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Variability in the Structural Polypeptides of Herpes Simplex Virus 1 Strains: Potential Application in Molecular Epidemiology

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This paper reports on the variability of structural polypeptides of 53 strains of herpes simplex virus 1 isolated from Italy, Uganda, South Africa, and various locations in the United States. Most strains were passaged a limited number of times at low multiplicity outside the human host; a few strains were characterized by numerous passages at variable multiplicities in cell culture and experimental animals. The acrylamide gel electrophoresis of polypeptides from purified virions revealed seven variable polypeptides. Virion polypeptides (VP) 7, 11, 13, 14, 15.2, and 23 were present in at least two isotypic forms characterized by fast and slow electrophoretic mobilities. VP8 could not be detected in three strains. In addition, VP13, 15.2, and 23 in some strains were either absent or comigrated with other polypeptides. A variety of tests showed that the variability in electrophoretic mobility of polypeptides was reproducible and could not be attributed to artifacts of purification, solubilization, or electrophoresis. Attempts to classify the strains on the basis of electrophoretic mobility of five or all seven variable polypeptides yielded 14 and 19 groups, respectively. The bulk of the strains (41 of 53) fell into six groups. Not all possible permutations of variable polypeptides were observed. Comparison of early and late passages of laboratory strains showed that in the few instances tested the variability could not be attributed to the propagation of the virus outside the human host. Clustering of strains on the basis of country of origin was not observed. Some clustering of isolates on the basis of site of isolation was observed, and the data do suggest that further analyses of isolates for evidence of a correlation between the site of localization on the human body and the structural polypeptides might be useful. Electrophoretic characterization of structural polypeptides has the potential of becoming a powerful tool for epidemiological studies of herpes simplex virus infections.

This paper deals with the variability of herpes simplex virus 1 (human herpesvirus 1 [HSV-1]) structural polypeptides. A previous publication (10) reported that virion polypeptides (VPs) of strain HSV-1 (F1), a prototype of HSV-1 passaged at low multiplicities in human cells a maximum of four times, formed at least 33 bands on electrophoresis in sodium dodecyl sulfate polyacrylamide gels. Comparisons showed that several HSV-1 strains characterized by numerous passages outside the human host differed in several of their structural polypeptides from each other and from HSV-1 (F1) and another isolate HSV-1 (F5) passaged in the same fashion. The available data were not sufficient to determine whether the variability was related to the passage history outside the human host or to inherent instability of some structural polypeptides in nature. The studies described in this paper were designed to survey the extent of variability of HSV-1 strains.

MATERIALS AND METHODS

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

Viruses. Two sets of HSV-1 strains were selected for these studies. The first, designated as the limited-passage strains, consisted of isolates from various locations in United States, Italy, Uganda, and South Africa. All of these strains were passaged a limited number of times in culture, usually at relatively low multiplicities. An average of three passages and a maximum of six passages at low multi-

plicities (0.001 to 0.01 plaque-forming units/cell) were necessary to prepare stock virus of high titer before purification of enveloped virions. The second set, designated as unlimited-passage strains, consisted of laboratory viruses whose history was either uncertain or known to include numerous passages outside the human host. Strains deliberately exposed to mutagenic agents have been excluded. The passage history, site of isolation, country of origin, and the number of independent purifications of the 53 strains are given in Table 1. HSV strains were identified as type 1 in all instances on the basis of neutralization and immunofluorescence tests (7, 14, 26) and in many instances on the basis of buoyant density of the deoxyribonucleic acid (DNA) (8,13). The procedures for preparation of HSV stocks were as described by Roizman and Spear (20), except that the cells were infected at low multiplicity.

Solutions and chemicals. Cells were infected in phosphate-buffered saline (6) supplemented with 1% inactivated calf serum and 0.1% glucose. Maintenance medium for infected cells was mixture 199 supplemented with 1% dialyzed calf serum. Labeled amino acids purchased from Schwarz/Mann, Orangeburg, N.Y., included L-[U-^{14}C]leucine, L-[U-^{14}C]isoleucine, and L-[U-^{14}C]valine, all with specific activities of approximately 300 mCi/mM. Labeling medium consisted of mixture 199 containing one-third the normal concentration of leucine, isoleucine, and valine and supplemented with 1% dialyzed calf serum. The concentration of labeled amino acids ranged from 0.2 to 0.3 μCi/ml of labeling medium.

Production of radiolabeled, enveloped virions. Roller-bottle monolayer cultures of HEp-2 cells, containing approximately 2×10^8 cells at confluency, were infected with an input multiplicity of 1 to 5 plaque-forming units of virus per cell, then incubated at 34 C in maintenance medium. The infected cells were labeled in 50 ml of labeling medium from 6 h postinfection. The cells were harvested at 48 h postinfection.

Purification of enveloped virus particles. The purification procedure employed in this study was that of Spear and Roizman (24) as modified by Heine et al. (10). All steps in the purification procedure were performed at 0 to 4 C. Cells labeled from 6 to 48 h postinfection were scraped off the glass surface and collected by low-speed centrifugation. The supernatant fluid was decanted and the infected-cell pellet was suspended in approximately 2 volumes of 1 mM phosphate buffer, pH 7.4. After swelling in ice for 30 min, the cells were disrupted with five strokes of a tight-fitting Dounce homogenizer, and sufficient 60% (wt/wt) sucrose was added to yield a final concentration of 0.25 M. The nuclei were pelleted by low-speed centrifugation, and the cytoplasmic extract from 2×10^8 to 4×10^8 cells was centrifuged through a 35-ml dextran T 10 density gradient (1.04 to 1.09 g/cm^3 prepared in 1 mM phosphate buffer) for 1 h at 25,000 rpm in the Beckman SW27 rotor. The enveloped nucleocapsids (virions) were found in the diffuse light-scattering band at the middle of the tube. The band was removed from the top of the gradient in a small volume (approximately 2.5 ml), diluted to 36 ml with 1 mM phosphate buffer, and centrifuged at 25,000 rpm for 1 h in the SW27 rotor to pellet the virions. The radio-labeled virions were resuspended in 0.2 to 0.5 ml of 1 mM phosphate buffer, depending on the pellet size, and stored at −70 C before solubilization and electrophoresis on polyacrylamide gels.

Polyacrylamide gel electrophoresis. The electrophoretic, staining, and autoradiographic techniques were as described previously (10, 24), except that the polyacrylamide gel slabs were cross-linked with *N,N'*-diallyltartardiamide (Aldrich Chemical Co., Milwaukee, Wis.) in place of the same weight of *N,N'*-methylenebisacrylamide. The stacker and separation gel contained 3 and 8% acrylamide, respectively. Before electrophoresis, the labeled virion proteins were denatured and solubilized by boiling for 2 min in a mixture containing sodium dodecyl sulfate and beta-mercaptoethanol. The solubilized proteins in 50-μl volumes were subjected to electrophoresis at a constant current of 8 mA.

The gels were stained with Coomassie brilliant blue, destained in acetic acid and methanol, and dried. Analyses of radioactive proteins were done by autoradiography with Cronex X-ray film.

The equipment employed in these studies permitted simultaneous electrophoretic separations of up to 12 samples in a single gel slab. To facilitate comparisons, the samples subjected to electrophoresis included HSV-1 (F1) and at least one other standard strain (HSV-1 [14]) placed either at the end slots and both at end and middle slots.

RESULTS

Extent of variation in the electrophoretic mobility of HSV-1 structural polypeptides. The basic observations we wish to document are (i) 53 strains varied in at least seven polypeptides, and (ii) two kinds of differences were observed. In most instances one or more of six HSV-1 (F1) polypeptides, i.e., VP7, VP11, VP13, VP14, VP15.2, or VP23, were replaced by a corresponding number of polypeptide bands that migrated either faster or slower than polypeptides they replaced. However, the absolute number of bands formed by the electrophoretically separated polypeptides remained constant. In these instances, we tentatively identified the bands containing the electrophoretically differentiable polypeptides on the basis of relative concentration of the polypeptides in the virion and by the observation that the number of forms of the variable polypeptides was generally two and at most three. For example, VP23 of strain A457 and A647 (Fig. 1) has the same electrophoretic mobility as VP23 of strain F1 (molecular weight, 36,000). Strain A222 (Fig. 2) differs from A457 and A647 in that VP23 (molecular weight, 36,000) is replaced by a more rapidly migrating polypeptide with an apparent molecular weight of 34,000. Of the 53 strains, 52 contained either the slow (36,000 molecular

TABLE 1. *Origin, properties, and classification of HSV-1 strains*

Group strain designation	Country of origin[a]	Site of isolation	Passage and history[b]	No. of independent purifications	No. of polyacrylamide gel runs	VP7 (mol wt)	Source and reference
Group A							
01	US(C)	Cornea	LP	2	2	128,000	7
02	US(C)	Cornea	LP	1	2		7
03	US(C)	Cornes	LP	2	2		7
04	US(C)	Cornea	LP	1	2		7
08	US(C)	Cornes	LP	1	2		7
A457	US(A)	Brain	LP	2	4		+
A647	US(A)	Brain	LP	2	4		+
J145(B)	SA	Liver	LP	3	6		2
Group B							
A222	US(A)	Genitals	LP	1	1		+[c]
J708	US(A)	Brain	LP	1	1		+
H389	SA	Lung	LP	3	3		2
117	I	Lip	LP	2	4		26
U5001	U	Mouth	LP	2	3		++
Sch	US(Cinn)	Brain	UP	1	2		25
89	I	Cornea	LP	1	1		*
91	I	Lip	LP	1	1		*
Group C							
F9	US(C)	Lip	LP	1	8		7
F11	US(C)	Skin	LP	1	4		7
63A	I	Mouth	LP	2	3	128,000	*
63B	I	Mouth	LP	1	1	128,000	*
14	I	Cornea	LP	2	4		*
466	I	Lip	LP	2	3		5
161	I	Lip	LP	1	1		1
Group D							
25	I	Cornea	LP	2	4		27
30	I	Lip	LP	2	3		*
HFEM	US(NY)	Lip	UP	2	2		**
STH2 HFEM	US(NY)	Lip	UP	1	2		***
V1004	US(A)	Mouth	LP	1	1		+
V1006	US(A)	Genitals	LP	1	1		+
P82M	US(A)	Genitals	LP	1	1		+
P82C	US(A)	Skin	LP	1	1		+
Group E							
VR3	US	Brain	UP	1	1		22
A201	US(A)	VR3	UP	1	1		14, 15
S78	US(A)	VR3	UP	1	1		14, 15
NT	US(B)	Skin	LP	1	6		11
mP	US	NT[d]	UP	3	3		11
MP	US	NT	UP	3	3		11
Group F							
F1	US(C)	Lip	LP	40	100		7
F5	US(C)	Lip	LP	3	3		7
Group G							
07	US(C)	Skin	LP	1	2		7
A548	US(A)	Trigeminal ganglia[e]	LP	2	2		+
J1052	US(A)	Genitals	LP	1	3		+
J738	US(A)	Genitals	LP	1	2		+
J145N	US(A)	Genitals	LP	1	2		+
Group H							
F7	US(C)	Skin	LP	1	2		7
A447	US(A)	Brain	LP	2	4		+

TABLE 1—*Continued*

Group strain designation	Country of origin[a]	Site of isolation	Passage and history[b]	No. of independent purifications	No. of polyacrylamide gel runs	VP7 (mol wt)	Source and reference
Group I							
2	I	Lip	LP	2	3	128,000	26
13	I	Lip	LP	3	10	128,000	26
A602	US(A)	Trigeminal ganglia[e]	LP	3	4		+
Group J							
35	I	Mouth	LP	3	5	128,000	26
Group K							
A428	US(A)	Trigeminal ganglia[e]	LP	3	5	128,000	+
Group L							
A1056	US(A)	Genitals	LP	1	2		+
Group M							
KOS	US(H)	Lip	UP	2	4	Trace amount of VP13	16
Group N							
U5098	U	Mouth	LP	3	4		++

[a] Abbreviations: US, United States; I, Italy; U, Uganda; SA, South Africa; B, Baltimore; C, Chicago; NY, New York; A, Atlanta; H, Houston; Cinn, Cincinnati.

[b] LP, Limited-passage history; UP, characterized by numerous passages outside the human host.

[c] +, Strain from A. Nahmias, Emory University, Atlanta, Ga.; ++, Andrew G. Dean, Communicable Disease Center, Atlanta, Ga.; *, M. Terni, University of Bologna, Bologna, Italy; **, P. Wildy, University of Birmingham, Birmingham, United Kingdom; ***, A. Buchan, University of Birmingham, Birmingham, United Kingdom.

[d] NT, Not tested.

[e] Human trigeminal ganglia were explanted in culture. The virus was collected after development of cytopathic effects.

weight) or fast (34,000 molecular weight) forms. The only exception was strain A428 (Fig. 3), which contained a form of VP23 characterized by an intermediate electrophoretic mobility corresponding to a molecular weight of 35,000. In a similar fashion, by comparing strains differing in one or at most two polypeptides we were able to identify fast- and slow-migrating forms of VP11, 13, 14, and 15.2. Autoradiograms of electrophoretically separated polypeptides of representative strains are shown in Fig. 1, 2, and 3. For the purpose of this report, we shall define the variable polypeptides as isotypic, i.e., common to HSV-1 but differentiating classes and subclasses within the serotype.

The absence of analogues of VP8, VP13, or of VP15.2 in a few strains constituted the second type of difference among strains (Fig. 1 and 2). For example, no analogues of VP8 were found in strains MP (10), V1004 (Fig. 2), and A1056 (Fig. 2). Similarly, analogues of VP15.2 could not be recognized in A447 and F7, whereas isotypic forms of VP13 were absent from nine strains. In the case of strain MP, it has recently been shown by Spear (Proc. Symp. Oncogenesis Herpesviruses, in press) that it does not have polypeptides with the immunologic specificity of VP8, and therefore it is likely that VP8 is missing and not simply comigrating with another polypeptide. In all other instances, and especially in the case of VP13 of strain A1056, it is conceivable that isotypic forms of the "missing" polypeptide are present but comigrate with another polypeptide.

Analysis of strains for covariant properties. To facilitate comparisons, we subdivided the strains into groups differing with respect to the electrophoretic mobility of VP11, 13, 14, 15.2, and VP23. We have excluded VP7 and VP8 from this scheme since their inclusion would add five additional groups each containing one strain. as shown in Table 2, this method of clustering yields 14 groups. The electrophoretic properties of the polypeptides of each strain can be readily deduced from Tables 1 and 2. It is noteworthy that most (41 of 53) strains cluster in six groups. Exclusion of VP15.2 as a criterion for classification would raise the total number of strains in these groups to 45; inclusion of VP7 into this scheme would decrease the number to 39. Clearly, irrespective of the method of classifying the 53 strains, the bulk falls into a few groups.

Based on five variable polypeptides with two, i.e., fast- or slow-migrating, isotypic forms we could expect many more groups than the 14 we

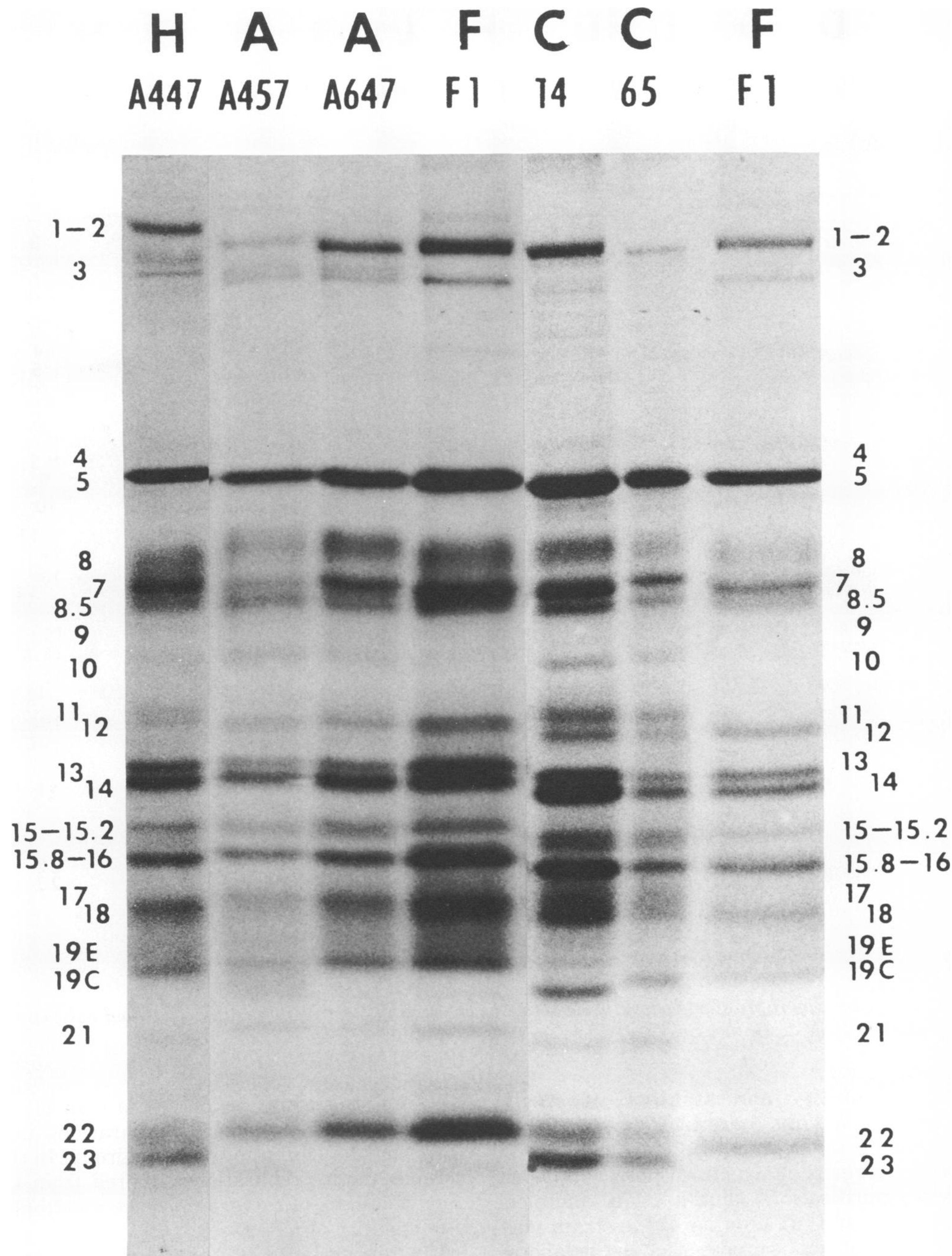

FIG. 1. *Autoradiograms of polyacrylamide gel slabs containing electrophoretically separated polypeptides of representative HSV-1 strains. The single letter at the top identifies the group in which the strain was tentatively placed. The designation below the letter identifies the strain. The origin and other data concerning the strains are listed in Table 1.*

actually observed. Clearly, some polypeptide combinations are either prohibited or low in incidence. For example, we have not observed strains with all five polypeptides migrating rapidly. Especially striking was the absence of strains with slow VP13 and fast VP14. On the other hand, strains with fast VP13 and slow VP14 could account for L, M, and N groups.

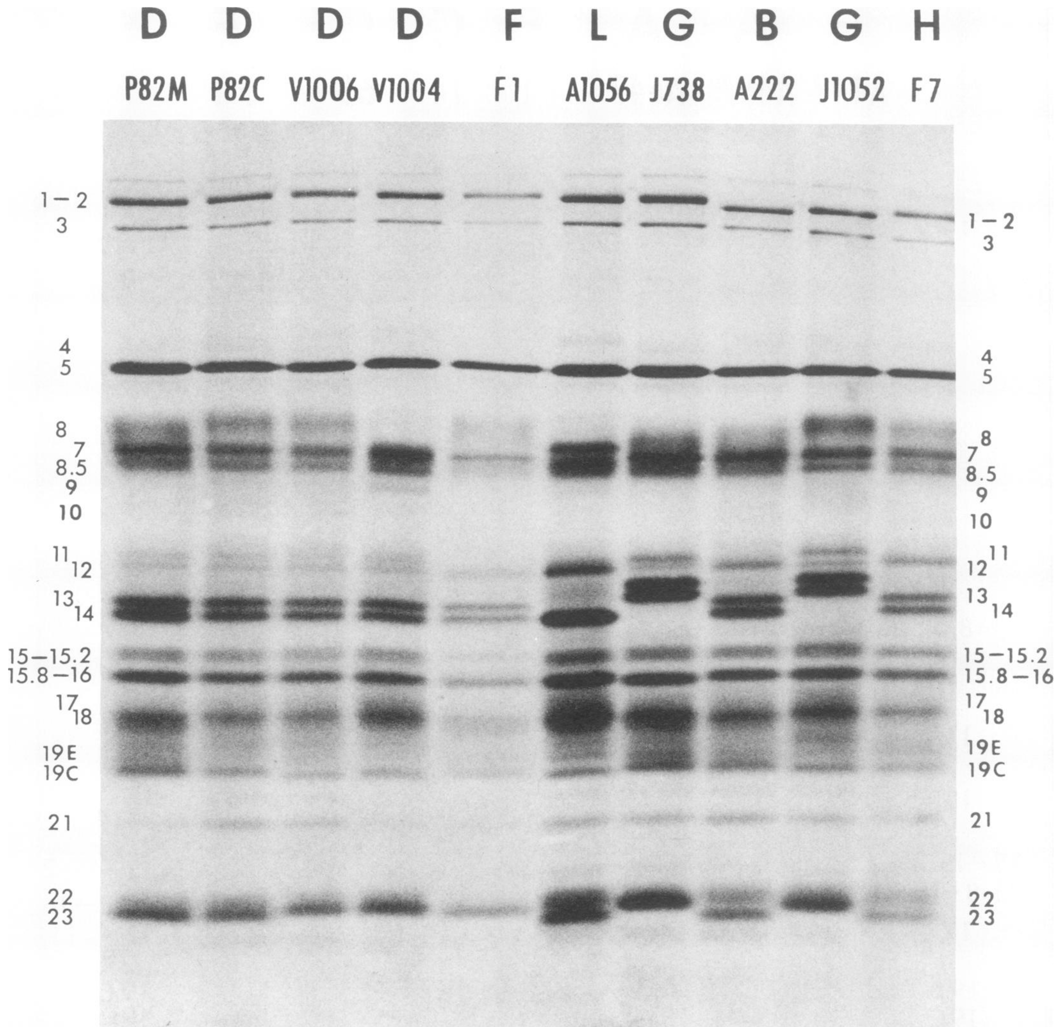

FIG. 2. *Autoradiograms of polyacrylamide gel slabs containing electrophoretically separated polypeptides of representive HSV-1 strains. See legend to Fig. 1.*

Reproducibility and stability of strain phenotype. We are concerned here with two problems: (i) the reproducibility of the electrophoretic patterns of structural polypeptides on repeated purification, solubilization, and electrophoresis, and (ii) stability of the strain phenotype on continuous propagation in the laboratory. The data bearing on these issues may be summarized as follows.

Of the 53 strains studied, 31 were independently purified and subjected to electrophoresis at least two times, and 23 strains were analyzed at least three times. In each instance, the electrophoretic profiles were identical. In one instance we unknowingly passaged and made duplicate stocks subsequently labeled 63A and 63B from the same isolate. Analyses of the purified virions from both preparations indicated that they fell into the same group. In this instance, both preparations differed from the other members of the group in the electrophoretic mobility of VP7.

Another measure of the reproducibility of polyacrylamide patterns emerged from examination of multiple isolates from the same patients. As shown in Table 3, we could not differentiate between the viruses isolated from lip and cornea, respectively, of patients 1 and 2. Similarly, we could not differentiate a strain isolated from a newborn (P82C) from strain P82M isolated from mother's urogenital tract (Fig. 2). In these instances, it could be predicted

that the corresponding pairs of isolates would be identical (15). However, strains isolated from the throat (V1004) and penis (V1006) of another patient, although classified in the same group, differed in that V1004 lacked VP8, whereas V1006 did not (Fig. 2), suggesting that they were not identical. In this instance no prediction could be made; the fact that the oral and genital isolates were not identical argues against autogeneous transmission (15).

With one exception all strains characterized by numerous passages outside the human host appear to cluster with limited passage strains. The exception is KOS, which is the sole constituent of group M. We cannot exclude the possibility that limited-passage strains identical to KOS exist or that many unlimited-passage strains have drifted genetically to another group characterized by a phenotype more advantageous to growth in cell culture. It is noteworthy that this study did not contribute additional evidence demonstrating changes in virion structural polypeptides on serial propagation beyond those published previously (10, 12, 17). Thus no difference was observed between strain mP, passaged more than 60 times in cell culture, and the parent strain NT (11) passaged more than three times on the chorioallantoic membrane and once in FL cells. Parental VR3 virus could not be differentiated from virus stocks prepared after five serial passages in mouse brain (strain S78) or in rabbit kidney

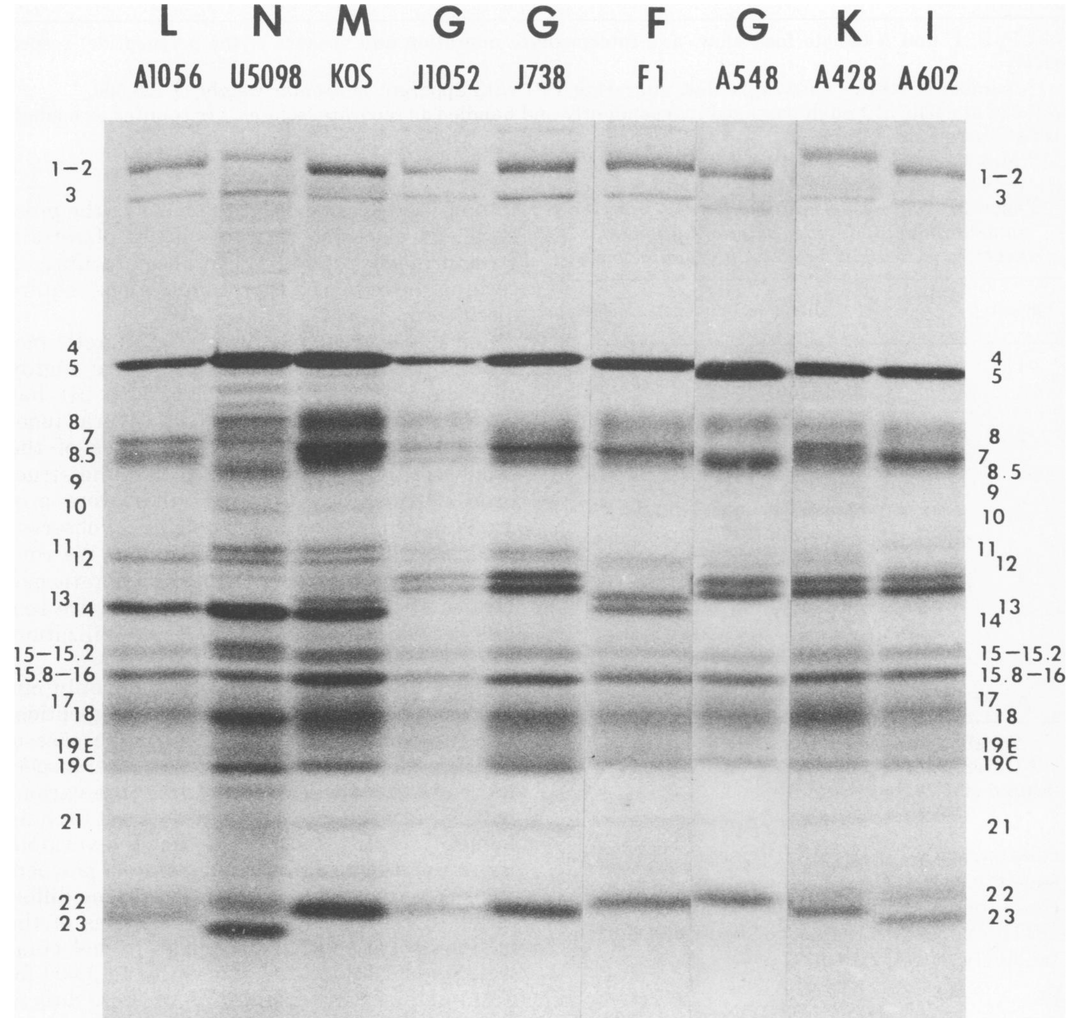

FIG. 3. *Autoradiograms of polyacrylamide gel slabs containing electrophoretically separated polypeptides of representative HSV-1 strains. See legend to Fig. 1.*

TABLE 2. *Tentative classification of HSV-1 strains according to variable polypeptide composition*

Group	No. of strains	Variable VPs: relative migration and mol wt (10^{-3})					
		VP11	VP13	VP14	VP15.2	VP23	Other
A	8	F[a] 94	F 82	F 80	S 72	S 36	S VP7 (128) (1/8)[b]
B	8	F 94	F 82	F 80	S 72	F 34	
C	6[c]	S 97	F 82	F 80	S 72	F 34	S VP7 (128) (1/6)
D	8	S 97	F 82	F 80	S 72	S 36	VP8 absent (1/8)
E	6	F 94	A	F 80	S 72	S 36	VP8 absent (1/6)
F	2	F 94	F 82	F 80	F 69	S 36	
G	5	S 97	S 91	S 89	S 72	S 36	
H	2	F 94	F 82	F 80	A	F 34	
I	3	S 97	S 91	S 89	S 72	F 34	S VP7 (128) (2/3)
J	1	F 94	S 91	S 89	S 72	F 34	S VP7 (128) (1/1)
K	1	F 94	S 91	S 89	S 72	I 35	S VP7 (128) (1/1)
L	1	F 94	A[d]	F 80	S 72	F 34	S VP7 (128) (1/1) VP8 absent (1/1)
M	1	S 97	A	F 80	S 72	S 36	
N	1	S 97	A	F 80	S 72	F 34	

[a] F, S, I, and A denote fast, slow, and intermediate migration and absence of the polypeptide, respectively.

[b] Number of strains showing a slow migrating VP7 with apparent molecular weight of 128,000.

[c] 63a and 63b, although passaged independently and handled as separate isolates, are counted as a single isolate here and in Table 3.

[d] May be comigrating with VP14.

TABLE 3. *Analyses of multiple isolates from the same individual or from pairs of individuals suspected of being infected with the same virus*

Patient	Strain no.	Site of isolation	Group
1	25	Cornea	D
	30	Recurrent lip	D
2	89	Cornea	B
	91	Recurrent lip	B
3	63a	Mouth (primary gingivostomatitis)[a]	C
	63b		
4	V1006	Genitals (penis)	D
	V1004[b]	Throat	D
5	P82M	Vulva (mother)	D
6	P82C	Neonatal herpes (child)	D

[a] Parallel passages of the same isolate.

[b] Differentiated from V1006 on the basis of the absence of VP8.

cells (strain A201). Similarly, strain HFEM passaged extensively in HeLa and HEp-2 cells could not be differentiated from the HFEM (STH2) parent that had not been passaged extensively in cell culture.

DISCUSSION

The data presented in this paper indicated that the 53 strains of HSV-1 can be classified into at least 14 groups on the basis of the presence and electrophoretic mobilities of certain structural polypeptides. Several comments concerning the data and their implications require discussion.

Our experience that the electrophoretic mobilities of structural polypeptides are highly characteristic and reproducible (10, 24) has been reinforced in this study by several independent analyses of the polypeptides of the same strains, and by evidence that the structural polypeptides of strains that were predicted to be identical based on clinical observations were in fact indistinguishable. We conclude that the differences in electrophoretic mobilities of the structural polypeptides are real and not artifacts of purification, solubilization, or electrophoresis.

With exception of VP8, of which only one form was recognized, the variable polypeptides are either nonglycosylated, as in the case of VP23, or minimally glycosylated. We suspect therefore that the isotypic forms of the various polypeptides differ largely with respect to chain length. It is also noteworthy that the variability in the electrophoretic migration of polypeptides is not continuous but discrete; the differences in the electrophoretic mobilities of the isotypes correspond to differences in molecular weights of 1,000 to 2,000 for VP23 to 9,000 for VP13 and 14. One implication of these data is that the function of these polypeptides is not affected by possible variations in conformation resulting from loss or gain of certian amino

acids. It is noteworthy that only VP23 is a component of the capsid—the viral substructure whose assembly could be expected to be most affected by conformational changes in its structural polypeptides. The rest of the polypeptides are components of the envelope (VP8) or of the structure designated as the tegument (18) and localized between the capsid and envelope (VP11, 13, 14, and 15.2). Dependence of proper assembly and stability of the subviral structures on the conformation of the polypeptides may explain the fact that only seven of the currently recognized 33 structural polypeptides are variable. It may also explain the fact that we were able to discriminate a maximum of 19 groups, i.e., much less than the number of possible groups that could result from random assortment of two to three isotypic forms of the seven variable polypeptides. Parenthetically, the variation in electrophoretic mobilities of viral polypeptide suggests that designations of polypeptides by their apparent molecular weight may be misleading and less useful than the numerical system employed by us (10, 24) but annotated with the apparent molecular weight.

The studies reported in this article support earlier reports concerning the variability of HSV-1 and HSV-2 strains from our as well as from other laboratories (7, 10, 12, 17, 20, 23, 28). The extent of variation and its significance is, however, not yet fully known. The recognition of at least 14 groups among 53 strains suggest that systematic analyses of many more strains will undoubtedly uncover additional groups. It is also likely that isotypic forms exist among nonstructural polypeptides as well, and moreover, variations in the apparent molecular weights of some structural polypeptides imply appropriate differences in base sequence in viral DNAs. Studies on the DNAs of a small number of HSV-1 and HSV-2 strains have in fact revealed differences in the restriction enzyme cleavage sites and in the size of some fragments (9). Moreover, the DNA of strain Schooler (group B) is characterized by a slightly lower density than that of F1 (group F) (E. Cassai, unpublished data). The potential significance of these findings arises from the fact that differences between HSV-1 and HSV-2 include localization on different parts of the human body in addition to structural and genetic differences (4, 21). Although the studies described in this article were not designed to investigate the possible relationship between structural polypeptides and site of localization in the human body, certain trends are readily apparent. Thus the distribution of strains into groups by country or origin (Table 4) failed to show clear evidence of geographic clustering, but the number of strains was too low to permit a definitive conclusion. On the other hand, the clustering of isolates from cornea in group A and from central nervous system in a few groups (Table 5) is impressive, but the significance of these findings is not clear since we cannot exclude the possibility of sampling errors. The data do sug-

TABLE 4. *Distribution of HSV-1 strains according to country of origin*

Country of isolation	Frequency distribution of isolates among groups													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
United States	7	3	2	6	6	2	5	2	1		1	1	1	
Italy		3	4	2					2	1				
Uganda		1												1
South Africa	1	1												

TABLE 5. *Distribution of HSV-1 strains according to site of isolation on human body*

Site of isolation	Frequency distribution of isolates among groups													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Ocular	5	1	1	1										
Oral		3	4	4		2			2	1			1	1
Trigeminal							1		1		1			
Brain	2	2			3			1						
Skin			1	1	3		1	1						
Genital		1		2			3					1		
Generalized infections	1	1												

gest that there might be a relationship between the biochemical properties of virus and its localization in the human body and that a more extensive survey might be warranted.

The inherent reproducibility of these experiments and apparent stability of the structural polypeptides suggest that analyses of structural polypeptides alone or in conjunction with analyses of fragments generated by restriction endonuclease cleavage of viral DNAs might serve as a powerful epidemiologic tool especially useful in tracing sources and chains of transmission of viral infection.

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ADDENDUM IN PROOF

After this work was completed, we received from F. Kapp (Hershey Medical Center, Hershey, Pa.) a new isolation of KOS strain from the recurrent labial infections of that individual. Analysis of the limited-passage new KOS strain indicated that it belongs in the D group.

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