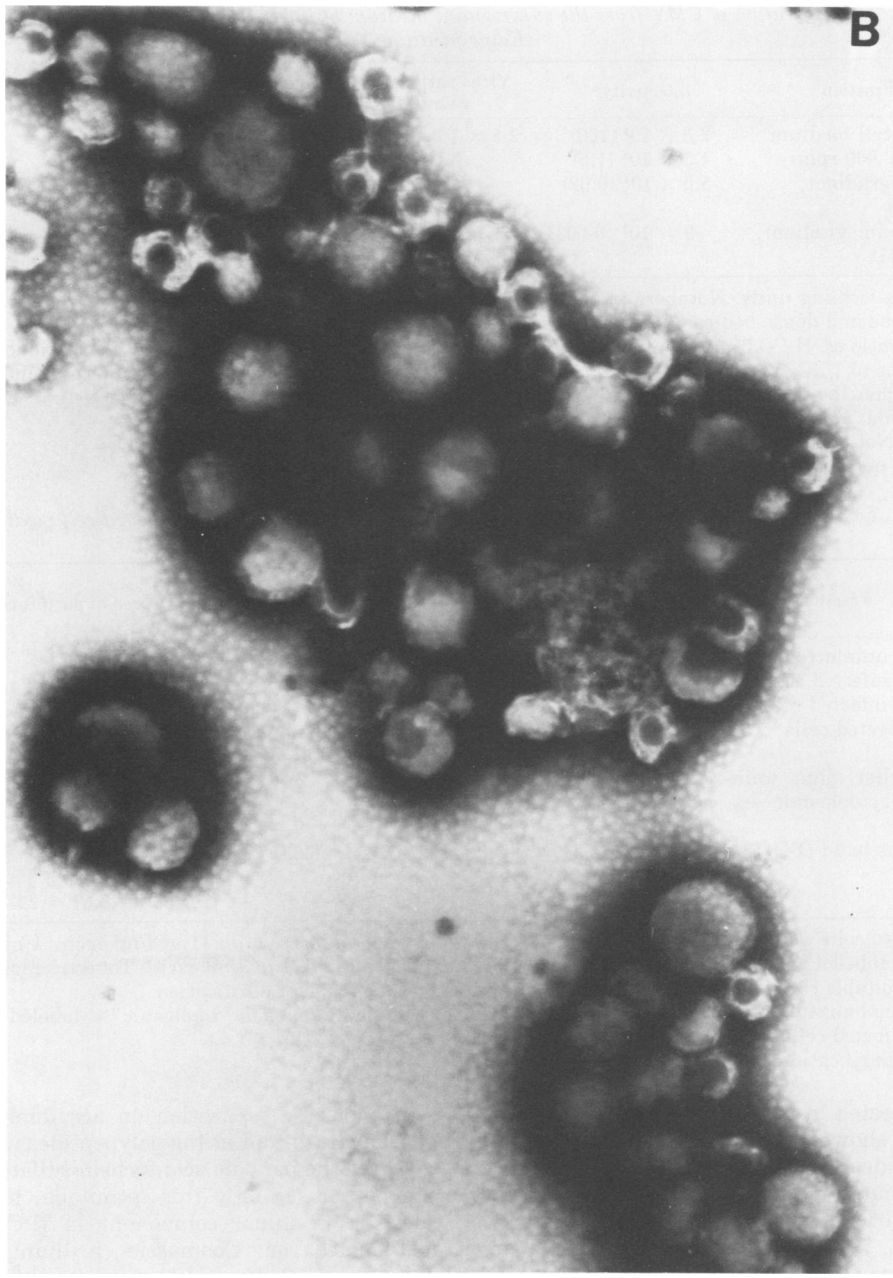

FIG. 1B

of dense bodies of size and density similar to virions is selected during the purification. This selected population of dense bodies is, therefore, difficult to separate from virions, and our best preparation of virions contained 10% contamination with dense bodies. To determine the polypeptide composition of virions, we therefore analyzed purified preparations of equivalent total protein content per physical particle that were composed of differing proportions of virions and dense bodies. An example of such a comparison is illustrated in Fig. 5, where absorbance tracings of labeled polypeptides separated from samples containing 30 or 60% CMV virions are shown, together with the polypeptides of purified herpes simplex type 1 virions. Nineteen polypeptides of molecular weights from >230,000 (no. 1) to 22,000 (no. 19)

TABLE 1. *Purification of CMV from the supernatant medium of infected cells maintained in complete Eagle medium*

Fraction	Infectivity[a]	Virus particle[b] count	Total protein content (g)	Total protein per particle (g)	$^3H/^{14}C$[c]
Infected-cell medium	2.8×10^8 (100)	2.5×10^{10} (100)	1.2×10^{-1}	4.8×10^{-12}	7.0
Pellet (20,000 rpm)	4.3×10^6 (1.5)	ND[d]	8.0×10^{-4}	—[e]	20.0
Velocity gradient, band B	5.6×10^4 (0.02)	ND	ND	—[e]	24.0
Equilibrium gradient, band BD	9×10^3 (0.003)	4×10^9 (16)	1.2×10^{-5}	3×10^{-15}	57.0

[a] Focus-forming units. Numbers in parentheses are percentages.
[b] Virions and dense bodies. Numbers in parentheses are percentages.
[c] The ratio of $^3H/^{14}C$ counts was determined in an experiment in which [^{14}C]leucine, [^{14}C]isoleucine, and [^{14}C]valine (0.1 μCi of each/ml) were added before infection, and the cells were washed, infected, and labeled with [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine (1 μCi of each/ml) at 24 to 96 h after infection. Degree of purification: 57/7 = 8.1-fold.
[d] ND, Not done.
[e] Data not available.

TABLE 2. *Two-step purification of virus from a mixture of [^{3}H]amino acid-labeled infected-cell medium and [^{14}C]amino acid-labeled material from uninfected cells*

Fraction	Total radioactivity		$^3H/^{14}C$	Degree of purification
	^3H	^{14}C		
Medium, uninfected cells[a]		1,969,999		
Medium, infected cells	8,850,000		4.49	
Pellet, uninfected cells		13,842		
Pellet, infected cells	477,686		34.47	7.68
Virus pellet plus uninfected cytoplasmic extract[b]	396,990	188,728	2.10	
Final virus band (BD)	13,584	218	62.31	29.67
				$7.68 \times 29.67 = 227.87$[c]

[a] Infected cells were labeled with [^{3}H]leucine, [^{3}H]isoleucine, and [^{3}H]valine (1 μCi/ml each). Uninfected cells were labeled with [^{14}C]leucine, [^{14}C]isoleucine, and [^{14}C]valine (0.1 μCi/ml each). Total trichloroacetic acid-precipitable counts of ^{3}H and ^{14}C were determined at each stage of purification.
[b] Artificial mixture of a pellet from infected ^{3}H-labeled cell medium and a cytoplasmic ^{14}C-labeled extract from uninfected cells.
[c] Total purification.

were detected in these preparations. From the tracings shown in Fig. 5 it can be seen that polypeptides 2, 8, 14, 16, 17, and 18 increase approximately twofold or more as the percentage of virions increases; conversely, polypeptides 5, 6, 7, and 9 increase with an increasing percentage of dense bodies. Results of planimetric analyses of the percent contribution of polypeptides in these preparations are presented in Table 3. On the basis of these and similar comparisons, polypeptides 2, 8, 14, 16, 17, and 18 appear to be represented predominantly or exclusively in virions and, conversely, polypeptides 5, 6, 7, and 9 appear to be represented predominantly in dense bodies; the remainder are apparently shared by both particle types.

Glycosylated virion polypeptides. A virus preparation rich in virions was subjected to electrophoretic separation on acrylamide gel. Carbohydrate-containing polypeptides were detected by the periodic acid fuchsin-sulfate staining method (18). By this technique, polypeptides 1A (a minor component in [^{14}C]amino acid-labeled or Coomassie brilliant blue-stained gels), 3, 10, and 11 were stained (Table 3). Polypeptides 3, 10, and 11 appeared to be shared by virions and dense bodies. Polypeptide 1A was not sufficiently prominent in amino acid-labeled or Coomassie brilliant blue-stained gels to permit its allocation.

DISCUSSION

Preparations of CMV virions free from host cell proteins are needed for biochemical studies of virion polypeptides as well as for immunological studies of virus envelope and nucleocapsid

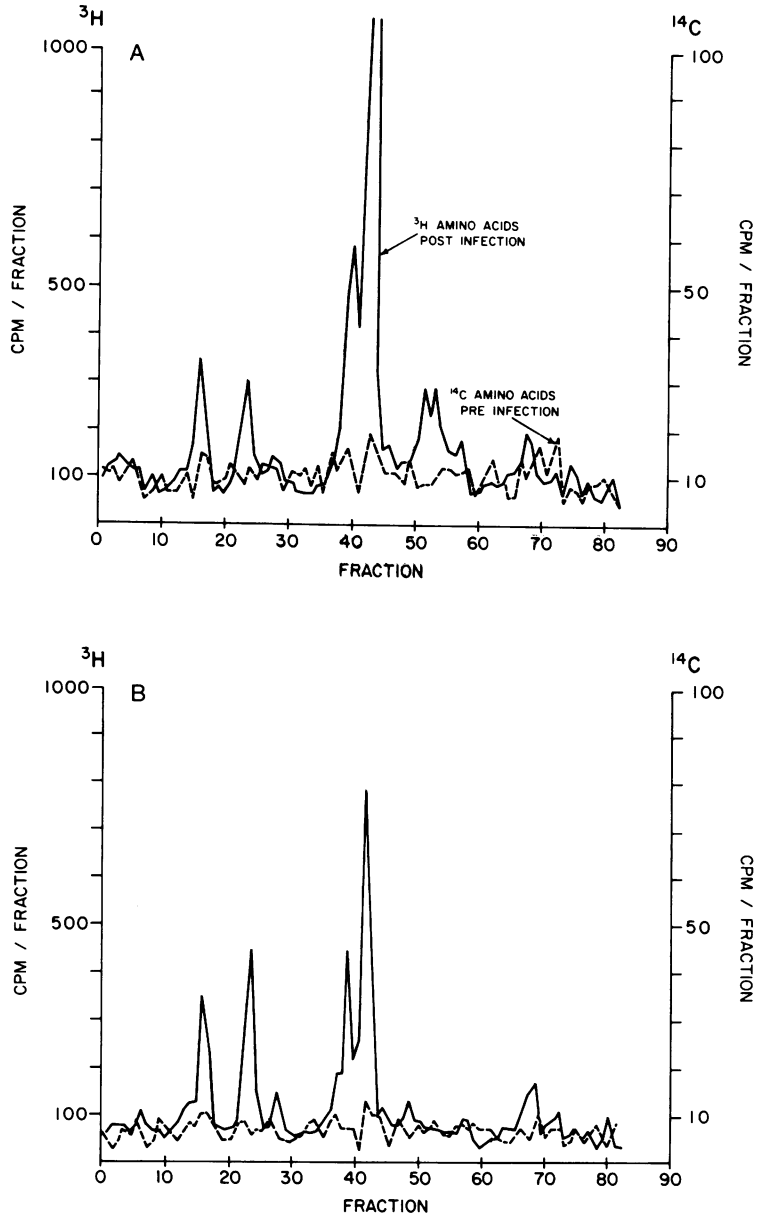

FIG. 2. *Electropherograms of partially purified (virus pellet [A]) and purified (fraction BD [B]) virus prepared from infected-cell medium of cells labeled with [^{14}C]amino acids before infection and [^{3}H]amino acids after infection. The gels were sliced into 1-mm sections and after treatment with NCS solubilizer were assayed by liquid scintillation spectroscopy.*

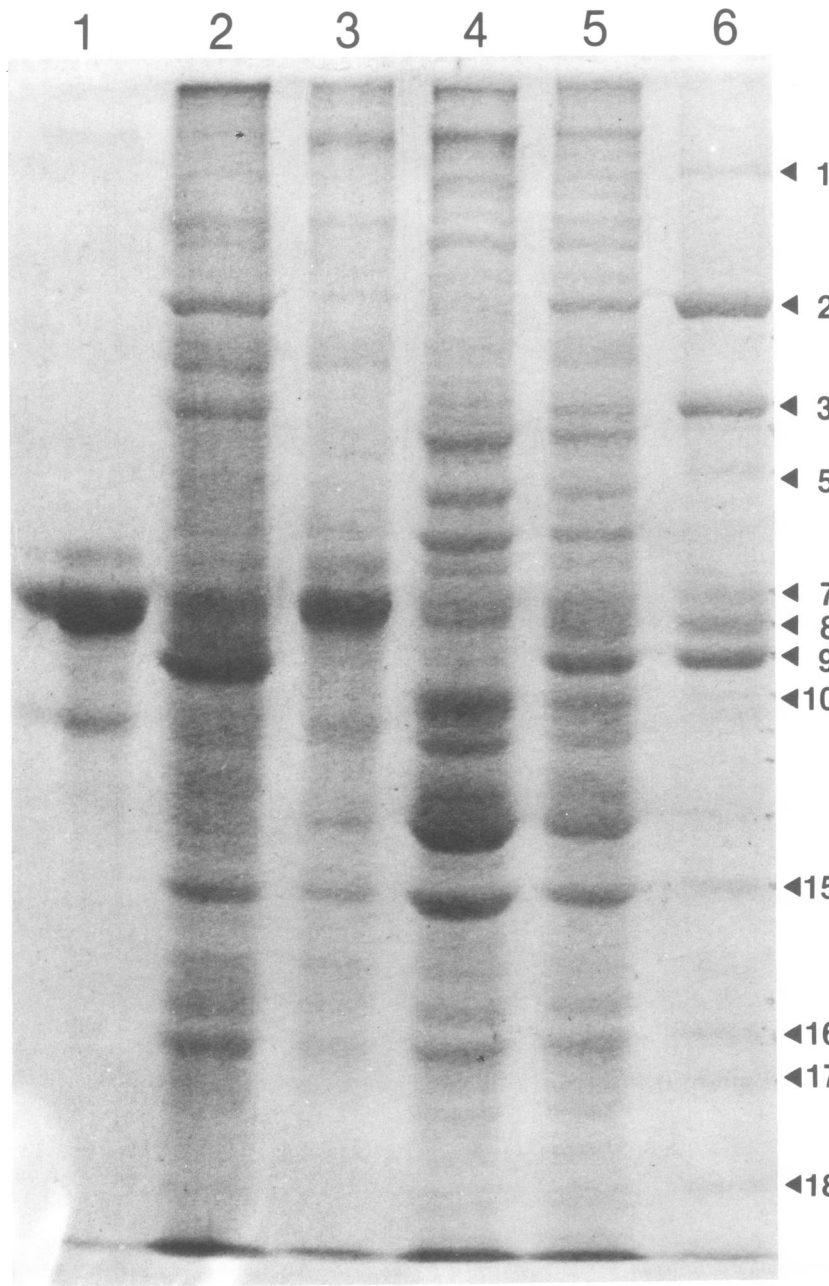

FIG. 3. *Photographs of Coomassie brilliant blue-stained acrylamide gel polypeptide profiles. (1) Maintenance medium (MEM-FCa2). (2) Pellet of infected-cell medium. (3) Pellet of uninfected-cell medium. (4) Cytoplasmic extract from uninfected cells. (5) Artificial mixture of the pellet of infected-cell medium with the cytoplasmic extract from uninfected cells. (6) Final virus preparation purified by velocity and equilibrium centrifugation (band BD).*

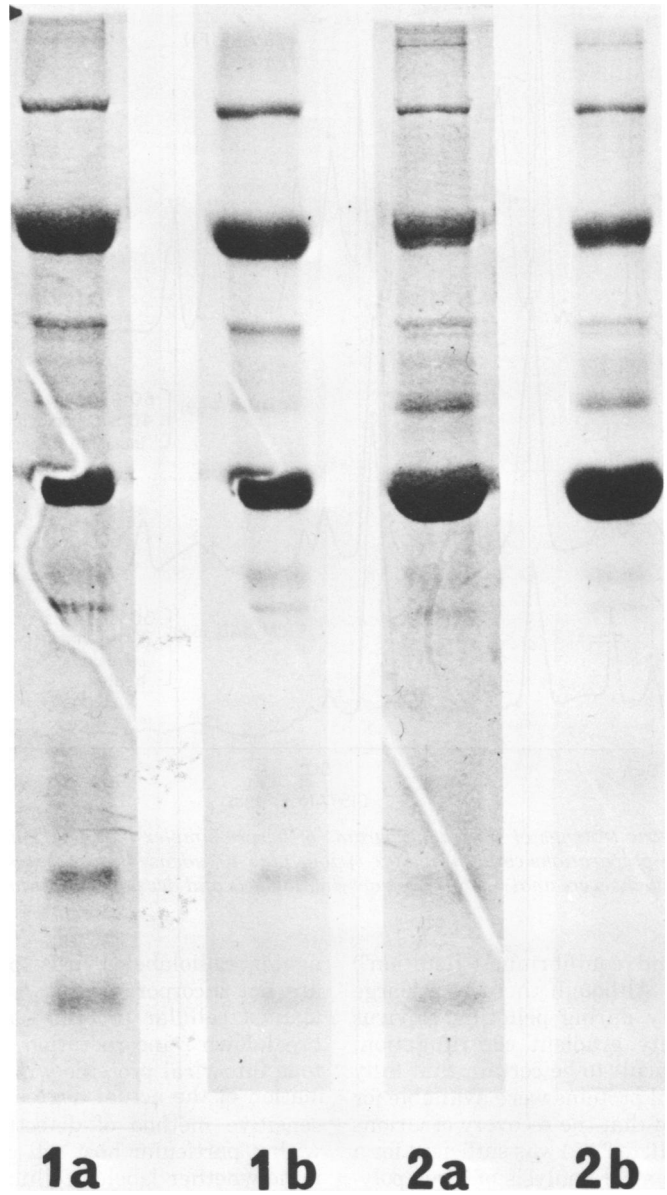

FIG. 4. *Comparison of Coomassie brilliant blue-stained polypeptide profiles (1a and 2a) with autoradiographic profiles (1b and 2b). Two virus preparations were used: that used in 1a and 1b contained 60% virions (fraction AC, Fig. 1A), and that used in 2a and 2b contained 30% virions (fraction AD, Fig. 1B).*

antigens. In this study, culture medium from infected cells was used as the starting material for virus purification. Other workers have concentrated CMV from infected-cell medium and subjected the virus to differential, sucrose, and CsCl gradient centrifugation (13, 23). In one study (13), virus preparations were shown to be free from host cell nucleic acids. In the other (23), virus preparations were found to be free from membranous materials and cellular debris by electron microscopy. The degree of contamination with soluble host cell proteins was not determined, and no quantitative data on the degree of purification or recovery were presented.

The purification procedure employed in the current study involves differential centrifugation, virus pelleting, and centrifugation of vi-

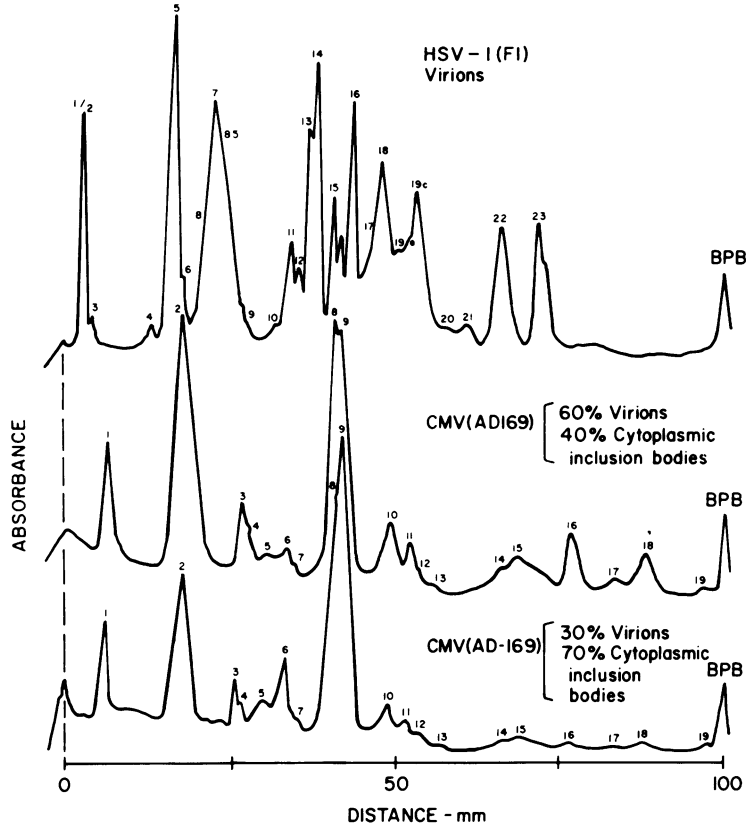

FIG. 5. *Densitometric tracings of electropherograms of herpes simplex type 1 virion polypeptides and of CMV polypeptides in preparations containing 60% virions plus 40% dense bodies and 30% virions plus 70% dense bodies. The proteins were analyzed on 7.5% acrylamide gels and developed by autoradiography of dried gels.*

rus on velocity and equilibrium ("flotation") sucrose gradients. Although there were large losses of infectivity during pelleting of virus and during velocity gradient centrifugation, which make it difficult to be certain that fully representative viral proteins were available for study, it is believed that the recovery of virions and dense bodies (10 to 20%) was sufficient for a meaningful biochemical analysis of virus polypeptides.

The extent of contamination of purified virus preparations with host proteins or components of the medium was studied by methods that are based on (i) the absence of isotopically labeled host cell proteins in the final preparation of virus and (ii) the reduction in protein content per virus particle. The first approach suggested an 8.1-fold purification when labeling was done in the same cells before and after infection and a 228-fold purification when infected and uninfected cells were labeled separately. As was pointed out by Spear and Roizman (24), the purification procedure used discriminates against radiolabeled virus-specific proteins that are not incorporated into virions, as well as against cellular proteins, and both this and breakdown reincorporation of preinfection isotope into viral proteins will lead to underestimation of the actual degree of purification. A sensitive method of detecting contamination with a particular host cell protein is to determine whether labeled cellular polypeptides comigrate electrophoretically with viral peptides. In the final preparation of virus, contaminating host cell material was either absent or was scattered diffusely throughout the electropherogram in a minute amount. Furthermore, the Coomassie brilliant blue-stained profile of the purified virus preparation corresponded precisely with the postinfection radioautograph, indicating little, if any, contamination. Estimates of changes in the protein content per particle during purification led to the conclusion that, in the final product, virus particles were purified 1,600-fold.

The purified preparations obtained in this

study contained a variable percentage of virions and dense bodies. Viral polypeptides were considered as mainly originating from virions or from dense bodies based upon their relative representation in preparations with an increasing or, in the second case, decreasing percentage of virions. Peptides shared by virions and dense bodies may vary by predominating in one or the other structure but also may be present in such small amounts that an accurate classification is not possible. In this regard we note the importance of separation of virions and dense bodies during the purification procedure and quantitation by electron microscopy of the degree of contamination of virions with dense bodies in the final product.

Although some CMV polypeptides do appear to be shared by virions and dense bodies, our present data clearly indicate that certain polypeptides are found predominantly or exclusively in the virion (polypeptides 2, 8, 14, 16, 17, and 18) and, conversely, that polypeptides 5, 6, 7, and 9 are predominantly or exclusively found in dense bodies. These conclusions are at variance with those of Sarov and Abady (23), who suggested that virions and dense bodies of the strain AD169 share all except one of their peptides with some variability in relative proportions. This conclusion requires, however, rigorous demonstration of the absence of cross-contamination, which was not presented by these workers. Our observations suggest that density and size separations in sucrose gradients are not sufficient to remove contamination with dense bodies similar in size to virions. It seems also relevant that we observed two polypeptides of very similar mobility, one apparently from virions (no. 8) and the other from dense bodies (no. 9), in the region of a single "shared" polypeptide described by Sarov and Abady (23). Moreover, their VP 2 (150,000 molecular weight) contributed 3.1% of the total protein in their virion preparation, whereas our no. 2 (150,000 molecular weight) contributed 15.8, 20.4, and 23.0% of the total protein in preparations containing 30, 60, and 70% virions and 70, 40, and 30% dense bodies, respectively. Differences in the polypeptide composition of virions and dense bodies that may depend on culture conditions also constitute a possible reason for the divergent results.

TABLE 3. *Molecular weight, glycosylation, and percent contribution of CMV polypeptides*

Peptide no.	Mol wt ($\times 10^{-3}$)	Glycosylation[a]	Percent contribution to preparation with:		Designation[b]
			(A) 60% virions	(B) 30% virions	
1	>230	−	7.3	6.6	Shared
1A	210	+	ND	ND	?
2	150	−	20.4	15.8	Virion
3	100	+	3.3	2.9	Shared
4	98	−	1.5	1.6	Shared
5	90	−	2.9	4.3	Dense body
6	85	−	3.1	5.7	Dense body
7	82	−			Dense body
8	70	−	12.1	6.5	Virion
9	68	−	7.6	20.7	Dense body
10	62	+	4.7	3.4	Shared
11	57	+	2.0	1.4	Shared
12	55	−	0.6	0.7	Shared
13	52	−	0.6	0.8	Shared
14	40	−	3.9	1.2	Virion
15	38	−	2.4	1.9	Shared
16	35	−	3.8	1.3	Virion
17	29	−	1.7	0.5	Virion
18	26	−	3.6	1.3	Virion
19	22	−	<0.5	<0.5	Shared

[a] Determined by periodic acid fuchsin-sulfate staining.

[b] Components increasing approximately twofold or more from the preparation B to A are designated as virion polypeptides, those increasing approximately twofold from A to B are designated as dense-body polypeptides, and those not changing significantly are designated as shared.

Despite these reservations, it should be emphasized that our own data indicate a substantial number of polypeptides common to virions and dense bodies and, notably, that these include glycosylated polypeptides. Independent evidence also suggests the existence of at least one antigenic determinant that is shared by the surfaces of virions and dense bodies (1). One attractive theory concerning the origin of dense bodies is that they are products of an aberrant assembly of virion structural proteins (23). Our data suggest that this aberrant assembly is not random with respect to virus-specific polypeptides. Preliminary experiments using, for dissociation of virus before gel electrophoresis, only 5% mercaptoethanol indicate that, among the polypeptides predominantly or uniquely found in the virion, polypeptide no. 2 is a major component of the virus capsid. All glycopeptides except possibly 1A, on the other hand, belong to the group of shared peptides. We therefore favor a more specific hypothesis on the origin of dense bodies and propose that they are products of aberrant assembly of the CMV envelope and tegument (21) polypeptides. The putative major CMV capsid polypeptide (no. 2) has a molecular weight of 150,000, which is close to the 155,000 molecular weight of the major polypeptide VP 5 of the capsid of herpes simplex virus (8, 12). Detailed comparison of CMV polypeptides with those of herpes simplex virus shows, however,

profound differences in the protein composition of these two herpesviruses.

The finding that the ratio of dense bodies to virions is influenced by the amino acid content of the culture medium suggests that the synthesis or assembly of viral proteins may be modulated by the concentration of small precursor molecules. Such modulation could be of importance in vivo as well as in vitro. In the course of human CMV infections, intranuclear inclusion formation is found primarily in renal, lung, and liver cells (10). In other cells such as leukocytes (7) and intestinal mucosal cells (5), infectious virus may be found only after extensive in vitro cultivation. These cells lack the characteristic cytopathic changes of CMV produced in vitro.

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