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Proteins Specified by Herpes Simplex Virus

XII. The Virion Polypeptides of Type 1 Strains

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The polypeptides from purified virions of a herpes simplex 1 (human herpesvirus 1) strain, F1, which had been passaged a limited number of times in cell culture after isolation, formed 33 bands on electrophoretic separation in polyacrylamide gels cross-linked with *N,N'*-diallyltartardiamide in contrast to a maximum resolution of only 24 to 25 bands in gels cross-linked with *N,N'*-methylenebisacrylamide. This increase in the number of bands was due chiefly to an improved separation of glycosylated polypeptides from nonglycosylated polypeptides with which they co-electrophoresed on methylenebisacrylamide cross-linked gels. Purified virions of HSV-1 [F1] had a protein/DNA mass ratio of 10.7 ± 0.96, and based on a DNA molecular mass of 85 × 10^6 to 100 × 10^6 the estimated weight of virion polypeptides ranges from 16.4 to 19.4 × 10^{-16} g. The number of molecules of each polypeptide per virion ranged from less than 50 to 1,500. Comparison of the virion polypeptides of two HSV-1 strains with similar isolation and limited passage history with those of four HSV-1 strains with histories of numerous passages outside the human host showed a number of nonrandom variations in virion polypeptides. Thus, although the virion polypeptides of two strains with similar isolation and limited passage history could not be differentiated, strains with extended passage histories differed markedly from each other and from the limited passage strains in the number and electrophoretic mobility of noncapsid polypeptides and notably in those of the envelope.

An earlier publication from this laboratory (22) reported the protein composition of purified virions of the F1 strain of herpes simplex 1 (human herpesvirus 1, HSV-1). From an electrophoretic analysis of virion polypeptides on polyacrylamide gels cross-linked with methylenebisacrylamide (MBA) it was concluded that HSV-1 [F1] virions contained at least 24 virion polypeptides (VP) numbered from VP1 to 24, and that trace amounts of host proteins remaining in purified virus preparations were adventitious contaminants and not structural constituents of the HSV-1 virion. In that study it was also shown that many virion polypeptides were glycosylated; however, attempts to identify glycosylated polypeptides by autoradiography of gels containing separated polypeptides labeled with [^{14}C]glucosamine were not entirely satisfactory. At least two amino acids labeled bands, VP7 and 8, were resolved in the region occupied by a single broad glucosamine labeled band. Similarly, two additional broad bands of glucosamine label were located at lower molecular weights in the region of VP10-16 and VP17-19 even though nine separate polypeptides bands were readily differentiated in the Coomassie brilliant blue-stained gels from which the autoradiograms were prepared as well as in autoradiograms of gels containing separated ^{14}C-amino acid-labeled polypeptides. The ratio of glucosamine label to protein therefore varied across each of these regions and was much lower for the region of bands VP10-16 than for VP7-8 or VP17-19. These observations suggested either the existence of protein bands with widely different degrees of glycosylation or a failure to separate glycosylated from nonglycosylated components, notably in the region of VP10-16. These alternative explanations had very different implications for the biosynthesis of glycosylated components and the topology of the virion. In addition, we have previously noted that virus strains which have been passaged a limited number of times in cell culture differ from a number of strains with extensive passage histories outside the human host with respect to immunologic specificity (5), virus growth and development (21), and effects on the social behavior of infected cells (5, 10, 15, 16). We therefore wished to determine whether

strains differing in their passage history had systematic differences in their virion polypeptide compositions.

In this paper we present additional data on the protein composition of HSV-1 virions. This data includes the separation of glycosylated and nonglycosylated polypeptides in the region of VP10–19 on polyacrylamide gels cross-linked with diallytartardiamide (DATD), estimates of the number of copies of virion polypeptides, and results of comparisons of the polypeptide composition of virions from HSV-1 strains passaged a limited number of times in cell cultures with those of HSV-1 strains with histories of numerous passages outside the human host.

MATERIALS AND METHODS

Cells. Human Epidermoid Carcinoma no. 2 (HEp-2) cells were grown at 37 C in Eagle minimal essential medium (EMEM) supplemented with 10% calf serum, 0.001% ferric nitrate, and 1% sodium pyruvate.

Viruses. The HSV-1 strains used in these studies were as follows: **HSV-1 [F1].** This strain was isolated in 1967 at the University of Chicago from a patient with a herpetic infection of the face and under the designation of HSV-1 [F] served as the prototype of HSV-1 strains in this laboratory in all publications subsequent to that of Ejercito et al. (5). The virus was isolated in HEp-2 cells and is being passaged in these cells at low multiplicities of infection a maximum of four times. The properties of the virus and its DNA have been published elsewhere (5, 6, 7, 11, 22).

HSV-1 [F5]. The isolation and passage history of this strain are similar to that of HSV-1 [F1]. By plaque morphology (5), it cannot be differentiated from HSV-1 [F1].

HSV-1 [mP]. This virus, originally designated as strain NT, was obtained in 1958 as an infected third passage chorioallantoic membrane. It was isolated in 1955 at the Johns Hopkins Hospital from a patient with eczema herpeticum. It was passaged some 20 times in FL cells and subsequently some 40 times in HEp-2 cells. Its plaque morphology is similar to that of HSV-1 [F1]. Some differences apparent on electron microscope studies of the development of this virus as compared to that of HSV-1 [F1] have been reported (21).

HSV-1 [MP] arose spontaneously in FL cells infected with HSV-1 [mP] (8). It was differentiated from the parent strain by the fact that it caused fusion rather than clumping of infected cells. The virus was plaque purified and passaged some 20 times in FL cells and nearly 60 times in HEp-2 cells. It has been previously shown to differ from its parent HSV-1 [mP] virus and from HSV-1 [F1] not only in its effect on the social behavior of cells (5, 15) but also in certain features of its development, sedimentation of its DNA (2), chromatographic behavior on brushite columns (19), immunologic specificity (5, 19), buoyant density in CsCl solutions (18), and in the virus-specific polypeptides binding to the membranes of infected cells (10, 16, 21).

HSV-1 [13] was obtained from R. Monservigi and M. Terni. The virus was isolated in Italy and passaged five times in HEp-2 cells prior to use in the experiments described in this paper. The virus was classified as a strain of HSV-1 on the basis of the buoyant density of its DNA, as determined by isopycnic banding in a Beckman model E analytical ultracentrifuge, and by serum neutralization tests as described by Ejercito et al. (5).

HSV-1 [13 v B4] is a plaque morphology variant obtained after BUdR mutagenesis of HSV-1 [13]. It was isolated and grown in HEp-2 cells and on these cells gives large syncytial plaques in contrast to the clumps of rounded cells produced by the parental virus.

HSV-1 [HFEM] was obtained from Peter Wildy by way of Gordon Plummer. It is derived from the HF strain originally isolated at the Rockefeller Institute in 1922 and was subsequently passaged in eggs, mice, HeLa, and in BHK-21 cells.

The procedure for the propagation of the HSV strains were as described previously (20).

Radiolabeling and purification of enveloped virus particles. HEp-2 cells infected at multiplicity of 5 to 10 PFU per cell were incubated at 34 C and labeled from 4 to 24 or 40 h postinfection with [^{14}C]leucine, [^{14}C] isoleucine, and [^{14}C]valine (0.3 μCi/ml), with [^{14}C]glucosamine (0.3 μCi/ml) or with [^{14}C]fucose (0.3 μCi/ml), in 100 ml of labeling medium per 2×10^8 cells. For ^{14}C-amino acids, the labeling medium was mixture 199 containing one-fourth of the normal concentration of leucine, isoleucine, and valine and supplemented with 1% dialyzed calf serum. For [^{14}C]glucosamine the labeling medium was EMEM containing one-half of the normal concentration of glucose and supplemented with 1% dialyzed calf serum. The labeling medium for [^{14}C]fucose was the same as that for glucosamine except that it contained the normal amount of glucose. The purification of the virions essentially followed the procedure previously described (22), except that virions obtained after dextran banding were used directly. Virions of comparable purity to those obtained previously by isopycnic banding of dextran-purified virions (22) could be obtained directly from dextran gradients providing that: (i) the Dounce homogenization was minimal, consistent with efficient cell breakage, and (ii) the cytoplasmic fraction from no more than 2×10^8 to 3×10^8 cells was loaded onto each 35-ml dextran-10 gradient. Electron microscopy of such virion preparations negatively stained with neutral phosphotungstate showed at least 95% unpenetrated enveloped virus particles (normally >98%).

Analytical procedures. Protein and DNA determinations. Protein concentration was determined by the procedure of Lowry et al. (13) with bovine serum albumin as calibrating standard. DNA concentration was determined by the diphenylamine technique as described by Burton (3) with either salmon sperm or *Micrococcus lysodeikticus* DNAs as calibrating standards. In the measurement of DNA, color development with the diphenylamine reagent was allowed to proceed at 28 C overnight. Under these conditions sialic acid, a potential reactant in the diphenylamine

procedure, did not affect the measurements when added in amounts sufficient to yield a ratio of sialic acid to virus protein of 0.02 (wt/wt) in the experimental reaction mixtures.

Polyacrylamide gel electrophoresis. The procedures for sample preparation and electrophoresis on sodium dodecyl sulfate-containing polyacrylamide gel slabs were as previously described (4, 12, 22) except that gels were polymerized at 26 to 28 C with DATD (1, 7) in place of *N,N'*-MBA. A somewhat higher gel strength was necessary in order to achieve the same migration of standard protein DATD cross-linked gels as compared with MBA cross-linked gels (compare Fig. 1A and E of this paper and our Fig. 2 with data of ref. 22). It should also be noted that gels cross-linked with DATD also expand more than MBA cross-linked gels under acid conditions of fixation and staining.

Quantitation of virion polypeptides. Virion polypeptides were quantitated by computer-aided planimetry of absorbance tracings of virion polypeptides separated on polyacrylamide gels and stained with Coomassie brilliant blue, or from absorbance tracings of autoradiographic images of electrophoretically separated polypeptides labeled with ^{14}C-amino acids. The methods employed have been described and illustrated elsewhere (9).

RESULTS

Separation of HSV-1 [F1] virion polypeptides on polyacrylamide gels cross-linked with DATD and with MBA. Figure 1 shows autoradiograms of electrophoretically separated HSV-1 [F1] virion polypeptides labeled with ^{14}C-amino acid in polyacrylamide gels cross-linked with DATD and MBA, and autoradiograms of virion polypeptides labeled with [^{14}C]fucose and [^{14}C]glucosamine, respectively, and separated on DATD cross-linked gels.

Comparisons of the autoradiograms of ^{14}C-amino acid-labeled polypeptides show that, although similar profiles are obtained in both types of gels, the resolution of the DATD cross-linked gels is somewhat superior. For example, whereas VP15 and 16 are resolved in both systems they are more clearly separated on DATD cross-linked gels (compare Fig. 1A with B and E.) Moreover, another polypeptide (VP15.8) is resolved only in the DATD cross-linked gels (Fig. 1B and E). A minor polypeptide designated VP6.5 became apparent in both bisacrylamide and DATD cross-linked gels. The electrophoretic mobility of this polypeptide is greater than VP6 but not as high as VP7 in bisacrylamide cross-linked gels. In gel cross-linked with DATD VP6.5 is obscured by VP8 and is visible only in virions lacking VP8 as described below (Fig. 4 and related text).

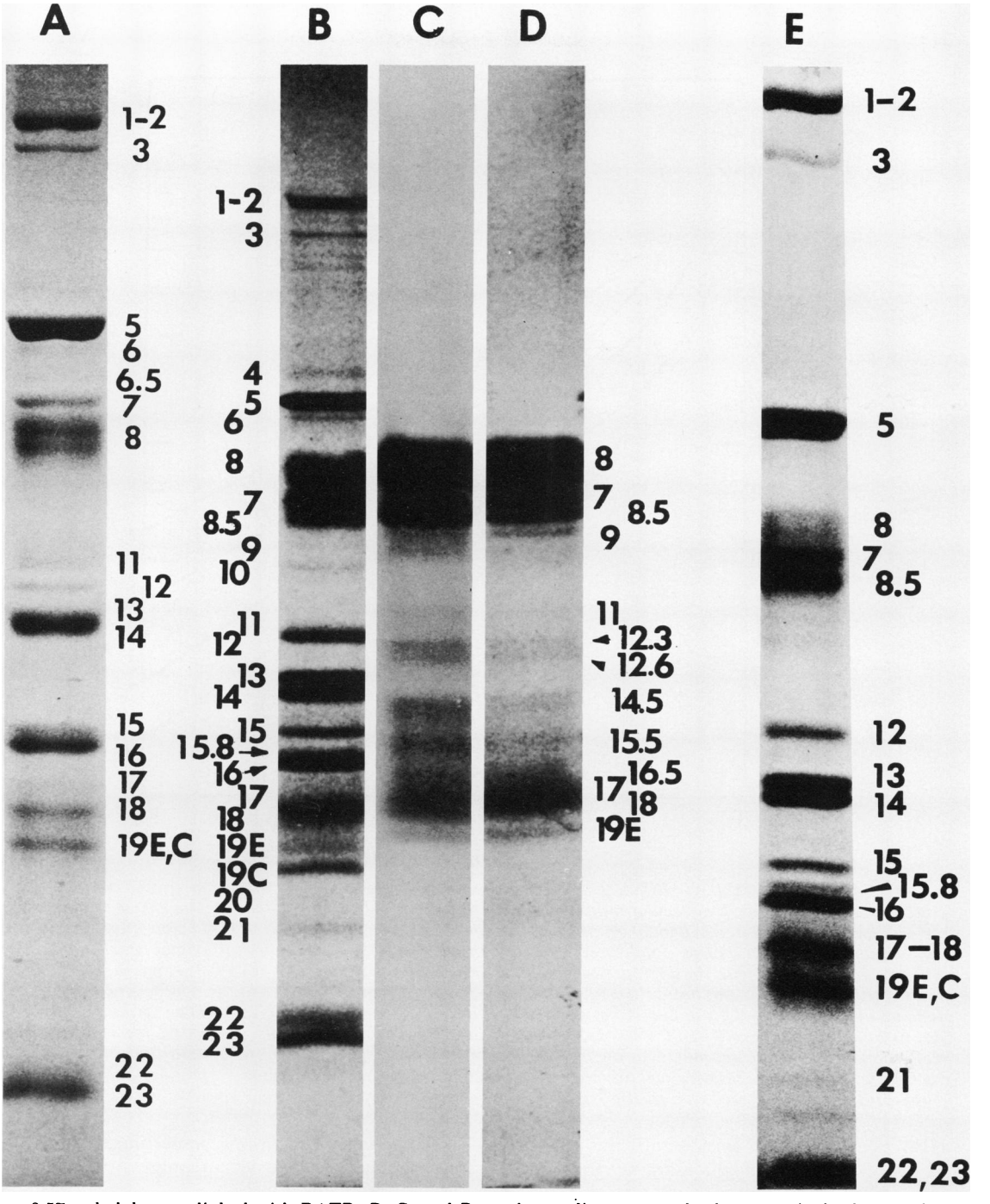
In general, the behavior of nonglycosylated polypeptides on DATD and MBA gels do not differ significantly, as evident from a comparison of the molecular weights of viral polypeptides determined on DATD gels (Fig. 2) with those previously (22) determined on MBA cross-linked gels and listed in Table 1. The difference between DATD and MBA polyacrylamide gels is in the sieving of glycosylated polypeptides. Specifically:

(i) The polypeptides forming bands 7 and 8 in MBA gels separated into three bands in gels cross-linked with DATD. In this instance VP8, characterized by a higher level of glycosylation, lower ratio of peak height to band width, and more rapid migration than VP7 in MBA, actually migrated more slowly than VP7 in DATD cross-linked gels containing less than 10% acrylamide. The new band, designated VP8.5, migrated more rapidly than VP7. On DATD gels VP7 was differentiated from VP8 and 8.5 by its lower ratio of glucosamine to amino acids and its higher ratio of peak height to width.

(ii) Comparisons of viral polypeptides in HSV-1 [F1] virions and capsids on MBA cross-linked gels (6) led to the prediction that virion band 19 consisted of two polypeptides, i.e., a nonglycosylated polypeptide designated 19C and contained in the capsid and a glycosylated polypeptide 19E contained in the virion proteins. These polypeptides were not separated on MBA gels but were readily separated on DATD gels of appropriate concentration (Fig. 1A and B).

(iii) In DATD cross-linked gels the minor bands of glucosamine-labeled polypeptides migrating between VP11 and VP15 did not comigrate with the major protein bands in this region, i.e., VP12, 13, 14, 15, and 16. These minor bands designated VP12.3, 12.6, 14.5, 15.5, and 16.5 (Fig. 1B, C, and D) are therefore minor glycoproteins. It is noteworthy, however, that the ratio of glucosamine to amino acids for these polypeptides was approximately the same as that for the major glycoproteins (VP8, 8.5, 17, 18, and 19E) and that this ratio was more than 4,000-fold higher than that for the major virion nonglycosylated polypeptides (e.g., VP5). The latter observation is in accord with the previous report from this laboratory (10) that infected

FIG. 1. *Autoradiograms of HSV-1 [F1] polypeptides separated on polyacrylamide gels. A, Polypeptides from virions labeled with ^{14}C-amino acids separated on a 7.75% gel cross-linked with MBA. B, Polypeptides from virions labeled with ^{14}C-amino acids separated on an 8.5% gel cross-linked with DATD. C and D, Polypeptides from virions labeled with D-[^{14}C]glucosamine and L-[^{14}C]fucose, respectively, subjected to electrophoresis on an*



8.5% gel slab cross-linked with DATD. B, C, and D are from adjacent sample slots of a single slab gel, but different exposure intervals were used in printing the photographs for this figure. Coomassie brilliant blue-stained polypeptides in B, C, and D were identical in amount and electrophoretic mobilities. E, Polypeptides from virions labeled with ^{14}C-amino acids and separated on a 7.75% gel cross-linked with DATD. The trace bands between VP1–2 and 3 and between VP3 and 4 of Fig. 1B were seen only in occasional preparations; together they account for only 0.3% of the total absorbance of this autoradiogram, i.e., well within the range of estimated amounts of host contaminants in our preparations of purified virions (23). Bands 15.8 and 16 were only separated on DATD cross-linked gels, the prolonged exposure necessary to reveal fainter bands has obscured VP15.8 by VP16 in 1B, but they can both be seen clearly in Fig. 1E. A comparison of Fig. 1B and 1E also serves to illustrate the importance of gel strength for the separation of glycosylated from nonglycosylated polypeptides of similar mobility. Thus, 19E and 19C were separated on the 8.5% DATD cross-linked gel but not on the 7.75% DATD cross-linked gel. We have found the 8.5% DATD cross-linked gel optimal for the separation of glycosylated and nonglycosylated polypeptides.

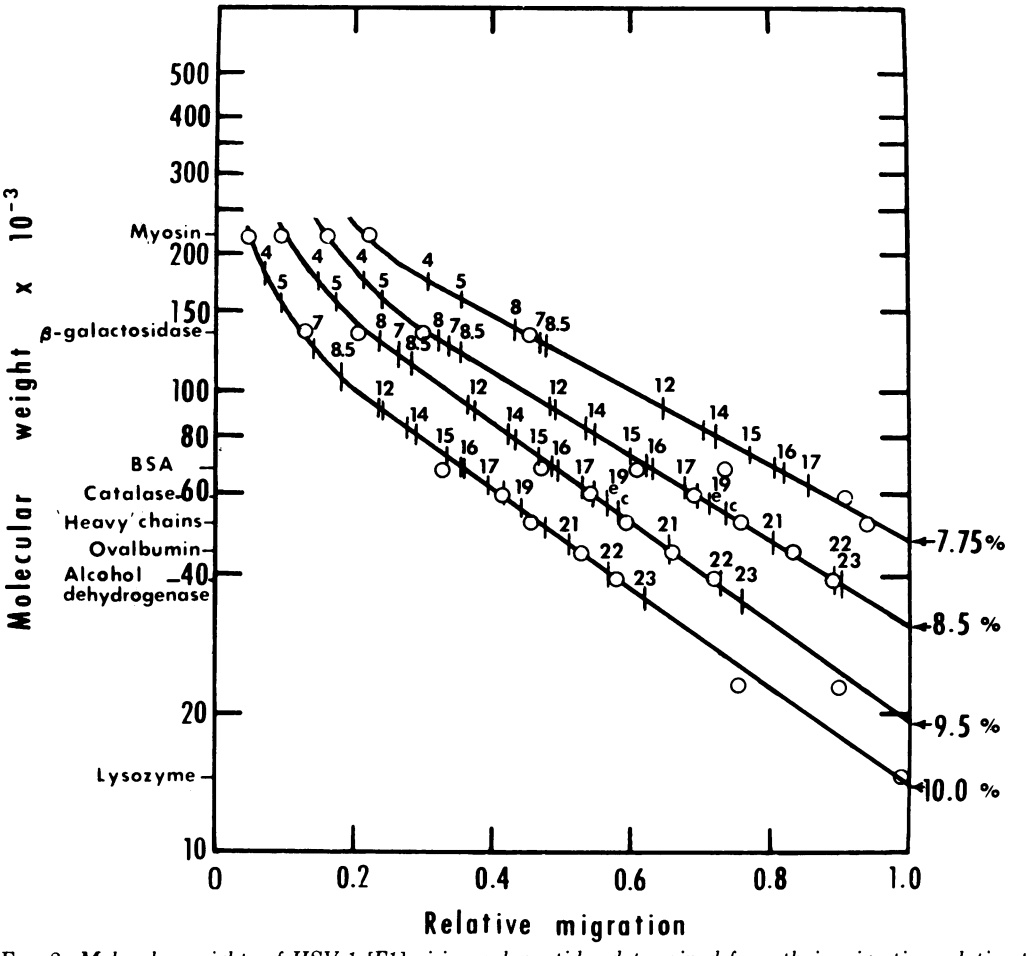


FIG. 2. *Molecular weights of HSV-1 [F1] virion polypeptides determined from their migration relative to protein standards of known molecular weight (open circles, identified at the appropriate molecular weight on the ordinate) on 7.75, 8.5, 9.5, and 10.0% polyacrylamide gels cross-linked with DATD. The position of virion polypeptides on the various gels is indicated by short vertical lines intersecting the curve connecting standard proteins and annotated with the appropriate numerical designation. The positions of VP1-3 are not shown, since at all of the gel strengths employed their mobilities were less than that of myosin and the relationship between distance of migration and log molecular weight was not linear in this range of mobilities. The position of minor glycosylated polypeptides 9, 12.3, 12.6, 14.5, and 15.5 are not shown to simplify the figure; however, their molecular weights were estimated in an identical fashion to those shown and are summarized in Table 1 together with the estimates obtained for other polypeptides. The migrations of standard proteins and of virion polypeptides were expressed as a fraction of the distance migrated by bromophenol blue dye.*

HEp-2 cells do not convert significant amounts of exogenous labeled glucosamine and fucose into amino acid precursors. The ratio of major to minor components was reproducible and was not changed by further purification of virions by isopycnic banding in sucrose density gradients. It is also significant that all glucosamine-labeled glycoproteins in the virion were also labeled with fucose and that the ratio of fucose to glucosamine label was similar for both minor (i.e., VP12.3, 12.6, 14.5) and major (i.e., VP8, 8.5, 17, 18) virion glycoproteins.

The current designations, molecular weights, and properties of the virion polypeptides are presented in Table 1.

Determination of virion protein:DNA mass ratio and estimation of total virion protein mass. Table 2 shows the results of chemical determination of protein and DNA content of five different virion preparations. Based on the average ratio of protein to DNA obtained from these determinations (10.7 ± 0.96) and the previously determined values which are in the range from 85 × 10^6 to 100 × 10^6 for the

TABLE 1. *Enumeration, quantitation, and glycosylation of virion polypeptides of HSV-1 [F1]*

Virion polypeptide[a]	Mol wt × 10^{-3}[a, b]		% Virion protein mass[c]		Molecules per particle[d]		Glycosylation	
	a	b	c	d	e	f	Glucosamine	Fucose
1-2	260-275	>260	3.3 (10)	3.5 (5)	<110-150	<120-150	–	–
3	260	>260	1.0 (8)	1.3 (4)	<30-40	<50-60	–	–
4	184	177	1.1 (8)	0.9 (7)	60-64	50-60	–	–
5	155	157	10.9 (10)	13.5 (7)	690-810	850-1,000	–	–
6	146	149	1.0 (10)		65-80		–	–
6.5		130		0.4 (2)		30-40	–	–
7		126	4.8 (8)		380-450		+?	+
8		129	6.5 (8)		500-590		+	+
8.5		119	4.1 (8)		340-400		+	+
(7 + 8 + 8.5)			18.3 (10)	11.8 (7)	1,440-2,140	930-1,100		
9	112	115		0.8 (5)		70-86	+	+
10	98	100		0.9 (3)		80-100	–?	–?
(9 + 10)			2.0 (6)	1.7 (5)	190-220	150-180		
11	93	94		2.5 (2)		260-310	+	+
12	87	91		2.2 (2)		240-280	–	–
(11 + 12)			3.8 (9)	4.7 (5)	410-480	500-600		
12.3		88	ND	ND			+	+
12.6		86	ND	ND			+	+
13	78	82	5.0 (6)		600-710		–	–
14	78	80	6.5 (6)		810-950		–	–
(13 + 14)			11.5 (10)	13.0 (7)	1,400-1,660	1,590-1,880		
14.5		76	ND	ND			+	+
15	71	73	2.9 (8)	3.6 (2)	390-460	490-580	–	–
15.5		70	ND	ND			+	+
15.8		69					+?	+?
16.0	65	68					–	–
(15.8 + 16.0)			6.9 (8)	5.9 (2)	1,000-1,200	850-1,010		
(15 + 15.8 + 16.0)			9.8 (10)	11.1 (7)				
17	59	62	3.6 (7)	3.5 (2)	580-680	560-660	+	+
18	57	59	5.4 (7)	4.1 (2)	860-1,020	690-810	+	+
19E	53	57	3.2 (7)	2.5 (2)	560-660	430-510	+	+
(17 + 18 + 19E)			12.3 (10)	10.3 (5)				
19C	53	55	3.8 (8)	4.2 (2)	680-808	760-890	–	–
20	50	51	1.5 (6)	1.8 (7)	300-350	350-410	+	+
21	44	47		1.1 (4)		230-270	–	–
21h				2.2 (4)		480-570	–	–
22	37	39	4.9 (6)	8.8 (2)	1,240-1,470	2,230-2,640	–	–
23	33	36	5.3 (6)	4.5 (2)	1,460-1,720	1,240-1,460	–	–
(22 + 23)			9.9 (8)	11.8 (7)				
24	25							

[a] Column a: Molecular weights of virion polypeptide as determined by Spear and Roizman (23) by electrophoresis on polyacrylamide gels cross-linked with MBA and calibrated with proteins of known molecular weight.

[b] Column b: Molecular weights of virion polypeptides determined by electrophoresis on calibrated polyacrylamide gels cross-linked with DATD (Fig. 2).

[c] Columns c and d: Contribution of each polypeptide to total virion protein mass determined by computer-aided planimetry (9) of absorbance scans of (*c*) autoradiograms and (*d*) absorbance scans of Coomassie brilliant blue-stained gels. The number of determinations on different gels contributing to each mean value are indicated in parentheses. Note that in some instances for groups of closely spaced polypeptides (e.g., VP7, 8, 8.5 and VP13, 14) the contribution of the group of components could be estimated under conditions where resolution was inadequate to allow accurate estimation of the amounts of each species within the group. Therefore, the mean values for the contribution of these groups of components were derived from a greater number of estimations than were the number of estimations of each polypeptide within the group. ND, Not accurately estimable; all these values were <1% of protein mass.

[d] Columns e and f: The number of molecules of each polypeptide or group of polypeptides per virion was calculated from their percentage in columns c (e) and d (f), respectively, based on the relationship:

$$\text{Molecules per virion} = \frac{\text{Total protein weight of virion} \times \text{percentage contribution of polypeptide to total virion protein}}{\text{Polypeptide molecular weight}}$$

For groups of more than one polypeptide the total number of molecules of all polypeptides within the group was calculated using the independently estimated values for the percentage contribution of the group to total virion protein and a value for the molecular weight which was the average of the molecular weights of the separated polypeptides. Upper and lower values for the number of molecules refer to values calculated for extremes of the range (16.4 to 19.4) × 10^{-16} g per virion for the weight of virion protein determined as described in the text.

TABLE 2. *Determination of protein/DNA ratio of purified virions of HSV-1 [F1]*

Determination	Virion preparation				
	1	2	3	4	5
Protein (mg/ml)	0.877	0.547	0.930	1.130	0.803
DNA (mg/ml)	0.087	0.049	0.080	0.124	0.070
Protein/DNA[a]	10.0	11.1	11.6	9.1	11.5

[a] Mean protein/DNA ratio ± SD = 10.7 ± 0.96.

molecular weight of viral DNA (11, 23), we calculated that the aggregate mass of the virion protein is 9.9×10^8 to 11.7×10^8, i.e., that the total weight of protein per virion is between 16.4 $\times 10^{-16}$ and 19.4 $\times 10^{-16}$ g. Recent values for the molecular weight of herpes simplex virus DNA determined by measurements of contour length with the electron microscope (P. Sheldrick, personal communication; G. Heyward, personal communication) or by co-sedimentation with T4 phage DNA (24) are closer to 100×10^6 and thereafter favor the upper value for the protein mass of the virion. This estimate is in agreement with determinations based on ratios of protein weight to particle count obtained with the aid of the electron microscope. However, the scatter in determinations of protein to particle ratios by electron microscopy was larger than that observed in the course of determinations of protein/DNA ratios.

Number of copies of virion polypeptides per particle. The average number of copies of each polypeptide in the virion may be computed from the molecular weight of the polypeptide, the total virion protein mass, and the fraction of the total virion protein mass constituted by each polypeptide. The estimates of the percentage of total virion protein contributed by each polypeptide are based on computer-aided planimetric analysis both of autoradiograms of polyacrylamide gels containing electrophoretically separated ^{14}C-amino acid-labeled polypeptides (Table 1, column c) and of Coomassie brilliant blue-stained gels (Table 1, column d). The computed number of copies based on each of these estimates are shown in columns e and f, respectively. Two comments should be made in connection with these data. A priori, both methods of estimation suffer from systematic errors. Radiochemical analyses suffer from two sources of error, i.e., errors due to the fact that the structural polypeptides do not all belong to the same coordinately regulated groups and, as documented elsewhere, they belong to classes whose temporal patterns of synthesis differ (7, 9). This source of error can be minimized by beginning the labeling interval early in infection. The second error would arise if polypeptides had large differences in the molar percentage of the amino acids used for labeling. However, no large differences were observed between autoradiograms of electrophoretically separated viral polypeptides labeled with a mixture of 13 ^{14}C-amino acids and those labeled with the three amino acids used in this study (9). Analyses of Coomassie brilliant blue-stained gels are free of these errors but suffer from the fact, documented elsewhere (6, 7, 22), that virion polypeptides differ in their affinity for this stain. For most polypeptides the agreement between the estimates obtained by the two techniques is quite good and it is notable that the exceptions are polypeptides 7, 8, 8.5, and 22 which, as documented elsewhere (6, 7, 22), differ from other virion polypeptides with respect to their affinity for Coomassie brilliant blue.

In general these estimates provide an explicit statement of the molecular complexity of the herpesvirion. In particular, they indicate that a number of major virion components are present in comparable molarities, i.e., VP5, VP19C, VP14, and VP15.8 + 16.0, and that although many components are present in more than 400 copies per virion, with the possible exceptions of VP22 and 23, none are represented in more than about 1,000 copies per particle. Based on either absorbance of autoradiograms or of Coomassie brilliant blue-stained gels (Table 1), we calculated that the polypeptides known to be in the virus capsid, VP5, 19C, 21, 23, and 24 (ref. 6, 7), make up 20 to 23% of the protein content of the virion. Of the remainder, 33 to 39% is made up of glycosylated polypeptides presumed to be in the virion envelope and 33 to 39% of nonglycosylated noncapsid, polypeptides whose location is uncertain (B. Roizman and D. Furlong, *Comprehensive Virology*, vol. 3, in press).

Comparison of virion polypeptides of several strains of HSV-1. The structural and biochemical studies reported from this laboratory in recent years have been done with strain HSV-1 [F1] annotated in Materials and Methods. Previous studies from our laboratory have shown that HSV-1 [F1] differs from many virus strains propagated serially in the laboratory for many years with respect to immunologic specificity, certain physical properties, viral development, and effect of the virus on the social behavior of infected cells. It was therefore of interest to determine the extent of differences in the structural polypeptides of virus strains

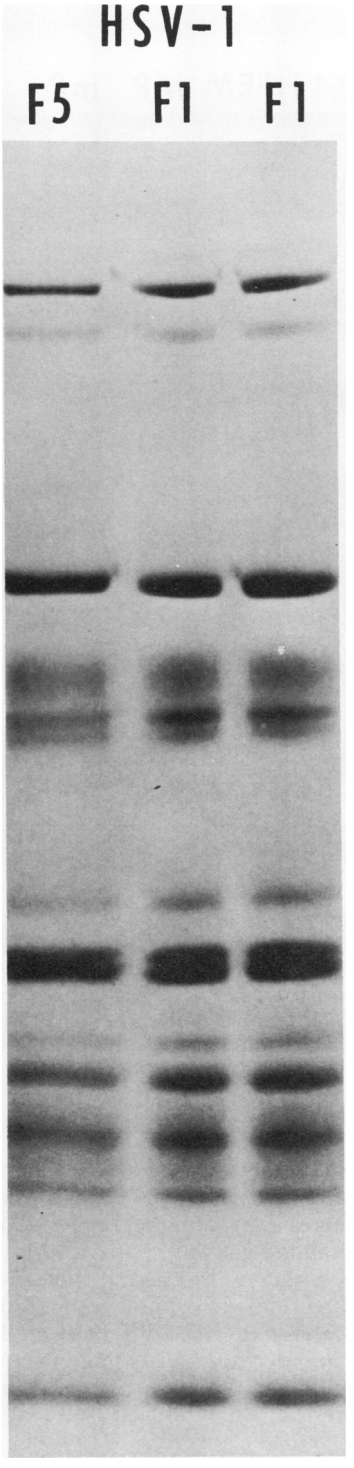


FIG. 3. *Autoradiogram of a polyacrylamide gel slab containing electrophoretically separated polypeptides from two independently purified preparations of HSV-1 [F1] virions and from purified virions of a virus strain with similar isolation and limited passage history, HSV-1 [F5]. All preparations were labeled with ^{14}C-amino acids.*

differing in their isolation and passage histories. The basis for this comparison is the observation that the electrophoretic profiles of virion polypeptides obtained from numerous independently purified preparations of HSV-1 [F1] in the same type of gel are identical. Figure 3 shows autoradiograms of electrophoretically separated virion polypeptides of two independently purified preparations of HSV-1 [F1] virions and those of strain HSV-1 [F5] characterized by a similar isolation and passage history. The main feature of the data presented in Fig. 3 is that the three autoradiograms cannot be differentiated with respect to the relative amounts and electrophoretic mobility of the virion polypeptides. In contrast, the autoradiogram of electrophoretically separated virion proteins of five HSV-1 strains (HSV-1 [13], HSV-1 [13 v B4] HSV-1 [HFEM], HSV-1 [mP], and HSV-1 [MP]), Fig. 4 and 5, demonstrate that the structural polypeptides of strains characterized by long histories of passage in cell culture, and in some instances in experimental animals, differ extensively from those of the HSV-1 [F1] strain. The autoradiograms in Fig. 4 are presented as they appeared on the intact gel slab and separately to aid in pinpointing those polypeptides which are either missing in one or more strains or which do not coincide in electrophoretic mobility with those of HSV-1 [F1]. Specifically, HSV-1 [mP] differed from HSV-1 [F1] in that VP13 and 15.8 were absent, whereas a new polypeptide appeared between VP15 and VP16. HSV-1 [MP] was identical to HSV-1 [mP] from which it was derived except that VP8 was absent or drastically reduced. In the absence of VP8 the minor virion polypeptide VP6.5 (VP6A of ref. 9) can be observed on DATD cross-linked gels (see HSV-1 [MP] and HSV-1 [13 ts B4] electropherograms of Fig. 4). This trace component is demonstrable in all virions so far examined by electrophoresis on MBA gels, where it migrates slower than VP7 (see Fig. 1A). Virions of HSV-1 [MP] also lacked VP15.8 and there appeared to be quantitative differences in the region of VP17 and 18 as compared with corresponding regions of HSV-1 [mP] and of HSV-1 [F1]. HSV-1 [HFEM] differed in several respects from HSV-1 [F1]. The polypeptides in the mobility range of VP11 and 12 were more widely separated and neither comigrated with those of HSV-1 [F1]; the amount of VP13 was reduced and an additional polypeptide was present in the region between VP16 and VP17. In addition,

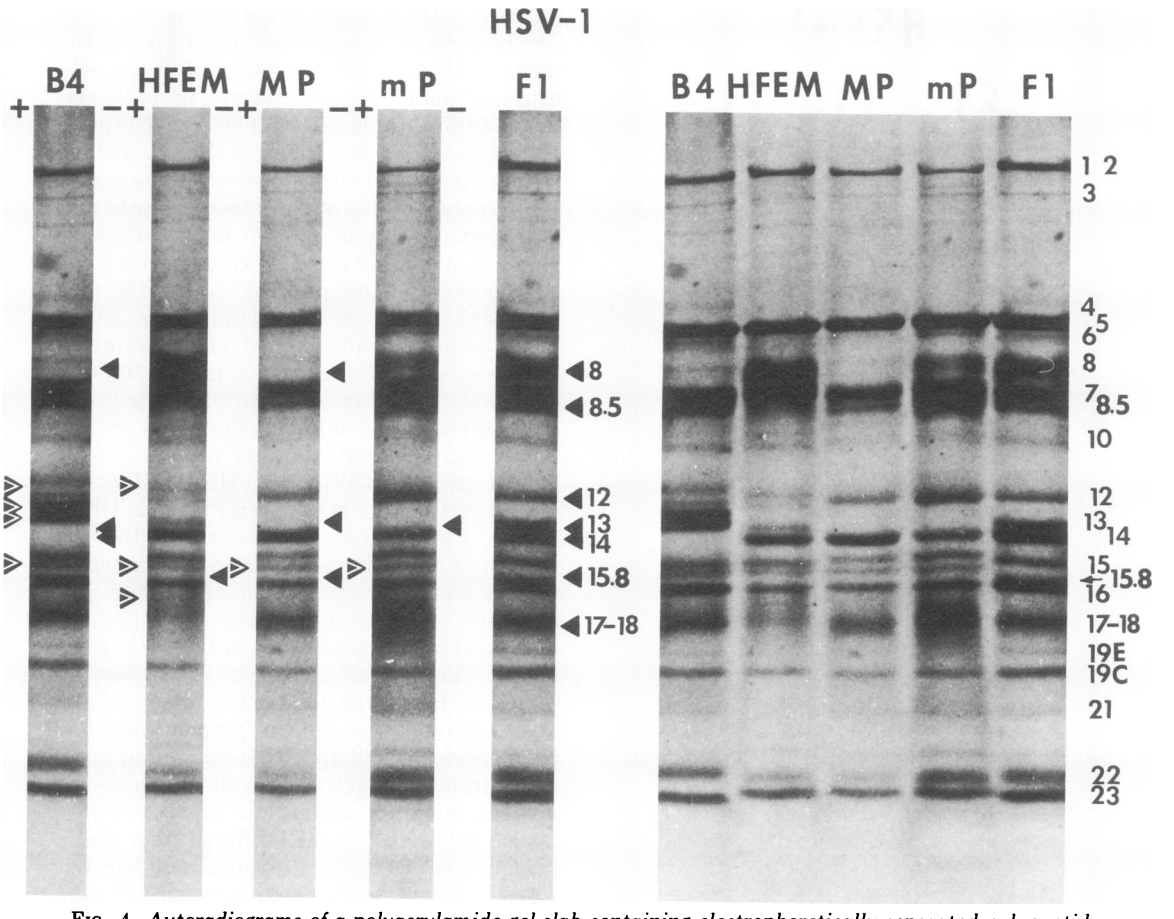


FIG. 4. *Autoradiograms of a polyacrylamide gel slab containing electrophoretically separated polypeptides from purified virions of HSV-1 [F1], HSV-1 [mP], HSV-1 [MP], HSV-1 [HFEM], and HSV-1 [13 ts B4] labeled with ^{14}C-amino acids. The intact autoradiogram is shown on the right together with the designations of HSV-1 [F1] virion polypeptides. To facilitate comparisons, the autoradiograms of each gel are also shown on the left. The arrows on the (−) side of each gel pinpoint the position of HSV-1 [F1] polypeptides absent from the various HSV-1 strains examined. The arrows on the (+) side indicate the polypeptides in virions of these other strains for which there is no electrophoretic analogue in the HSV-1 [F1] virions. The arrows against the gel containing the electrophoretically separated HSV-1 [F1] polypeptides indicate all polypeptides whose electrophoretic analogue is absent in one or more of the other HSV-1 strains tested.*

VP18 was either reduced in amount or absent from the HFEM. The electrophoretic profile of HSV-1 [13 v B4] proteins diverged more from that of HSV-1 [F1] polypeptides than did those of the other virus strains. Thus, VP8, 13 and 14 were missing, polypeptides in position 11 and 12 were even more widely separated than those in the corresponding region of the HSV-1 [HFEM] electropherogram, and two additional polypeptides were observed in the region between VP12 and 13 of HSV-1 [F1]. The additional polypeptide observed in the region between VP15 and 16 in the other strains was also present; and lastly, VP22 and 23 were separated by a significantly larger distance than the corresponding polypeptides of HSV-1 [F1]. Figure 5 shows autoradiograms of the polypeptides of the parent HSV-1 [13] strain and of HSV-1 [F1] subjected to electrophoresis in different slots of a single polyacrylamide gel slab, but with an intervening sample removed to permit identification of the polypeptides. It is noteworthy that HSV-1 [13] and HSV-1 [13 v B4] differ only in the region of the major virion glycoproteins, VP7–8.5. These differences may be the consequences of mutagenesis of the viral DNA with BUdR.

DISCUSSION

This paper deals with three aspects of protein composition of HSV-1 virions. It is convenient to discuss each of these topics separately.

Number of polypeptide species in the

HSV-1 virion. In an earlier paper in this series (22) our laboratory reported that polypeptides contained in HSV-1 [F] virions formed 24 bands on electrophoresis through polyacrylamide gels cross-linked with MBA. In this paper we are reporting that we have resolved several additional species of polypeptides by the use of polyacrylamide gels cross-linked with DATD. The separations of nonglycosylated polypeptides obtained in those gels were in general comparable to those previously obtained on MBA cross-linked gels, and the agreement between the molecular weights estimated in this and preceding studies were excellent (Table 1, columns a and b). The main advantage of the DATD cross-linked gels was their ability to separate a number of minor, glycosylated proteins from the nonglycosylated polypeptides with which they comigrated on MBA cross-linked gels. These analyses on DATD gels have shown that the HSV-1 virion contains at least 33 electrophoretically distinct species of polypeptides. Several comments should be made in connection with this observation. Although the 33 polypeptides do not exceed the potential informational content of HSV-1 DNA, we cannot exclude the possibility that not all polypeptide moieties of the 33 glycosylated and nonglycosylated polypeptides are primary gene products. It may be that the virion contains both a nonglycosylated precursor and its glycosylated product or the precursor and products from cleavage of a glycosylated polypeptide. However, the improved separation of minor glycosylated polypeptides has shown that they do not differ significantly in the extent of their glycosylation from that of the major glycoproteins. Thus, if minor glycosylated polypeptides represent a glycosylated subset of other nonglycosylated virion polypeptides, it is apparent that glycosylation is as extensive on this subset as on the major glycoproteins.

Molar concentrations of virion polypeptides. The data presented in Table 1 are of interest from several points of view. First, they illustrate the large disparities between the molar concentrations of certain polypeptides. These are evident, for example, from comparisons of the number of molecules per virion of VP4 or VP6 as with those of VP5, 14, 16, 22, and 23. Second, it is of interest to note that at least two capsid (VP5 and 19C) and two nonglycosylated, noncapsid (VP14, 16) polypeptides are present in nearly identical molarities suggesting possible interaction between each pair in the corresponding substructures of the virion. On the other hand the data point to some of the problems encountered in the attempts to estimate the molar concentrations of certain polypeptides. We refer specifically to the wide differences in the estimates of VP8, 8.5, and 22 obtained from analyses of the stained gels and from autoradiograms of such gels. We have al-

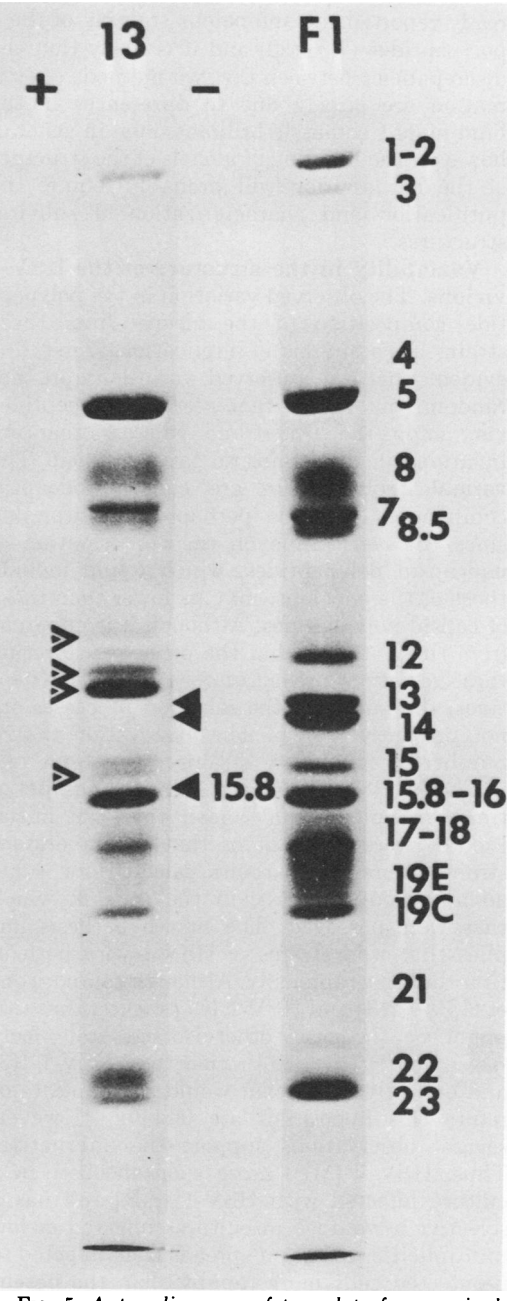


FIG. 5. *Autoradiograms of two slots from a single polyacrylamide gel slab containing electrophoretically separated polypeptides from purified virions of HSV-1 [13] and HSV-1 [F1] labeled with ^{14}C-amino acids. The print of the autoradiogram was labeled as in Fig. 4.*

ready reported the anomalous staining of these polypeptides (7, 6, 22) and it is likely that the discrepancies between the two methods of estimation are largely due to differences in the binding of Coomassie brilliant blue. In general, however, the building of models of the structure of the herpesvirion will probably require the purification and characterization of subviral structures.

Variability in the structure of the HSV-1 virions. The observed variation in the polypeptide composition of the virions from these strains has a number of implications. First, it is evident that the observed variations are not random, but rather that certain polypeptides vary among the virus strains, whereas others are invariant in all strains so far examined. The variable polypeptides are chiefly noncapsid components. This is perhaps not surprising since the constraints on the conformation of noncapsid polypeptides, which would include those of the envelope, may be lower than those of capsid polypeptides. Although it is apparent from these studies that the pressures selecting these variations are not uniform for all polypeptides, the nature of the selection processes are not presently known. The observation that a number of laboratory strains differ from two isolates of HSV-1 passaged a limited number of times in culture may mean that the initial isolates were different or that the laboratory strains represent variants selected for some advantageous property in the cells in which they were passaged. The first hypothesis implies that wild strains of HSV-1 vary both in time and geographically. Although comparisons of HSV-1 [13] and HSV-1 [F1] suggest that this might be the case, other isolates from Italy resembled HSV-1 [F1] rather than HSV-1 [13] and definitive data that would substantiate or refute this hypothesis are lacking. However, several observations support the alternative. Thus, HSV-1 [MP] arose spontaneously in a culture infected with HSV-1 [mP] and has a selective advantage in cultures infected at low multiplicities in that it spreads from infected to uninfected cells more rapidly than the parent strain (8). Another example is the selection of spontaneous mutants (HSV-1 [MPdk$^+$1p] and HSV-1 [MPdk$^+$sp]) capable of multiplying in dog kidney cells from cultures of these cells abortively infected with the HSV-1 [MP] virus (17). The mutants differed from the parent HSV-1 [MP] with respect to immunologic specificity, buoyant density in CsCl solution, and stability at 40 C—all of which are consistent with altered virion structure.

Finally, the data have some important practical implications since concurrent with, or related to, the variations in the noncapsid components are two other properties of the virus. Thus, the virion may exhibit altered immunologic specificity as has been clearly demonstrated for HSV-1 [MP] which is neutralized equally well by both anti HSV-1 and HSV-2 sera, whereas the parent HSV-1 [mP] virus is more readily neutralized by anti HSV-1 than by anti HSV-2 sera (5, 14). Moreover, cells infected with the laboratory strain differ from those infected with HSV-1 [F1] in details pertaining to development as evident from electron microscope studies (21) and from the nature of the social interaction (i.e., tight clumps, loose clumps, polykaryocytosis) of infected cells among themselves (5, 16). The obvious implications are that virus strains used as antigenic reagents in seroepidemiologic studies must be shown to be identical with the strains circulating in the population being analyzed since misleading data may otherwise result. Moreover, biochemical studies should include sufficient information on the virus strain being used to assess the validity of comparisons of data generated in different laboratories.

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