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Herpes Simplex Virus Products in Productive and Abortive Infection

II. Electron Microscopic and Immunological Evidence for Failure of Virus Envelopment as a Cause of Abortive Infection

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Herpes simplex virus strain MP*dk*$^-$ multiplies in HEp-2 cells, but not in dog kidney (DK) cells. Strain MP*dk*$^+$*sp*, a multistep mutant of MP*dk*$^-$, multiplies in both HEp-2 and DK cells. Stabilized lysates of productively infected cells yield three macromolecular aggregates of viral deoxyribonucleic acid and protein banding in CsCl gradients at densities of 1.285 g/cm^3 (α), 1.325 g/cm^3 (β), and 1.37 to 1.45 g/cm^3 (γ). Similar lysates from abortively infected cells yield only the β and γ bands. Electron microscopic examination revealed that (i) the α band contained enveloped nucleocapsids, whereas the β band contained naked nucleocapsids and particles tentatively identified as internal components of the nucleocapsids, and that (ii) the enveloped virions and reduplication of cellular membranes observed in thin sections of productively infected cells were absent from abortively infected cells. Studies of the surface antigens of infected cells in a cytolytic system described previously revealed that abortively infected cells contained approximately 10-fold less virus-induced surface antigen than did productively infected cells. From these and other data published previously, we concluded that infectious MP*dk*$^-$ virions are not made in DK cells because (i) functional viral products necessary for the envelopment of the nucleocapsid are not made, and (ii) capsid proteins and some nonstructural products specified by the virus malfunction.

The papers in this series concern the nature and characteristics of viral products made in productive and abortive infection of animal cells with herpes simplex virus. Specifically, MP*dk*$^-$ strain of herpes simplex virus multiplies in human (HEp-2) cells but not in dog kidney (DK) cells (1). The first paper in this series (20) dealt with the question of whether viral deoxyribonucleic acid (DNA) and protein previously (1, 2) shown to be made in abortively infected cells aggregate to form particles with properties of the virion and of subviral particles. In these experiments, lysates of productively infected HEp-2 cells and of abortively infected DK cells were centrifuged to equilibrium in CsCl density gradients. The results show the following. (i) CsCl was found to be deleterious in that it caused considerable disassembly of virions and loss of infectivity. (The virion may be stabilized and disassembly prevented by treating the cell lysate with formaldehyde.) (ii) Centrifugation of stabilized lysates of productively infected HEp-2 cells yielded three bands of viral material containing viral DNA and designated α, β, and γ, with buoyant densities of 1.285, 1.325, and 1.37 to 1.45 g/cm^3, respectively. (iii) Centrifugation of stabilized lysates of abortively infected DK cells yielded only β and γ bands; particles banding in the α position were missing. It was noted that untreated β particles formed in abortively infected cells were less stable than those made in HEp-2 cells and became disassembled more readily on centrifugation in CsCl solution.

The purpose of this paper is to report that MP*dk*$^-$ virus failed to produce infectious progeny in DK cells because the nucleocapsid was made but was not enveloped. The data indicate that (i) enveloped nucleocapsids are absent from lysates and from thin sections of abortively infected DK cells; (ii) abortively infected DK cells do not show the reduplication of membranes characteristic of productively infected cells (6, 18); (iii) abortively infected DK cells contain less virus-induced new surface antigen than do productively infected HEp-2 cells (8, 13).

MATERIALS AND METHODS

Solutions. The composition of the solutions and media used in this study were described in the preceding paper of this series (20).

Radiochemicals. Thymidine methyl-^{3}H (specific activity 15 c/mmole) was obtained from Schwarz Bio Research, Orangeburg, N.Y. Cells were labeled in Eagle's minimal essential medium (3).

Cells. The HEp-2 cell line was originally obtained from Microbiological Associates, Inc., Bethesda, Md. The continuous line of DK cells has been carried in our laboratory since 1963. The pertinent properties of these cell lines have been described (2, 11).

Virus. The pertinent properties of the macroplaque (MP*dk*$^-$) and microplaque (mP*dk*$^-$) strains of herpes simplex have been described elsewhere (5, 11, 12, 14). The viruses were assayed in HEp-2 cells under a liquid overlay and were scored in terms of plaque-forming units (PFU). Most of the studies on abortive infection of DK cells with herpes simplex were done with the MP*dk*$^-$ mutant. The mP*dk*$^-$ strain was used in the tests for altered immunological specificity of abortively infected cells. The MP*dk*$^+$*sp* strain is a multistep mutant of the MP*dk*$^-$ strain capable of multiplying in both DK and HEp-2 cells, but not nearly as well as does MP*dk*$^-$ virus in HEp-2 cells. Pertinent properties of the MP*dk*$^+$*sp* mutant are described elsewhere (11).

Preparation of labeled infected and uninfected cell lysates. The procedure was described in detail elsewhere (20).

Formaldehyde fixation. The procedure for stabilization of cell lysates with formaldehyde was the same as described previously (20).

Isopycnic centrifugation in CsCl solution. The procedures were the same as described previously (20). Briefly, all centrifugations were done in SW-39 rotors at 15 C and 100,000 × *g* for 44 hr. The density range, approximately 1.20 to 1.60 g/cm^3, was spanned by use of three tubes with overlapping density gradients. At the end of the centrifugation, the bottom of the tube was pierced. Five fractions (2 drops) were collected on the prism of a Zeiss refractometer and 18 fractions (5 drops) were collected into saline-citrate solution (SCS). The density of the CsCl (grade 99.9% pure; A. D. MacKay Inc., New York, N.Y.) was determined by reading the refractive index of the 2-drop fractions. Samples of 100 μliters were withdrawn from 5-drop fractions collected in 1 ml of SCS and were precipitated with 7.5% trichloroacetic acid on membrane filter discs (type HA; Millipore Corp., Bedford, Mass.). The discs were washed, dried, and immersed in toluene base scintillation fluid. Tritium disintegrations were counted in a Packard scintillation spectrometer. A portion of the fractions collected in SCS was dialyzed against Veronal buffer for assay of antigenic mass by complement fixation. Another portion of the SCS fraction was dialyzed against distilled water for subsequent examination by the negative staining technique.

Antibody. Serum 1, a rabbit convalescent serum used for complement-fixation tests, was the kind gift of Albert B. Sabin (University of Cincinnati, Cincinnati, Ohio). The serum was obtained from a rabbit which developed extensive skin lesions after intracutaneous injection of virus prepared in rabbit kidney cells. Serum 2 was prepared in a rabbit against boiled infected cell stroma. The preparation and properties of this serum were described previously (9, 16, 17). Briefly, 72 hr-infected HEp-2 cells were washed, suspended in distilled water, and incubated for 1 hr at 40 C. At that time, the suspension was acidified to *p*H 1 with HCl and was incubated for an additional 1 hr. The sediment obtained after two such extractions was washed three times with phosphate-buffered saline (PBS), resuspended in buffer, and sealed in glass ampoules. These were weighted and submerged in boiling water for 90 min. After determining that the preparation was free of residual infectivity, it was used for immunization of rabbits.

Before use, sera 1 and 2 were absorbed as follows: approximately 5-ml amounts of the sera were inactivated at 50 C for 30 min, then absorbed three times with a lysate obtained from 1 cc each of packed uninfected HEp-2 and DK cells and once with 0.5 cc of packed sheep red blood cells. The absorptions were done with constant shaking at 37 C; each absorption lasted 1 hr. The sera were then centrifuged at 49,000 × *g* for 2.5 hr. The supernatant fluids were collected and stored at −10 C.

Absorbed serum 1 was used to determine, by complement fixation (CF), the distribution of viral antigen in the CsCl density gradients. In CF tests, it reacted at a dilution of 1:160 with infected cell lysates and was used at a 1:40 dilution. At this dilution, serum 1 reacted with antigens remaining in the supernatant fluid after centrifugation (49,000 × *g* for 2 hr) and with pelleted, washed virus (A. B. Sabin, *personal communication*). The serum did not react with uninfected cell lysates. This and other rabbit convalescent sera reacted to a higher titer with naked nucleocapsids (band β) than with enveloped nucleocapsid (band α), and were used to localize rather than quantitate the antigen recovered from the various gradients. Thus, our findings concur with the conclusions of Watson and Wildy (24) that the antigenic determinants of naked and enveloped nucleocapsids differ. Absorbed serum 2 was used for the assay of surface antigens on infected cells. In neutralization tests, 10^{-3} ml of absorbed serum was necessary to reduce by 50% the titer of 600 PFU of virus in a 1-ml volume (16). In cytolytic tests for the assay of viral antigens on the surface of infected HEp-2 cells, 4.4×10^{-3} ml of adsorbed serum was necessary to injure 50% of 1,200 infected cells suspended in a 1-ml volume (16).

CF tests. The CF procedure was the same as previously described (20).

Electron microscopy of negatively stained preparations. The fractions collected following isopycnic centrifugation in CsCl density gradients were dialyzed against distilled water. A drop from each fraction was placed on the carbon substrate of a Formvar-coated grid. After 1 min, the excess fluid was blotted with filter paper, and a drop of 4% aqueous solution of sodium silicotungstate was added for 15 sec. The excess stain was then blotted, and the preparations were examined at 40 kv and 100,000 × magnification with

the aid of an electron microscope (Associated Electrical Industries Ltd., Harlow, Essex, England).

Electron microscopy of thin sections. Monolayer cultures were fixed for 30 min with 2.5% glutaraldehyde in Eagle's minimal essential medium (3) containing 1% calf serum, 24 hr after infection. The cells were scraped off the glass with a rubber policeman, washed in PBS, and fixed in 1% buffered osmium tetroxide and formaldehyde. They were then dehydrated in graded alcohols, permeated with propylene oxide, and embedded in Epon or in Araldite. Sections were cut with a Porter-Blum microtome equipped with a diamond knife, mounted on uncoated grids, and stained with uranyl acetate and lead citrate. Sections were examined at 50 kv and 10,000 to 30,000 × magnification.

RESULTS

Characteristics of macromolecular aggregates in stabilized lysates of productively and abortively infected cells. In the first paper of this series (20), it was shown that macromolecular aggregates containing viral DNA and antigen in lysates of HEp-2 cells productively infected with MP*dk*⁻ virus localize, on isopycnic centrifugation in CsCl solutions, in three bands designated as α, β, and γ. The macromolecular aggregates from stabilized lysates of DK cells abortively infected with MP*dk*⁻ virus localize in β and γ bands only; no viral aggregates were found at the density corresponding to the α band. The purpose of the experiments reported here was twofold. First, we wanted to determine whether lysates of DK cells productively infected with MP*dk*⁺*sp* mutant resemble those of productively infected HEp-2 cells or those of abortively infected DK cells. To this end, we compared the distribution of macromolecular aggregates containing viral DNA and antigen in lysates of HEp-2 cells infected with MP*dk*⁻ virus, DK cells infected with mP*dk*⁻ virus, and DK cells infected with MP*dk*⁺*sp* virus. The second objective was to estimate the relative amounts of α and β macromolecular aggregates in lysates from infected HEp-2 and DK cells. To this end, we measured the relative amounts of viral DNA, labeled with ^{3}H-thymidine, contained in the aggregates localizing in the α and β bands by summing the number of ^{3}H counts in each band of viral material.

The results of one series of experiments are summarized in Fig. 1. As reported previously (20), macromolecular aggregates, consisting of labeled DNA and CF antigen in stabilized lysates of HEp-2 cells infected with MP*dk*⁻ virus, localized on isopycnic centrifugation in bands designated as α, β, and γ. On the basis of bouyant density determinations, we previously showed that macromolecular aggregates in the α and β bands contained viral DNA, whereas the macromolecular aggregates in the γ band contained

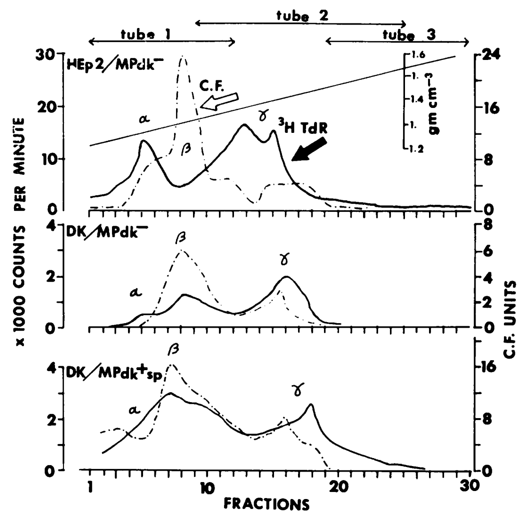

FIG. 1. *Distribution of macromolecular aggregates containing DNA labeled with ^{3}H-thymidine (TdR), and complement-fixing (CF) antigen on isopycnic centrifugation in CsCl solutions of formaldehyde-treated lysates of herpesvirus-infected cells. Determinations were made with HEp-2 cells productively infected with the MPdk⁻ strain, DK cells abortively infected with the MPdk⁻ strain, and DK cells productively infected with the MPdk⁺ sp strain. Each distribution is reconstructed from data obtained from three overlapping gradients. The density range covered by each gradient is shown by arrows labeled tube 1, tube 2, and tube 3. Symbols: solid line, ^{3}H-thymidine; dashed line, CF activity.*

both host (HEp-2) and viral DNA (20). On the basis of radioactivity measurements in this and other experiments, it was estimated that the α band contained approximately three times the amount of viral DNA contained in the β band. Macromolecular aggregates containing viral DNA and antigen in stabilized lysates of abortively infected DK cells banded in CsCl solutions at densities corresponding to those of β and γ bands only. Previously, it was shown that the small amount of labeled material banding at the density characteristic of the α band contained host (DK) DNA; viral DNA and CF antigen were absent (20). Moreover, in accord with this finding, material containing DK cell DNA in stabilized lysates of uninfected DK cells also banded at a density corresponding to that of the α band (20). The macromolecular aggregates in lysates of DK cells infected with MP*dk*⁺*sp* virus resembled those of HEp-2 cells infected with MP*dk*⁻ virus with respect to CF activity, but not with respect to distribution of aggregates containing viral DNA. Thus, aggregates containing viral DNA formed two bands. The first localized at a density of 1.415 g/cm^3, approxi-

mating the γ band obtained from lysates of DK cells infected with MP*dk*$^-$ virus. The second band peaked at a density of 1.325 (β region) but encompassed the region of both the α and β bands. We conclude that the aggregates containing viral DNA localizing in the β band exceeded those contained in the α band and that both α and β aggregates containing MP*dk*$^+$*sp* viral DNA are heterogeneous with respect to physical properties.

Electron microscopic studies of α, β and γ macromolecular aggregates banded in CsCl solutions. Two series of experiments were done. In one series, we examined negatively stained samples of α, β, and γ bands obtained from stabilized lysates of productively and abortively infected cells. About 500 particles in each band were counted and classified on the basis of their structure. Approximately 82% of the particles in band α from lysates of HEp-2 cells productively infected with MP*dk*$^-$ virus were enveloped nucleocapsids (25), 160 to 180 mμ in diameter (Fig. 2a, 2b); the remainder were naked nucleocapsids, 80 mμ in diameter (Fig. 2c). Band β consisted predominantly of naked nucleocapsids (60%); the remainder were particles consisting of a round body, 20 to 25 mμ in diameter impervious to negative stain, and surrounded by a beaded structure, 5 to 8 mμ in width (Fig. 2d, 2e). These particles were also seen in freshly prepared lysates of infected cells but not in stabilized lysates of uninfected cells, or in material from nonstabilized lysates of infected cells banding at the density corresponding to the β band. In accord with the distribution of viral DNA (Fig. 1), viral particles were 5 to 8 times more numerous on grids containing α material than on those containing material from the β band. Band γ yielded primarily debris identified as belonging to the host on the basis of comparisons with material from stabilized lysates of uninfected cells banding at the same density. In addition, band γ contained sodium silicotungstate-impervious particles, 20 to 25 mμ in diameter, similar to those observed in the β band but without the beaded structure. Nucleocapsids, enveloped or naked, were absent from the γ band. Enveloped nucleocapsids were not seen in fresh lysates of DK cells infected with MP*dk*$^-$ virus or in material from stabilized lysates of these cells at a density corresponding to the α band. The β and γ bands contained particles similar to those observed in corresponding bands obtained from stabilized lysates of infected HEp-2 cells. Enveloped nucleocapsids were present in lysates of DK cells infected with MP*dk*$^+$*sp* virus and in material banding at a density corresponding to that of the α band. The β and γ bands contained particles identical to those observed in corresponding bands obtained from lysates of MP*dk*$^-$ infected HEp-2 cells.

In the second series of experiments, we examined the α, β, and γ bands obtained after centrifugation of unstabilized lysates of HEp-2 and DK cells infected with MP*dk*$^-$ virus. The γ band obtained from unstabilized lysates of either abortively or productively infected cells lacked recognizable virus structures. The α and β obtained from lysates of infected HEp-2 cells showed that (i) band α contained enveloped nucleocapsids; the envelopes of these nucleocapsids were for the most part distorted in shape (Fig. 2f), but the nucleocapsids contained within the envelope appeared to be intact; (ii) band β contained numerous partially disrupted nucleocapsids. These nucleocapsids ranged from a small minority lacking a few capsomeres to numerous particles with only a few outer capsomeres still projecting from an inner structure impermeable to negative stain (Fig. 2g, 2h). In size and appearance, the structure seen in this band was similar to the beaded structure seen in the β band obtained from stabilized lysates. A similar structure, designated as the "core" of the virion, was described by Wildy et al. (25).

We conclude from the electron microscopic studies of negatively stained preparations that (i) band α contains predominantly enveloped nucleocapsids, and (ii) band β contains predominantly naked nucleocapsids and a small number of particles tentatively identified as being a structural component of the nucleocapsid. (iii) We have not identified the aggregates in the γ band containing viral DNA. At this time, we are not certain that the particle seen in the γ band obtained from stabilized lysates of infected cells is, in fact, a virion precursor. (iv) The observations made in this study confirm the earlier conclusion that centrifugation in CsCl results in disaggregation of herpes simplex virus (20). Qualitatively, the effects of CsCl appear to be far more pronounced on naked nucleocapsids than on enveloped nucleocapsids. (v) On the basis of the data presented in this and the preceding section, it seems clear that HEp-2 cells productively infected with MP*dk*$^-$ virus yield predominantly enveloped nucleocapsids. DK cells productively infected with MP*dk*$^+$*sp* virus yield an excess of naked nucleocapsids over enveloped ones. Lastly, DK cells abortively infected with the MP*dk*$^-$ mutant yield predominantly, if not exclusively, naked nucleocapsids. It is noteworthy that the relative amounts of enveloped virions are in good agreement with the yields of infectious virus. Infected HEp-2 cells yield, on the average, 200 PFU of MP*dk*$^-$ virus per cell; under optimal

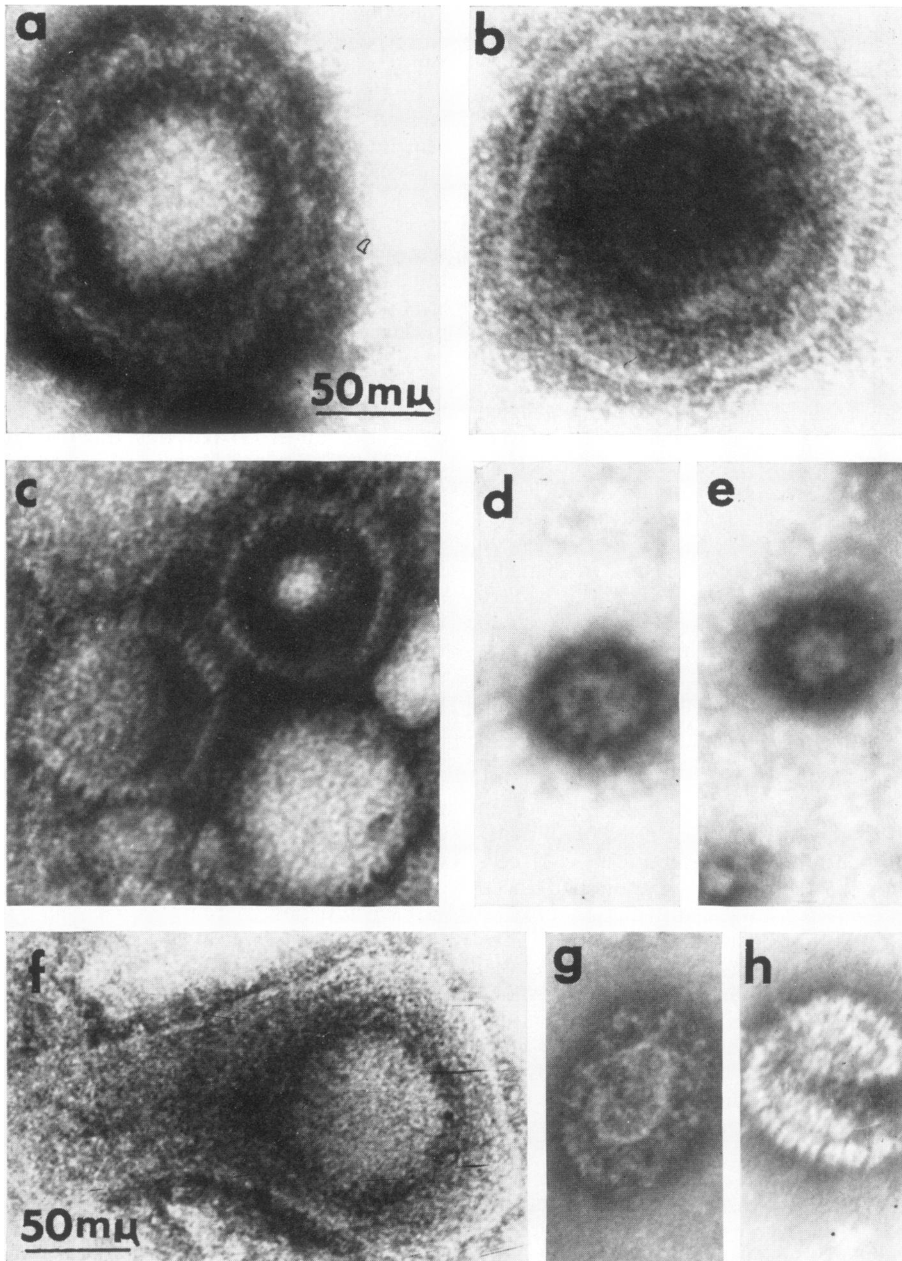

FIG. 2. *Electron photomicrographs of negatively stained preparations of herpes simplex virus. (a) Enveloped virion showing detail of nucleocapsid surface; (b) enveloped virion showing detail of the structure of the envelope, (c) unenveloped nucleocapsids; (d, e) subviral particle tentatively identified as a nucleoid surrounded by an inner capsid; (f) enveloped virion banded on isopycnic centrifugation in CsCl and showing distortion of the virion envelope; (g, h) partially disrupted nucleocapsids recovered after isopycnic centrifugation in CsCl. Magnification of b-e is the same as a. Magnification of g-h is the same as f.*

conditions, DK cells yield only 20 PFU of MP*dk*$^+$*sp* virus per cell (15). No infectious progeny can be detected in DK cells infected with MP*dk*$^-$ virus (1).

Electron microscopic studies on thin sections of HEp-2 cells infected with MPdk$^-$ *virus.* Electron microscopic studies on several preparations of 24 hr-infected HEp-2 cells revealed three pre-

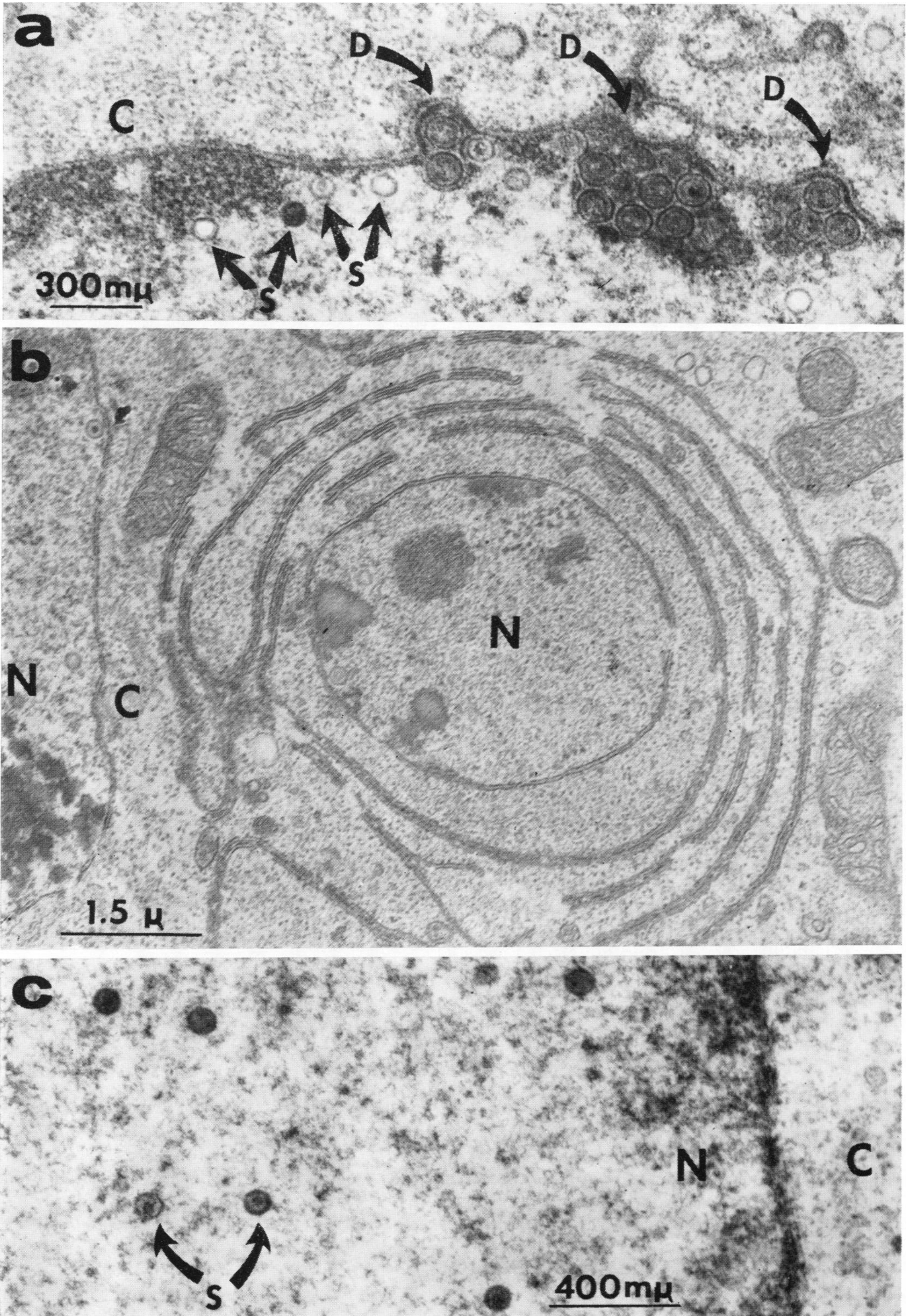

FIG. 3. *Electron photomicrographs of thin sections of cells 18 hr after infection with herpes simplex virus.* (*a*) *HEp-2 cell infected with MPdk⁻ strain showing the accumulation of viral particles with one electron opaque shell in the nucleus and of particles with two electron opaque shells between the layers of the nuclear membrane;* (*b*) *proliferation of membranes around the nucleus in a HEp-2 cell infected with the MPdk⁻ strain;* (*c*) *DK cell infected with MPdk⁻ strain showing the accumulation in the nucleus of particles with single electron opaque shell. Abbreviations: N, nucleus; C, cytoplasm; S, particle with single electron opaque shell; D, particle with two electron opaque shells.*

viously reported features (4, 6, 7, 18, 25) which are characteristic of productively infected cells: (i) particles 80 mμ in diameter consisting of a single electron opaque shell were present in the nucleus (Fig. 3a); (ii) particles 150 mμ in diameter consisting of two electron opaque shells were present between the inner and outer layers of the nuclear membrane (Fig. 3a) and in cytoplasmic vacuoles; (iii) reduplication of areas of the nuclear membranes occurred in all cells. Frequently, numerous shells of reduplicated membranes surrounded the nuclei of infected cells (Fig. 3b).

Electron microscopic examination of thin sections of DK cells abortively infected with MP*dk*$^-$ virus revealed intranuclear particles consisting of a single electron opaque shell (Fig. 3c). Reduplication of membranes and intracellular particles consisting of two electron-opaque shells were not observed in any of the numerous thin sections examined with the electron microscope. The results are in agreement with the conclusion reached in the preceding section that, in abortively infected DK cells, nucleocapsids are made but are not enveloped.

Alteration in immunological specificity of abortively infected cells. Previously, it was shown that 2 hr-infected HEp-2 cells injured by anti-HEp-2 cell antibody and guinea pig complement fail to produce plaques when seeded on monolayer cultures of HEp-2 cells (8, 13). Antibody alone or complement alone were ineffective (13). Similarly, rabbit antibody against infected HEp-2 cells and complement injured 2, 24, and 48 hr-infected cells. However, sera absorbed with uninfected cells injured 24 and 48 hr-infected cells only (13). These and other experiments (8, 9, 16) led to the conclusion that (i) 2 hr-infected cells cannot be differentiated from uninfected cells with respect to immunological specificity, and (ii) cells infected for 12 hr or longer acquire a new immunological specificity.

The purpose of the experiments described here was to determine whether DK cells abortively infected with MP*dk*$^-$ virus acquire a new immunological specificity in a manner analogous to that of productively infected HEp-2 cells. The cytolytic test (8) was redesigned as follows. HEp-2 cells infected with mP*dk*$^-$ virus (indicator cells) were mixed with varying amounts of DK cells infected with MP*dk*$^-$ virus (competing cells). The cells in the artificial mixtures were then exposed to 5 C′ HEp-2_{50} units of complement and to an amount of antibody sufficient to reduce the mP*dk*$^-$ plaque counts by approximately 50%. After incubation for 1 hr at 37 C in Veronal buffer, the infected cells were appropriately diluted in maintenance medium consisting of mixture 199, 1% calf serum, and 0.3% human pooled γ globulin and were plated on monolayer cultures of HEp-2 cells. If the infected DK cells acquired a new immunological specificity, we expected that the new antigen on the surface of these cells would compete with the antigen on the surface of the indicator cells for the limiting antibody and, hence, the HEp-2 cells infected with mP*dk*$^-$ virus would be spared. The following controls were included: (i) MP*dk*$^-$ infected DK cells, antibody, and complement; (ii) MP*dk*$^-$ infected DK cells, antibody, and heat-inactivated complement; (iii) mP*dk*$^-$ infected HEp-2 cells, antibody, and complement, but without abortively infected DK cells; (iv) mP*dk*$^-$ infected HEp-2 cells, antibody, and heat-inactivated complement; (v) mP*dk*$^-$ infected HEp-2 cells, antibody, complement, and various amounts of HEp-2 cells infected with MP*dk*$^-$ virus (competing cells known to contain new antigen on their surface); and (vi) uninfected DK cells. The plaques produced by the indicator cells infected with mP*dk*$^-$ virus were readily differentiated from the plaques produced by competing cells (12).

The results of one series of experiments with 18 hr-infected cells are summarized in Fig. 4. The capacity of cells to react with anti-infected cell antibody is deduced from the residual mP*dk*$^-$

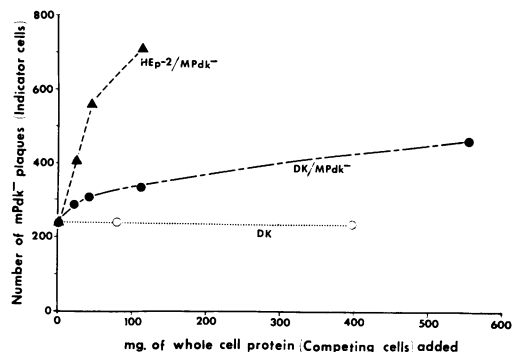

FIG. 4. *Comparison of the extent of alteration in cell determinant antigen of abortively and productively infected cells. HEp-2 cells infected with mPdk$^-$ virus (indicator cells) were mixed with anti-infected cell serum, complement, and various amounts of (i) HEp-2 cells productively infected with MPdk$^-$ virus (competing cells known to develop new antigens on their surface), (ii) DK cells abortively infected with MPdk$^-$ virus (competing cells being tested), or (iii) uninfected DK cells (negative control). The cells were diluted and plated on HEp-2 monolayer cultures after 1 hr of incubation at 37 C. Competing cells containing new surface determinant antigens bind the limited antibody, spare the indicator cells, increase the mPdk$^-$ plaque count. The ineffectiveness of uninfected HEp-2 cells in competing for the antibody was reported elsewhere (18).*

plaque count plotted as a function of total competing cell protein added to the reaction mixture. From the data presented in Fig. 4, it is estimated that HEp-2 cells infected with MP*dk*$^-$ virus contained at least 10 times more virus-induced antigen on their surface than did abortively infected DK cells.

DISCUSSION

The data presented in this paper indicate that DK cells abortively infected with MP*dk*$^-$ virus lack functional components for the envelopment of nucleocapsids. This conclusion is based on the following evidence: (i) enveloped nucleocapsids (α particles) were absent from lysates and from thin sections of abortively infected cells, and (ii) abortively infected DK cells contained less virus-induced surface antigen than did productively infected HEp-2 cells. Previously (8, 16), it has been shown that the antigen conferring immunological specificity on the enveloped herpesvirus nucleocapsid and the new antigenic determinant on the surface of productively infected cells are probably identical. The question that arises from these data is whether the structural components of the envelope are absent because they are not made or whether they are made but are defective and cannot be utilized.

Electron microscopy of thin sections of productively infected cells show that herpesviruses are enveloped by the nuclear membrane (4, 6, 7, 18). The envelope contains lipids, products specified by the host, and products specified by the virus. This conclusion is based on the following evidence. (i) The virus is sensitive to ether and chloroform (14, 25) and to lipases (Spring and Roizman, *unpublished data*). (ii) Enveloped nucleocapsids (but not naked nucleocapsids) are specifically agglutinated by antibody prepared against uninfected cells (24). (iii) The buoyant density of the virus is determined in part by the host species in which the virus is grown (19). (iv) The immunological specificity of the herpesvirion is genetically determined by the virus (11, 14). The hypothesis in best agreement with the available data is that the nucleocapsid is enveloped by a layer of the nuclear membrane modified by viral proteins inserted in it. With this interpretation of the structure of the envelope in mind, we tentatively conclude that, in abortively infected DK cells, envelopment fails because the structural components of the envelope are not synthesized. This conclusion is based on the following evidence. (i) Thin sections of abortively infected DK cells do not show the reduplication of nuclear membranes (Fig. 3b) characteristic of productively infected cells (18). (ii) There is an apparent correlation between the extent of envelopment and the presence of a characteristic species of polyribosomes in infected cells. Elsewhere it has been shown that, in HEp-2 cells productively infected with the MP*dk*$^-$ mutant, the sedimentation coefficient of the peak fraction was 270*S* (21–23). The polyribosomes extracted from abortively infected DK cells were predominantly 220*S*. Finally, HEp-2 and DK cells infected with the MP*dk*$^+$*sp* mutant contain nearly equal amounts of 270*S* and 220*S* polyribosomes (23). The relative amount of 270*S* polyribosomes recovered from productively and abortively infected cells shows an impressive correlation with the relative amount of enveloped nucleocapsids (α particles) recovered from formaldehyde-treated cell lysates (Fig. 1). We may speculate that the proteins made on the 270*S* polyribosomes are responsible for the reduplication of cellular membranes and for the modification of the membranes preceding envelopment. The conclusion that 270*S* polyribosomes and envelopment are causally related must be considered tentative, pending demonstration that viral envelope proteins are made on these polyribosomes.

Should subsequent studies on the identity of the proteins made on 270*S* polyribosomes confirm this conclusion, it would follow that infection of DK cells with MP*dk*$^-$ virus fails for two reasons. First, as suggested here, some products specified by the virus may not be made at all. The absence of envelopes may account for the lack of infectivity of the MP*dk*$^-$ progeny made in DK cells. Secondly, as shown elsewhere (10, 11, 20, 22), some proteins specified by the virus are made but they malfunction. The synthesis of malfunctioning proteins was apparent in complementation studies in DK cells doubly infected with the MP*dk*$^+$*sp* and MP*dk*$^-$ mutants (10, 11). Malfunctioning proteins may also account for the observations reported previously (20) that MP*dk*$^-$ nucleocapsids made in DK cells are less stable in CsCl than those made in HEp-2 cells, as well as for the slow and readily preventable inhibition of DK cell macromolecular synthesis (22).

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