Similarities and Differences in the Development of Laboratory Strains and Freshly Isolated Strains of Herpes Simplex Virus in HEp-2 Cells: Electron Microscopy

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HEp-2 cells infected with two laboratory strains (mP and MP) and two freshly isolated strains (F and G) of herpes simplex virus were fixed at intervals between 4 and 50 hr postinfection and sectioned, and were then examined with the electron microscope. These studies revealed the following. (i) All four strains caused identical segregation of nucleoli and aggregation of host chromosomes at the nuclear membrane. (ii) The development of MP virus could not be differentiated from that of its parent mP strain. (iii) There were quantitative differences between laboratory (mP) and freshly isolated (F) type 1 strains. Thus, cells infected with F contained numerous nuclear crystals of nucleocapsids and relatively few cytoplasmic structures containing enveloped nucleocapsids. Conversely, cells infected with mP or with MP virus contained numerous cytoplasmic structures with enveloped nucleocapsids and relatively few nuclear crystals of nucleocapsids. (iv) There were qualitative differences between type 2 strain (G) isolated from genital lesions and type 1 strains. Thus, cells infected with the G strain contain numerous filaments in nuclei and unenveloped and partially enveloped nucleocapsids in the cytoplasm. Of particular interest is the finding that cytoplasmic membranes in apposition to nucleocapsids were thickened and bent as if they were enveloping the particle. The significance of the qualitative differences in the development of the four strains is discussed.

The purpose of this paper is to report both quantitative and qualitative differences in the development of laboratory and naturally occurring strains of herpes simplex virus as observed with the aid of the electron microscope. The circumstances which led to this work are as follows. A recent survey of the multiplication of herpesviruses (28) revealed considerable differences in the results of electron microscopic studies of various phases of herpesvirus development. Thus, differences have been reported in the mode of entry of herpesviruses (6, 16, 23), in the site of envelopment of herpesviruses (8, 11, 22, 33, 35), and in the mode of egress of the virus from infected cells (22, 33). The differences in the results obtained in these studies are in some instances considerably greater than would be expected from mere variation in techniques or in interpretation of data. The question arose whether the reported differences in herpes simplex virus development might in fact all be correct and reflect variations in the development of the virus strains used in

these studies. This hypothesis is tenable since both laboratory and naturally occurring herpes simplex virus strains do vary with respect to many properties. Thus, with respect to naturally occurring viruses, it has been suspected for many years that genital (*herpes genitalis*) and nongenital (*herpes facialis*), etc., infections are caused by different variants of herpes simplex virus. These suspicions were confirmed by Scheneweiss (32) and by Plummer (26), who showed that freshly isolated viruses fall into two groups, 1 or 2, based on immunologic specificity, and also by Dowdle et al. (9) who showed that viruses isolated from nongenital infections fall largely into type 1, whereas genital lesions yield type 2 virus. The differences in immunological specificity between prototypes of types 1 and 2 virus have since been confirmed (10). In addition, prototypes of types 1 and 2 viruses have been shown to differ with respect to the guanine plus cytosine content of their deoxyribonucleic acid (DNA) (13), effect on social behavior of infected cells (10), site of infection and mode of

transmission in man (9), thermostability (11, 27), ability to grow in chick cells, and other biological properties (12, 27). Laboratory strains have been known for many years to differ with respect to their effects on cells (14, 15), buoyant density, thermostability, immunological specificity, and surface properties (10, 28-30). As would be expected, the variation among laboratory strains is greater than that found in nature (10), even though it should be pointed out that viruses with properties intermediate between types 1 and 2 have been isolated from herpetic lesions (Terni and Roizman, *J. Infec. Dis.*, *in press*).

This paper describes the development of four virus strains in HEP-2 cells. The variants were carefully chosen for the following properties. Strain mP was originally isolated from a recurrent eruption on the face. After isolation (15), the virus was passaged several times in chick embryo cells and approximately 300 times in FL and HEP-2 cells; it was plaque-purified six times. The virus causes cells to round up and form tight clumps. Strain F is a prototype of type 1 virus. It was isolated from a recurrent eruption of the face and passaged in HEP-2 cells three times. The F and mP strains cannot be differentiated with respect to effects on the social behavior of cells and immunological specificity. The G strain is a prototype of type 2 (10) and causes the cells to round up and form loose clumps, readily differentiated from those formed by cells infected with F or with mP strains. It was isolated and passaged in HEP-2 cells four times. The MP strain is a spontaneous mutant of the mP strain (15) and has been used in this laboratory for many years. The MP strain causes fusion of cells. It has been passaged some 200 times in HEP-2 cells and has been plaque-purified eight times. In neutralization tests, the immunological specificity of the MP strain is intermediate between that of the type 1 (F) and type 2 (G) prototypes (9, 10, 26).

MATERIALS AND METHODS

Virus strains. The method for the propagation of the virus strains, the procedure for assay, and properties of the various strains, etc. have been described elsewhere in detail (10, 31).

Cells. HEP-2 cells were originally obtained from Microbiological Associates, Bethesda, Md. Rapidly growing HEP-2 cells were exposed to virus at a multiplicity of 50 to 75 plaque-forming units per cell at 37 C for 2 hr. The cells were then washed, overlaid with maintenance medium consisting of mixture 199-1% calf serum, and incubated at 37 C.

Electron microscopy. Procedures for fixation and embedding were published elsewhere (33). The blocks were sectioned on a Porter-Blum MT-2 ultramicrotome and were doubly stained with uranyl acetate and lead citrate. Sections were examined on an AEI EM-

TABLE 1. *Grids and cells examined*

Virus strain	Material	Time of fixation postinfection		
		4 hr	18 hr	50 hr
mP	Grids	8	20	15
	Cells	16	58	32
MP	Grids	8	20	15
	Cells	15	62	35
F	Grids	6	20	15
	Cells	18	70	28
G	Grids	6	20	15
	Cells	11	50	41

6B electron microscope equipped with a liquid nitrogen-cooled specimen chamber.

Experimental design. Most of the studies were done on cells fixed at 4, 18, and 50 hr after infection with each of the virus strains. The numbers of cells and grids examined is shown in Table 1. In addition, we examined cells fixed at 6, 8, 16, and 24 hr after infection. The number of cells fixed and examined at these intervals varied from 10 to 80.

RESULTS

Nucleus of the infected cell. Virus strains used in this study caused very similar alterations in the nuclei of cells infected for 4 hr. Differences in nuclear structures and in the pattern of multiplication appeared between 8 and 18 hr postinfection.

(i) At 4 hr after infection, all organized, pre-existing structures appeared displaced to the nuclear membrane. The center of the nucleus became electron translucent (Fig. 1A, 1B, 2A). Topologically, the electron translucent mass corresponded to the intranuclear inclusion characteristic of herpesvirus-infected cells (7).

(ii) All herpes simplex virus strains used in this study caused disaggregation of nucleoli. The disaggregation was similar to that reported by Barski and Robineaux (2), Love et al. (18), and Bedoya et al. (3). At 4 hr after infection, the components of the nucleolus were still in close proximity to each other (Fig. 1C). At 8 hr the components were scattered near the nuclear membrane. The components of the nucleolus consisted of one or more grainy, spherical bodies with high affinity for metal salts (hence, electron opaque) and one larger, irregularly shaped body with a lower affinity for metal salts.

(iii) Two sets of structures appeared in the nuclei of cells infected with all strains. One set, present at 4 hr after infection, consisted of granular structures irregular in shape; it was usually

FIG. 1. Thin sections of HEp-2 cells infected with herpes simplex virus. (A) 18 hr after infection. Bar = 5 nm. (B) 4 hr after infection. Bar = 4 nm. (C) 4 hr after infection. Photomicrograph shows several components of disaggregated nucleolus. Bar = 6 nm. (D) 18 hr after infection. Photomicrograph shows several structures near the nuclear membrane. Bar = 5 nm. Letter abbreviations: c, cytoplasm; n, nucleus; i, aggregate of cell chromosomes; ii, remnant of nucleolus; iii, structure associated with naked nucleocapsids similar to that shown on Fig. 2D and 3B; iv, another structure associated with nucleocapsids similar to that shown in Fig. 2C and 3E.

FIG. 2
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FIG. 3. Thin sections of nuclear inclusions of HEP-2 cells infected for 18 hr with herpes simplex virus. (A) Densely staining structure near naked nucleocapsids. (B) Granules arranged into densely staining, lattice structure. (C) Naked nucleocapsids near loosely packed aggregates of granules. (D) Filaments arranged in parallel arrays. (E) Tightly packed aggregates of granules. (F) Naked nucleocapsids arranged in crystal array. Bars = 300 nm.

FIG. 2. Thin sections of HEP-2 cells at 18 hr after infection with herpes simplex virus showing nuclear inclusions associated with naked nucleocapsids. (A) Electron translucent mass fills the center of the nucleus of one cell. Section of the nucleus of another cell contains rosette structure associated with naked nucleocapsids. Bar = 5 nm. (B) Naked nucleocapsids within a rosette structure consisting of densely staining material in the nucleus of infected cell. Bar = 500 nm. (C) Aggregation of granules and naked nucleocapsids near the periphery of the nucleus. Bar = 300 nm. (D) Granular structure with densely staining halo seen in association with naked nucleocapsids. Bar = 400 nm. Letter abbreviations are given in Fig. 1.

located in apposition to the nuclear membrane (Fig. 1D). The second set was prominent between 8 and 24 hr after infection and was located at the periphery of the electron translucent mass filling the nucleus (Fig. 2A, B, C, D). Structures in this set were characteristically associated with naked nucleocapsids. We are not certain whether this set comprises three different or one pleomorphic structure. One structure consisted of electron opaque masses irregular in shape and arranged in the form of a rosette (Fig. 2A, 2B, 3A). Another structure consisted of dense aggregates of electron opaque grains 15 nm in diameter (Fig. 2D, 3E). Both structures were described by Watson et al. (41). The third structure consisted of an electron translucent patch containing naked nucleocapsids (Fig. 2C, 3C) and numerous granules 25 nm in diameter. The aggregates of large and small granules were frequently present in cells infected with the F and MP strains, whereas the electron opaque rosettes were often seen in cells infected with either the G or mP strains (Table 2).

(iv) Several nuclear structures were characteristic of cells infected with either the F or the G strains. In confirmation of reports by Murphy et al. (24) and by Couch and Nahmias, (5), we found filaments resembling microtubules and a lattice-like structure in nuclei of cells infected with the G strain (Fig. 3D). Filaments were frequently arranged in parallel bundles.

Nucleocapsids arranged in crystalline arrays were present in cells infected with all strains, but they were particularly prominent in cells infected with the F strain (Table 2). The nucleocapsid crystals were present in the nuclei of cells infected with the F strain as late as 50 hr after infection, i.e., at the time when the nuclear membranes were no longer intact and the cells appeared to be disintegrating. It is noteworthy that at 50 hr post-infection the relative frequency of naked nucleocapsids in cells infected with F strain was considerably greater than in cells infected with MP or mP strains. By this time after infection, the nuclear membrane has broken down, and naked nucleocapsids are rarely seen undergoing envelopment.

In cells infected with all strains, nucleocapsids were seen in apposition to thickened areas of the inner lamellae of the nuclear membrane and in invaginations of the nuclear membrane projecting into the cytoplasm. On the basis of similar data, it has been concluded that nucleocapsids are being enveloped at the nuclear membrane (8, 22, 34).

Cytoplasm of infected cells. Two characteristic features of herpes simplex virus infection are the reduplication of the nuclear membrane (20, 22) and the appearance of membrane-bound tubules in the cytoplasm (33). In the current study, these

features were present in all cells infected with the mP, F, or MP strains but were less prominent in cells infected with the G strain.

Naked nucleocapsids were frequently present in the cytoplasm of cells infected for 18 to 50 hr with the G strain (Fig. 4A, B, C, D). The origin of these particles is uncertain; we found no breaks in the nuclear membrane that would account for the presence of naked nucleocapsids in the cytoplasm and no evidence that they resulted from the uptake and uncoating of released particles. It is noteworthy that wherever the nucleocapsids were in close proximity to cytoplasmic membranes, the portion of the membrane in apposition to the nucleocapsid appeared to be thickened. It is also of interest to note that cells infected with the G strain and incubated for 50 hr prior to fixation were found to contain almost exclusively unenveloped nucleocapsids located in the cytoplasm; the nuclei contained relatively few particles.

Enveloped nucleocapsids (Fig. 5F) were present in the space between the lamellae of the nuclear membrane, in the extracellular fluid, or in the cytoplasm within structures delineated by a single membrane. The relative amounts of enveloped particles in cells infected for 18 hr are shown in Table 3.

TABLE 2. Frequency of various intranuclear inclusions seen in thin sections of HEp-2 cells infected with different strains of herpes simplex virus

Inclusion	Strains ^a			
	mP	MP	F	G
Nucleolar disaggregation (Fig. 1A, B, C).....	4	4	4	4
Electron opaque granular structures near nuclear membrane (Fig. 1D, 2A)....	4	4	4	4
Lattice structures (Fig. 3B)...	0	0	0	2
Densely staining material in rosettes (Figs. 3A, 2A, B)	3	2	2	3
Aggregates of granules 15 nm in diameter (Figs. 1D, 2C, 3C)	2	3	3	2
Aggregates of granules 25 nm in diameter (Figs. 2C, 3C) ..	2	3	3	2
Naked nucleocapsids in crystalline array (Fig. 3F) ..	1	1	4	1
Filaments (Fig. 3D)	0	0	0	3

^a Key: 4, present in all infected cells; 3, present in more than half of the infected cells; 2, present in less than half of the infected cells; 1, present, but very infrequently; 0, never seen.

FIG. 4. Electron photomicrographs of thin sections of HEP-2 cells infected for 50 hr with the G strain of herpes simplex virus. (A) Naked nucleocapsids present in the cytoplasm in apposition to membranes. Bar = 300 nm. (B) Naked nucleocapsids at the cell surface. Bar = 150 nm. (C, D) Cytoplasmic nucleocapsids in apposition to cytoplasmic membranes. Bars = 200 nm. Letter abbreviations are given in Fig. 1.

TABLE 3. *Observed frequency of virus particles in HEp-2 cells infected with different strains of herpes simplex virus*

Particle designation in Fig. 5	Description	Virus strain ^a				Cell compartment ^b
		mP	MP	F	G	
A	Capsid with electron translucent core	0	0	0	0	C
		1	2	3	1	N
		0	0	0	0	E
B	Capsid with electron opaque core	0	0	0	3	C
		3	3	2	2	N
		0	0	1	1	E
C	Capsid with densely staining electron opaque core	0	0	0	4	C
		1	1	1	0	N
		0	0	0	0	E
D	Capsid with ribbon shaped core	0	0	0	3	C
		0	0	0	1	N
		0	0	0	1	E
E	Capsid with an inner envelope	0	0	0	2	C
		1	1	1	1	N
		0	0	0	0	E
F	Enveloped particle in extracellular fluid and space between lamellae of nuclear membrane	0	0	0	0	C
		1	1	1	1	N
		4	4	4	4	E
Not illustrated	Enveloped particle inside structure delineated by membrane	4	4	2	3	C
		0	0	0	0	N
		0	0	0	0	E

^a For key, see Table 2.

^b C, cytoplasm; N, nucleus; E, extracellular fluid.

Particles present in infected cells. Six types of particles were present in cells infected with the various strains (Fig. 5). The frequency with which they are seen in the nucleus, cytoplasm, and extracellular fluid of cells infected with the four strains of herpes simplex virus is described in Table 3.

Naked nucleocapsids, 100 nm in diameter, appeared first in the nucleus (Fig. 5A, B, C). Enveloped nucleocapsids (Fig. 5D, E, F) were seen subsequently in the cytoplasm and extracellular fluid.

It has been reported by several workers (22, 41) that the core of the naked nucleocapsid differs in enveloped nucleocapsids as viewed in stained, thin sections. We have also observed differences between cores of naked and enveloped nucleocapsids of both types 1 and 2 strains. However, we have also found that the core of type 2 enve-

loped or partially enveloped nucleocapsid has a ribbon-shaped core which differs from that of type 1 virions.

DISCUSSION

We have shown the following. (i) All herpes virus variants share the ability to aggregate the chromatin at the nuclear membrane. (ii) The laboratory strains mP and MP, which differ in physical properties and in their effects on social behavior of infected cells, cannot be differentiated with respect to intracellular development. (iii) There are quantitative differences in the development of mP, a type 1 laboratory strain, and F, a type 1 virus passaged in cell culture a limited number of times. (iv) There are both quantitative and qualitative differences between G, a type 2 virus passaged a limited number of times in cell culture, and the type 1 strains.

Disaggregation of host nucleoli and the aggregation of host chromatin at nuclear membrane. The disaggregation of nucleoli and margination of chromatin occur early, within the first 4 hr after infection, and coincide with the inhibition of host DNA and ribonucleic acid (RNA) synthesis and with the inhibition of processing of ribosomal RNA. The inhibition of host DNA, (4), RNA (40), and protein synthesis (39), as well as the changes in the structure of the nucleoli and host chromatin have been reported in cells infected with herpesviruses and have been studied in detail (27a). The finding that all of the virus strains used in this study cause identical alteration in the structure and localization of nucleoli and chromatin reinforce the conclusion presented earlier (1) that inhibition of host metabolism may be a prerequisite to virus multiplication.

Qualitative differences in the development of laboratory and recently isolated type 1 strains. The major consistent differences observed among the F, mP, and MP strains were the frequency of intranuclear viral crystals and cytoplasmic structures containing fully enveloped nucleocapsids. Virus crystals have been described previously (19, 21). The crystals were present in all nuclei of cells infected with the F strain but were rarely seen in cells infected with the laboratory strains. Conversely, the cytoplasmic structures containing enveloped nucleocapsids were most frequent in cells infected with the laboratory strains. We interpret this as an indication that (i) numerous passages of virus in HEp-2 cells have resulted in the selection of virus variants with increased ability to multiply in these cells and that (ii) freshly isolated strains are partially restricted in HEp-2 cells, the restriction having something to do with

FIG. 5. Thin sections of particles seen in HEp-2 cells infected with herpes simplex virus. For description, see Table 3. Bars = 100 nm.

membrane synthesis or modification. It should be pointed out that restrictions at the level of membrane synthesis or modification have already been demonstrated in cells infected with herpes simplex (28, 37) and with other viruses (17). Our reason for viewing HEp-2 cells as being partially restrictive to freshly isolated strains may be summarized as follows.

(i) As indicated in detail elsewhere (36; B. Roizman, S. B. Spring, and J. Schwartz, *Fed. Proc.*, *in press*), naked nucleocapsids are not infectious. Nucleocapsids enclosed by an inner envelope or by both inner and outer envelopes are infectious. In type 1 strains, naked nucleocapsids and nucleocapsids with the inner envelope are assembled in the nucleus. In confirmation of the reports of numerous investigators, the nucleocapsids acquire the outer envelope from the inner lamella of the nuclear membrane. Nucleocapsids with an inner envelope are hydrodynamically smaller than particles with both inner and outer envelopes but are larger than naked nucleocapsids. With respect to intranuclear crystals, the distances between particle centers in the crystals indicate that they consist of naked nucleocapsids. It is not evident that naked nucleocapsids are withdrawn from the crystal and enveloped. The finding of numerous crystals in the nucleus at 50 hr after infection suggests that they are not. It seems likely that the formation of crystals reflects the failure of envelopment rather than a particular affinity of the nucleocapsids for each other.

(ii) In a preceding publication (33) we showed that the cytoplasmic structures containing the enveloped nucleocapsids are ducts probably derived from the cytoplasmic reticulum and connecting at

one end with the outer lamella of the nuclear membrane and at the other end with the cytoplasmic membrane. Biochemical studies (Spear, Keller, and Roizman, *unpublished data*) indicate that the smooth endoplasmic reticulum becomes modified after infection. It is noteworthy that the ducts are absent from DK cells abortively infected with the MP strain. A decrease in the frequency of the ducts may reflect the inability, total or in part, of the virus to modify the membranes of the endoplasmic reticulum.

Qualitative differences between types 1 and 2 strains. In this paper, we have reported three qualitative differences in the development of types 1 and 2 viruses. Specifically, (i) the cores of type 2 virions appear in thin section to be structurally different from those of type 1 virions. (ii) The cytoplasm of cells infected with type 2 virus contains numerous naked and partially enveloped nucleocapsids. (iii) As previously reported (5, 24), nuclei of cells infected with type 2 virus exhibit a lattice-like structure and filaments absent from nuclei of cells infected with type 1 virus. We do not know the significance of the ribbon-like appearance of the core of the type 2 virion or the function of the lattice structure and the filaments. However, in the light of the numerous studies on the envelopment of herpesviruses, the most intriguing finding to us is the presence of naked and partially enveloped nucleocapsids in the cytoplasm. Two points should be noted.

(i) The mere finding of unenveloped or partially enveloped nucleocapsids is not in itself of particular significance. It could be argued that (i) nucleocapsids of type 2 virus are assembled in part in the cytoplasm, (ii) the nuclear membrane of cells

infected with type 2 virus is particularly fragile, (iii) the ducts are fragile and the naked nucleocapsids present in the cytoplasm represent degradation products of enveloped nucleocapsids, or (iv) the virus is taken into the cell and unenveloped just as rapidly as it is released. The cytoplasmic particles contain cores; there are no obvious breaks in the nuclear membrane, and we found no evidence of virus uptake late in infection. Nevertheless, none of the possibilities listed above is readily excluded. It is significant, however, that wherever nucleocapsids are found in the proximity of cytoplasmic membrane (endoplasmic reticulum, plasma membrane, etc.), the portion of membrane immediately opposite the nucleocapsids is thickened and appears to be enveloping the nucleocapsid.

(ii) Although naked nucleocapsids are not uncommon in the cytoplasm of cells infected with type 1 strains, particularly late in infection, the finding of nucleocapsids partially enveloped at cytoplasmic membranes relatively early in infection is not a common finding. Epstein (11) and Siminoff and Menefee (35) concluded on the basis of relatively similar evidence that herpes simplex virus can be enveloped in the cytoplasm. This conclusion has been disputed by several workers (7, 8, 25, 34). It is clear from the data presented in this paper that Epstein, Siminoff, and Menefee probably worked with a type 2 strain, whereas the others probably studied type 1 strains. The question still remains whether the type 2 virus is enveloped in the cytoplasm, particularly since it could be argued that the structure of the nucleocapsid is such that it modified membranes with which it comes in contact without necessarily becoming enveloped. If the nucleocapsids can be enveloped by cytoplasmic membranes, the question arises as to how the particles get to these membranes. In a recent paper, Stackpole (38) also found naked and partially enveloped herpesviruses in the cytoplasm of cells from Lucké tumors of frogs. Stackpole concluded that the nucleocapsids were first enveloped by the inner lamella of the nuclear membrane, unenveloped by the outer lamella of the same membrane, and finally enveloped by membranes of the Golgi apparatus. Although we cannot, on the basis of static pictures, deduce whether type 2 virus also follows the complex sequence of events described by Stackpole, it is nevertheless clear from the foregoing that the development of several herpesviruses both naturally and in artificial systems of the laboratory involves the presence of naked nucleocapsids in the cytoplasm and that type 2 herpes simplex viruses differ from type 1 strains with respect to specific features of intracellular development.

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LITERATURE CITED

1. Aurelian, L., and B. Roizman. 1965. Abortive infection of canine cells by herpes simplex virus. II. The alternative suppression of synthesis of interferon and viral constituents. *J. Mol. Biol.* 11:539-548.
2. Barski, G., and R. Robineaux. 1959. Evolution of herpes simplex lesions observed *in vitro* by phase contrast cinematography. *Proc. Soc. Exp. Biol. Med.* 101:632-645.
3. Bedoya, V., A. S. Rabson, and P. M. Grimley. 1968. Growth *in vitro* of herpes simplex virus in human lymphoma cell lines. *J. Nat. Cancer Inst.* 41:635.
4. Ben-Porat, T., and A. S. Kaplan. 1965. Mechanism of inhibition of cellular DNA synthesis by pseudorabies virus. *Virology* 25:22-29.
5. Couch, E. F., and A. J. Nahmias. 1969. Filamentous structures of type 2 *Herpesvirus hominis* infection of the chorio-allantoic membrane. *J. Virol.* 3:228-232.
6. Dales S., and H. Silverberg. 1969. Viroplex of herpes simplex virus by HeLa cells. *Virology* 37:475-480.
7. Darlington, R. W., and C. James. 1966. Biological and morphological aspects of growth of equine abortion virus. *J. Bacteriol.* 92:250-257.
8. Darlington, R. W., and L. H. Moss, III. 1968. Herpesvirus envelopment. *J. Virol.* 2:48-55.
9. Dowdle, W. R., A. J. Nahmias, R. W. Harwell, and F. P. Pauls. 1967. Association of antigenic type of herpesvirus *hominis* with site of viral recovery. *J. Immunol.* 99:974-980.
10. Ejercito, P. M., E. Q. Kieff, and B. Roizman. 1968. Characterization of herpes simplex virus strains differing in their effect on social behavior of infected cells. *J. Gen. Virol.* 3:357-364.
11. Epstein, M. A. 1962. Observations on the mode of release of herpesvirus from infected HeLa cells. *J. Cell Biol.* 12:589-597.
12. Figueroa, M. E., and W. E. Rawls. 1969. Biological markers for differentiation of herpes-virus strains of oral and genital origin. *J. Gen. Virol.* 4:259-267.
13. Goodheart, C. R., G. Plummer, and J. L. Waner. 1968. Density difference of DNA of human herpes simplex viruses types 1 and 2. *Virology* 35:473-475.
14. Gray, A., T. Tokumaru, and T. F. Scott. 1958. Different cytopathogenic effects observed in HeLa cells infected with herpes simplex virus. *Arch. Gesamte Virusforsch.* 8:60-76.
15. Hoggan, M. D., and B. Roizman. 1959. The isolation and properties of a variant of herpes simplex producing multinucleated giant cells in monolayer cultures in the presence of antibody. *Amer. J. Hyg.* 70:208-219.
16. Hummeler, K., N. Tomassini, and B. Zajac. 1969. Early events in herpes simplex virus infection: a radioautographic study. *J. Virol.* 4:67-74.
17. Klonk, H., and P. W. Choppin. 1969. Lipids of plasma membranes of monkey and hamster kidney cells and of parainfluenza virions grown in these cells. *Virology* 38:255-268.
18. Love, R., A. S. Rabson, and P. Wildy. 1964. Changes in the nucleolus of normal and neoplastic cells infected with riboviruses and deoxyviruses. *Acta Unio Int. Contra Cancrum* 20:1384-1387.
19. Melnick, J. L., E. R. Rabin, and A. B. Jensen. 1968. Intracellular herpesvirus aggregate in the form of a pentagonal dipyramidal crystal-like structure. *J. Virol.* 2:78-80.
20. Morgan, C., S. A. Ellison, H. M. Rose, and D. H. Moore. 1954. Structure and development of viruses as observed in

- the electron microscope. I. Herpes simplex virus. *J. Exp. Med.* 100:195-202.
21. Morgan, C., E. P. Jones, M. Holden, and H. M. Rose. 1958. Intranuclear crystals of herpes virus observed with the electron microscope. *Virology* 5:568-571.
 22. Morgan, C., H. M. Rose, M. Holden, and E. P. Jones. 1959. Electron microscopic observations on the development of herpes simplex virus. *J. Exp. Med.* 110:643-656.
 23. Morgan, C., H. M. Rose, and B. Mednis. 1968. Electron microscopy of herpes simplex virus. I. Entry. *J. Virol.* 2:507-516.
 24. Murphy, F. A., A. K. Harrison, and S. G. Whitfield. 1967. Intranuclear formation of filaments in herpes virus *hominis* infection of mice. *Arch. Gesamte Virusforsch.* 21:463-468.
 25. Nii, S., C. Morgan, and H. M. Rose. 1968. Electron microscopy of herpes simplex virus. II. Sequence of development. *J. Virol.* 2:517-536.
 26. Plummer, G. 1964. Serological comparison of the herpes viruses. *Brit. J. Exp. Pathol.* 45:135-141.
 27. Plummer, G., J. L. Waner, and C. P. Bowling. 1968. Comparative studies of type 1 and type 2 "herpes simplex" viruses. *Brit. J. Exp. Pathol.* 49:202-208.
 - 27a. Roizman, B. 1969. Herpesviruses—a biochemical definition of the group, p. 1-7. In B. Benacerraf et al. (ed.), *Current topics in microbiology and immunology*, vol. 49. Springer-Verlag, Heidelberg.
 28. Roizman, B., and L. Aurelian. 1965. Abortive infection of canine cells by herpes simplex virus. I. Characterization of viral progeny from cooperative infection with mutants differing in ability to multiply in canine cells. *J. Mol. Biol.* 11:528-538.
 29. Roizman, B., and P. R. Roane, Jr. 1961. A physical difference between two strains of herpes simplex virus apparent on sedimentation in cesium chloride. *Virology* 15:75-79.
 30. Roizman, B., and P. R. Roane, Jr. 1963. Demonstration of a surface difference between virions of two strains of herpes simplex virus. *Virology* 19:198-204.
 31. Roizman, B., and P. G. Spear. 1968. Preparation of herpes simplex virus of high titer. *J. Virol.* 2:83-84.
 32. Scheneweiss, K. E. 1962. Serologische Untersuchungen zur Typendifferenzierung des herpes virus *hominis*. *Z. Immunoforsch.* 124:24-48.
 33. Schwartz, J., and B. Roizman. 1969. Concerning the egress of herpes simplex virus from infected cells: electron and light microscope observations. *Virology* 38:42-49.
 34. Siegert, R. S., and D. Falke. 1966. Electron microscopic investigation of the development of herpes *hominis* virus in culture cells. *Arch. Gesamte Virusforsch.* 19:230-249.
 35. Siminoff, P., and M. G. Menefee. 1966. Normal and 5-bromo-deoxyuridine-inhibited development of herpes simplex virus. An electron microscope study. *Cell Res.* 44:241-255.
 36. Spring, S. B., and B. Roizman. 1968. Herpes simplex virus products in productive and abortive infection. III. Differentiation of infectious virus derived from nucleus and cytoplasm with respect to stability and size. *J. Virol.* 2:979-985.
 37. Spring, S. B., B. Roizman, and J. Schwartz. 1968. Herpes simplex virus products in productive and abortive infections. II. Electron microscopic and immunologic evidence for failure of virus envelopment as a cause of abortive infection. *J. Virol.* 2:384-392.
 38. Stackpole, C. W. 1969. Herpes-type virus of the frog renal adenocarcinoma. I. Virus development in tumor transplants maintained at low temperature. *J. Virol.* 4:75-93.
 39. Sydiskis, R. J., and B. Roizman. 1967. The disaggregation of host polyribosomes in productive and abortive infection with herpes simplex virus. *Virology* 32:678-686.
 40. Wagner, E. K., and B. Roizman. 1969. Ribonucleic acid synthesis in cells infected with herpes simplex virus. I. Patterns of ribonucleic acid synthesis in productively infected cells. *J. Virol.* 4:36-46.
 41. Watson, D. H., P. Wildy, and W. C. Russell. 1964. Quantitative electron microscope studies on the growth of herpes virus using the techniques of negative staining and ultramicrotomy. *Virology* 24:523-538.