JOURNAL OF VIROLOGY, Dec. 1973, p. 1347-1365

Vol. 12, No. 6
Printed in U.S.A.

Proteins Specified by Herpes Simplex Virus

XI. Identification and Relative Molar Rates of Synthesis of Structural and Nonstructural Herpes Virus Polypeptides in the Infected Cell

ROBERT W. HONESS AND BERNARD ROIZMAN

Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

Received for publication 14 August 1973

Analyses of polypeptides made in HEp-2 cells infected with herpes simplex virus type 1 by high-resolution polyacrylamide gel electrophoresis revealed the synthesis of at least 49 infected cell polypeptides (ICP) ranging in molecular weight from 15,000 to 280,000. Evidence for virus specificity based on increased rates of synthesis postinfection, immunological specificity, and viral control of mobility and rate of synthesis was available for 47 of the ICP. These 47 polypeptides can account for 75% of the virus genetic information assuming a DNA molecular weight of 10^8 and asymmetric transcription. On the basis of their mobility relative to virion proteins, the ICP were classified as structural (S, 23 polypeptides), nonstructural (NS, 16 polypeptides), and unassigned (U, 10 polypeptides). Analysis of the synthesis of the ICP revealed the following. (i) Rapid posttranslational cleavages of HSV proteins were not detected; in parallel experiments rapid posttranslational cleavages were readily demonstrated in poliovirus-infected cells and these were blocked by protease inhibitors. (ii) Slow posttranslational changes in the mobility of at least two polypeptides were observed. (iii) Analysis of the rates of synthesis of ICP examined at four intervals postinfection revealed regulation of the pattern and amount of ICP synthesized. ICP formed six classes (A to F) differing in their kinetics of synthesis. S and NS ICP were distributed nonrandomly among these classes. Thus, of the sum of S protein amino acid sequences apportioned among these kinetic classes, 47%, constituting class A and comprising "late" structural proteins, were characterized by progressively increasing rates of synthesis until at least 12 h postinfection; whereas "early" structural proteins constituting class C, amounting to 31% of the total amino acid sequences, were synthesized with initially increasing rates until 4 h postinfection and with declining rates thereafter. NS polypeptides and remaining S polypeptides were distributed among the other kinetic classes—B, D, E, and F. Control of protein abundance was evident in that the polypeptides were not made in equimolar amounts. However, S and NS polypeptides could not be differentiated on the basis of their molar rates of synthesis. The bulk of the detected polypeptides did not differ by more than eightfold in their molar rates of synthesis.

Herpesvirus replication requires the implementation of two programs, the transcription of viral DNA and the translation of viral messages. Recent papers (6, 7, 21) from our laboratory have documented the existence of temporal controls of viral DNA transcription and controls of molar abundance of the RNA transcripts accumulating in the infected cell. In this paper we report the results of experiments designed to outline the time of synthesis and abundance of the translational products of the virus.

As a preface to this report, it seems desirable to summarize the background and some of the problems encountered in this work.

Previous attempts to analyze the polypeptides made in infected cells, defined as infected cell polypeptides (ICP), both in this laboratory (23) and in others (1, 10) are currently of limited value for two main reasons. First, the techniques of polyacrylamide gel electrophoresis employing the continuous buffer system (19), combined with the estimation of radioactivity within slices of fractionated gels, were inadequate to resolve the large number of proteins made in the herpesvirus-infected cell. Second, the procedure then used for the purification of

herpesvirus particles was inadequate (14), and hence no meaningful classification of proteins as either structural or nonstructural could be made. The availability of techniques for the purification of enveloped nucleocapsids (virions) (24), for high resolution electrophoresis of proteins on polyacrylamide gels (3, 12), and for quantitative assay of separated proteins (5) has enabled us to undertake the present studies.

The major difficulty encountered in the current studies of ICP is the differentiation between polypeptides specified by the virus and the host, although the bulk of host proteins ceases to be made after infection (23). Compelling evidence that a polypeptide is virus specific is its synthesis in a cell-free amino acid incorporating system programmed by mRNA complementary to viral DNA. Since this is not yet feasible, we have defined ICP as virus specific if they meet one or more of the following criteria, i.e., (i) stimulation in the rate of synthesis postinfection in the face of general decline in host protein synthesis; (ii) variations in properties of ICP as a function of the virus strain infecting the cell, and (iii) immune precipitation of ICP by antisera reactive solely with virus antigens.

In a similar manner, the differentiation between structural and nonstructural polypeptides ideally requires the rigorous demonstration of chemical identity between the structural proteins of the virus, previously designated (24) as virion proteins (VP), and their putative analogues among the ICP. In practice we have temporarily defined ICP as structural or nonstructural solely on the basis of electrophoretic mobility relative to VP, pending a more complete characterization of these proteins.

MATERIALS AND METHODS

Solutions and chemicals. Maintenance medium for infected cells was mixture 199 supplemented with 1% calf serum. Labeling medium consisted of mixture 199 containing one-tenth the usual amounts of leucine, isoleucine, and valine and supplemented with 1% dialyzed calf serum. Labeled amino acids were purchased from Schwartz/Mann, Orangeburg, N. Y., and were as follows: L-isoleucine-*U*-^{14}C, L-leucine-*U*-^{14}C, and L-valine-*U*-^{14}C, all with specific activities of approximately 300 mCi/mmol; L-isoleucine-^{3}H, L-leucine-^{3}H, and L-valine-^{3}H with specific activities from 1 to 30 Ci/mmol; and a mixture of thirteen ^{14}C-labeled amino acids (reconstituted protein hydrolyzate, composed of alanine, arginine, aspartic acid, glutamic acid, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine with specific activities of about 150 to 450 mCi/mmol). Tolylsulfonyl-lysyl chloromethyl ketone (TLCK), a specific inhibitor of trypsin, and tolylsulfonyl-phenylalanyl chloromethyl ketone (TPCK), a specific inhibitor of chymotrypsin activities (20) were obtained from Nutritional Biochemical Co., Cleveland, Ohio.

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

Viruses. Most of the experiments described in this paper were done with the F prototype of HSV-1 [HSV-1(F)]. Other strains analyzed in some of the studies were HSV-1(mP) and HSV-2(G) corresponding to a laboratory strain of HSV-1 and the G-prototype of HSV-2, respectively. The origin and some properties of these strains have been reported elsewhere (4). Procedures for production of HSV strains were as described by Roizman and Spear (17) with the exception that HSV-1(F) and HSV-2(G) have been passaged a maximum of four times at low multiplicity prior to their use in the high-multiplicity infections described in this study. Titrations of infectious virus were performed as described by Roizman and Roane (15).

An attenuated vaccine strain of poliovirus type 1 was employed as a positive control in experiments dealing with rapid posttranslational cleavages of virus polypeptides. Media and procedures employed for infection and radiolabeling of poliovirus-infected HEp-2 cells were identical to those described for HSV-infected cells.

Purification of isotopically labeled, enveloped virus particles. Infected cells were labeled from 4 to 24 or 30 h postinfection with either ^{3}H-leucine, isoleucine, and valine (1 to 1.5 μCi of each amino acid per ml of labeling medium) or ^{14}C-leucine, isoleucine, and valine (0.08 μCi of each amino acid per ml) in 50 ml of labeling medium per 2×10^{8} infected cells. Virions were purified from labeled cells essentially as described by Spear and Roizman (24), except that the flotation through the discontinuous sucrose gradient, which followed the banding in a dextran-10 gradient, was replaced by a second dextran-10 gradient sedimentation. Particles purified by this modified procedure had a polypeptide profile similar to that obtained using the original procedure. Particles purified in this way consisted of approximately 95% of enveloped particles (unpenetrated by neutral phosphotungstate negative stain) with particle to infectivity ratios of approximately 100.

Labeling of proteins synthesized by infected and uninfected cells. Confluent HEp-2 cell monolayers in tissue culture flasks (approximately 2×10^{6} cells per flask) were exposed to 20 PFU of virus per cell (in 20 ml of maintenance medium) or were mock infected with 2.0 ml of this medium. Inoculated cultures were incubated with constant agitation for 1 h at 37 C, and thereafter virus or mock inocula were decanted. The monolayers were then rinsed (5.0 ml of maintenance medium per flask), replenished with 5.0 ml of maintenance medium per flask, and reincubated at 37 C. For labeling, the cultures were rinsed and then replenished with labeling medium containing ^{14}C-leucine, isoleucine, and valine (0.3 to 1.7 μCi of each amino acid per ml). At the end of the labeling period the cells were either: (i) rinsed with ice-cold phosphate-buffered saline (3×5.0 ml/flask) to terminate incorpora-

tion ("pulse") and then stripped from the flask, denatured and solubilized by heating at 80 C with a small volume of 1% sodium dodecyl sulfate (SDS) for subsequent trichloroacetic acid precipitation or 2% SDS, 5% β-mercaptoethanol, 0.05 M Tris-hydrochloride (pH 7.0) for subsequent polyacrylamide gel electrophoresis, or (ii) rinsed (3 × 5.0 ml), replenished with maintenance medium (at 37 C), and reincubated in the absence of exogenous labeled precursors prior to terminating incorporation, as above ("pulse chase").

The times at which labeled precursors were added and removed, etc., are stated as the times in hours after the addition of the virus inoculum.

Radio-immune precipitation. The radio-immune precipitation reactions for analysis of herpesvirus-specific polypeptides were performed as described by Honess and Watson (J. Gen. Virol., in press). Briefly, the supernatant fluid obtained after sedimentation (35,000 rpm, 1 h, in a Beckman SW40 rotor) of a homogenate of infected HEp-2 cells, which had been labeled with ^{14}C-amino acids from 4.5 to 6.5 h postinfection, was mixed with an excess of a rabbit hyperimmune serum specific for herpes simplex virus antigens and incubated at +4 C overnight. The resulting immune precipitate was sedimented (3,500 rpm for 15 min), washed thoroughly by resuspension and sedimentation in ice-cold phosphate-buffered saline, and finally resuspended and solubilized by heating in 2% SDS, 5% β-mercaptoethanol, and 0.5 M Tris-hydrochloride (pH 7.0) at 80 C for 10 min. The solubilized immune precipitate was then subjected to electrophoresis on a polyacrylamide gel. The immune sera, kindly donated by P. Wildy and D. Watson, were hyperimmune rabbit sera prepared by inoculation with rabbit kidney cells infected with HSV-1, as has been described in detail (27). These sera gave up to 12 precipitin bands in agar gel radial immunodiffusion tests against infected HEp-2, RK13, or BHK-21 cells, but had no demonstrable reaction against uninfected cells or components of tissue culture media, and did not precipitate any significant radioactivity from an artificial mixture of labeled uninfected cells and unlabeled infected cell antigen samples (R. W. Honess and D. H. Watson, J. Gen. Virol., in press).

Analytical procedures. Incorporation of radioactive amino acids into acid-insoluble material was determined by precipitation with cold 5% trichloracetic acid. The precipitates were collected on membrane filters (Millipore Corp. type GA, 2.5 cm) and washed, and the radioactivity was estimated.

Polyacrylamide gel electrophoresis. The electrophoretic, staining, and autoradiographic techniques were as described previously (W. Gibson and B. Roizman, J. Virol., submitted for publication, 24), employing the discontinuous buffer system (2, 13) modified by the inclusion of SDS (3, 12).

Densitometry of stained gels and autoradiograms and computer-aided analysis of absorbance tracings. Absorbance tracings of stained gels and autoradiograms were obtained with a Gilford recording spectrophotometer equipped with a gel scanner attachment. The voltage output from the photocell was also recorded by a General Automation 16/45 digital computer and displayed as a plot of absorbance (voltage) as a function of distance migrated (time of scan) on a Tektronix oscilloscope. The computer was programmed to expand regions of the initial profile as determined by the operator and to compute the areas under the tracing for individual bands defined by vertical lines set parallel to the absorbance (voltage) axis. The results of a typical analysis of proteins made in HSV-1(F)-infected HEp-2 cells are shown in Fig. 1; the upper panel shows the complete profile and the regions (A, B, C) selected for expansion are shown in the three lower panels. The lines intersecting the expanded profiles in the lower panels demarcate the areas of the bands calculated by the computer. The bands are numbered in accordance with the designations of HSV-1(F) ICP which are described in Results. The peak of radioactivity migrating furthest towards the anode (the right side of all profiles shown in this paper) in this and subsequent gels represents material too small to be sieved at a particular gel strength and which therefore migrates with the free-SDS front (3) close to the bromophenol blue (BPB) dye marker. In the 8.5% gel shown in Fig. 1 all ICP smaller than ICP 45 migrate in this position.

RESULTS

Enumeration and differentiation between structural and nonstructural polypeptides synthesized in the infected cell. In the first series of experiments the mobilities of labeled ICP and VP were compared by electrophoresis either as an artificial mixture (coelectrophoresis) or separately, but on the same polyacrylamide gel slab (parallel electrophoresis). Both techniques (Fig. 2 and 3) showed that there were more ICP than there were VP and that the relative mobilities of ICP and VP were the same, whether obtained by a combination of coelectrophoresis and autoradiography (Fig. 2a and b) or by autoradiography of parallel separation on slab gels (Fig. 3). Mobilities of ICP relative to VP were independent of polyacrylamide gel concentration (Fig. 3).

However, as has been noted previously (24), autoradiography of whole gels resolved more bands than measurements of radioactivity in gel slices. For example, in Fig. 2a a maximum of eight bands was defined by variation in the ^{14}C-radioactivity of fractions 15 to 40, and a number of these peaks were defined by elevation of radioactivity in a single slice. In contrast, 12 well resolved bands were apparent in the autoradiogram of the corresponding region (Fig. 2b). On the basis of these results, autoradiography of parallel separations was the method chosen for subsequent experiments.

Analyses such as those shown in Fig. 2 and 3 have shown that cells infected with HSV-1(F) synthesize at least 49 polypeptides, whereas under the same conditions only 27 bands were resolved in separations of proteins from purified virions. These ICP were assigned numbers from

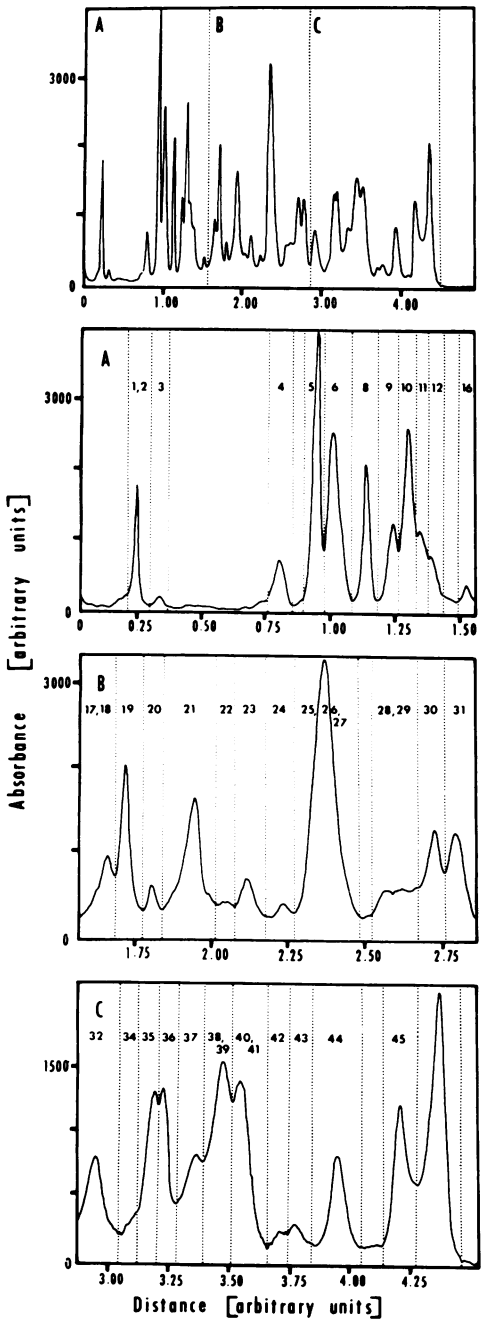

FIG. 1. *Illustration of computer planimetry of the proteins separated in polyacrylamide gels. The top panel shows the absorbance tracing of an autoradiogram of HSV-1(F) ICP labeled with ^{14}C-amino acids from 3.5 to 8.7 h postinfection. A, B, and C indicate those regions of the profile shown in the expanded form in the lower three panels. In each panel the areas bounded by the abscissa, absorbance profile, and the broken lines parallel to the absorbance axis were calculated by the computer. The numbers above the profile refer to the numerical designation of the ICP contained in each band. The arbitrary units on the ordinate are related to absorbance by the equation 1.0 absorbance unit equals 1,139 arbitrary units. One arbitrary unit of distance (abscissae) was equal to 2.0 cm.*

1 to 49 in order of descending molecular weight, and independent of the established numbering system for virion proteins (i.e., VP 1–24 [24]).

One objective of this study was to classify the ICP as structural and nonstructural and to examine their synthesis. For the purposes of this paper the basis for the classification was as follows. (i) All parallel-run comparisons on which assignments were made included proteins of purified virions in amounts sufficient to permit the detection of even those VP present in small amounts (e.g., VP 4, VP 6, and VP 9). (ii) An ICP with the same electrophoretic mobility as a VP was considered an analogue of this VP and was designated a "structural" (S) polypeptide. (iii) ICP with electrophoretic mobilities differing from those of VP, but migrating under a broad VP band, were designated "unassigned" (U) polypeptides. (iv) ICP with electrophoretic mobilities clearly different from those of VP were designated "nonstructural" (NS) polypeptides.

Table 1 (columns 1 to 4) summarizes the enumeration and classification of ICP by the above criteria. Of the 49 ICP, 10 were considered unassigned, 16 were nonstructural, and the remainder were structural.

Evidence for the virus specificity of ICP. Evidence that most of the ICP we have identified are specified by viral genetic information is based on three types of experiments dealing with comparisons of polypeptides synthesized in infected and uninfected cells, the specific precipitation of ICP by antisera reacting solely with virus-specific antigens, and comparisons of HSV-1(F) ICP with ICP made in cells infected with other strains and variants of herpes simplex virus.

Comparison of polypeptides synthesized in infected and uninfected cells. Figure 4 shows a typical result of a comparison of the polypeptides synthesized over a short period in infected and uninfected HEp-2 cells. In this experiment replicate cultures of HEp-2 cells were labeled with ^{14}C-amino acids from 4.5 to 5.0 h after infection with HSV-1(F) or after mock infection. Equivalent samples of labeled infected and uninfected cells were then harvested, solubilized, and subjected to electrophoresis on 8.5% acrylamide gels. Visual examination of gels stained with Coomassie brilliant blue re-

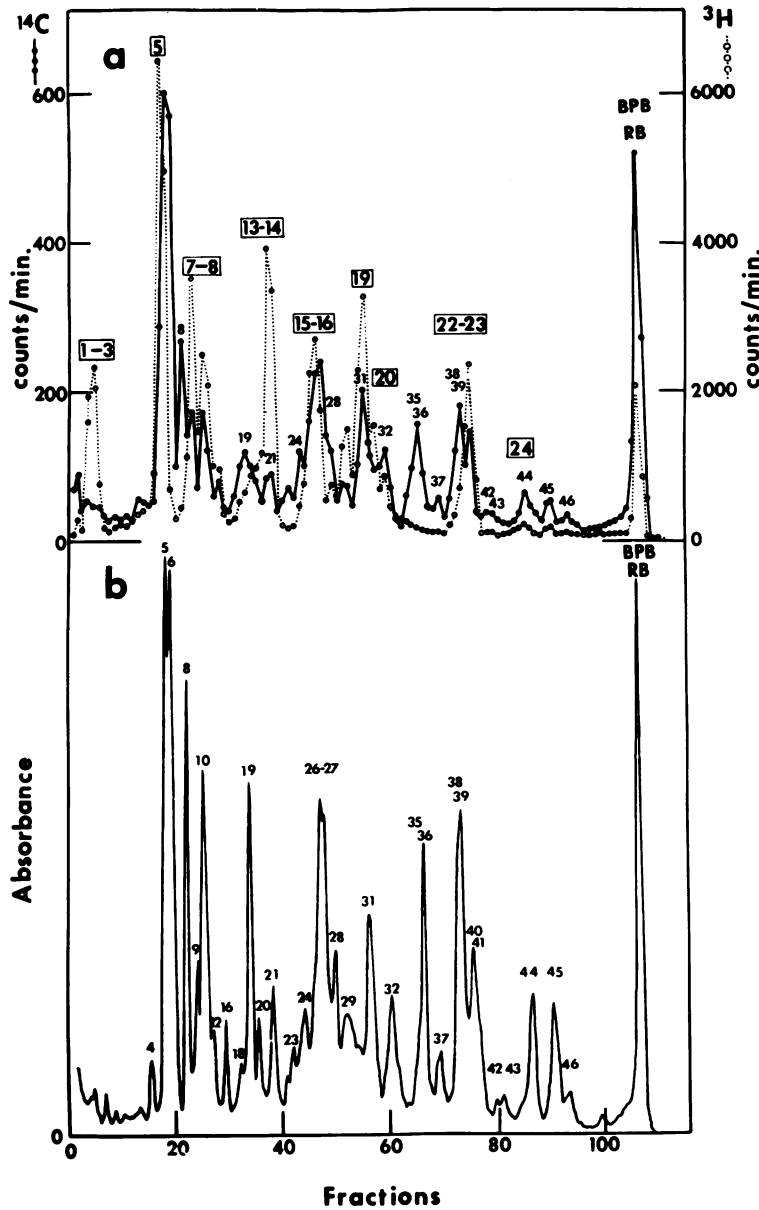

FIG. 2. *Distribution of radioactivity in a sliced gel (a) and the autoradiogram of an identical polyacrylamide gel (b) containing electrophoretically separated proteins from ^{3}H-amino acids-labeled purified virions and an infected cell lysate labeled with ^{14}C-amino acids from 4.25 to 6.5 h postinfection. In the radioactivity profile of the sliced gel (upper part of Fig. 2a), polypeptides of the virion (^{3}H-amino acids, open circles and broken line) are indicated by the larger numerals enclosed within rectangular frames, and the infected cell polypeptides (ICP), labeled with ^{14}C-amino acids, are indicated by the closed circles, the solid line, and smaller numerals. The lower part of the figure (b) is an absorbance tracing of the autoradiographic image (i.e., ^{14}C-amino acids—ICP) of an identical gel. The small numerals on this profile are those of the ICP. Electrophoresis on 8.5% cylindrical gels.*

vealed a large number of bands, too closely spaced to be well resolved by densitometry. Neither visual inspection nor densitometry revealed any significant difference between the stained profiles of electrophoretically separated proteins of total infected or uninfected cells

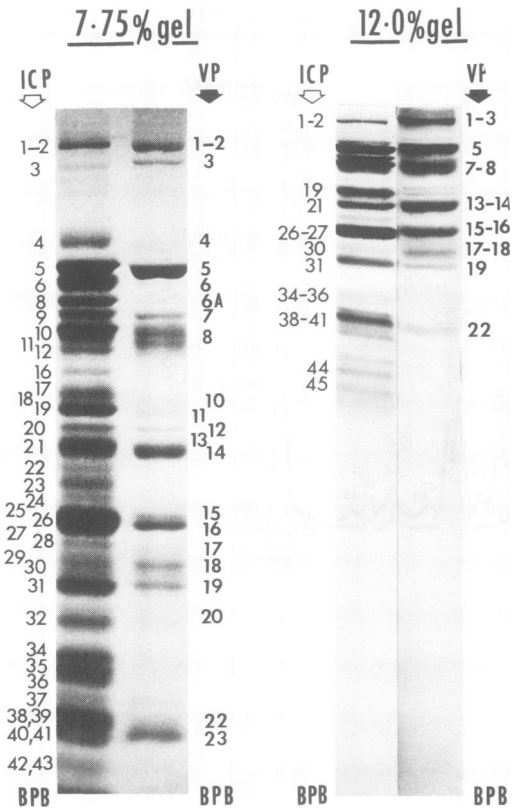

FIG. 3. *Autoradiographic comparisons of electrophoretically separated ^{14}C-amino acids-labeled virion polypeptides (purified from cells and labeled from 3 to 20 h postinfection) and polypeptides of infected cells labeled with ^{14}C-amino acids from 3.5 to 8.7 h postinfection. Solubilized labeled virions and labeled infected cells were subjected to electrophoresis in parallel on 7.75% and 12% slab gels. These were then processed for autoradiography. The figures are reproductions of photographs of the original autoradiogram, and therefore suffer from loss of resolution. The darker area at the top of the 7.75% gel is due to uneven illumination during photography. The 12% gel was printed with different exposure periods for each of the two samples.*

(Fig. 4, middle tracing). However, the autoradiographic images of these gels could be readily differentiated (Fig. 4, top and bottom tracings). The distribution of ^{14}C-amino acids-labeled uninfected cell proteins coincided with that of total protein (middle and bottom tracings of Fig. 4), with the majority of radioactivity migrating with polypeptides less than 50,000 in molecular weight. In contrast, infected cell lysates contained fewer labeled polypeptides and their distribution in the gel did not reflect the distribution of the total cell protein. A large proportion of ^{14}C-amino acid incorporation was into components with molecular weights greater than 50,000.

In view of the very large number of polypeptide species synthesized in uninfected cells for nearly every ICP there is a component in the uninfected cell characterized by the same or similar electrophoretic mobility. Attempts to differentiate between polypeptides synthesized in infected and uninfected cells solely on the basis of direct comparisons of their electrophoretic mobilities therefore seemed unprofitable. However, the fact that the profiles of proteins synthesized are different may be used as evidence for selective inhibition or stimulation (induction) to account for these differences. Quantitative comparisons of amino acid incorporation over similar regions of infected and uninfected cell profiles (top and bottom tracings of Fig. 4) indicated a higher rate of synthesis in the infected cell over regions of ICP other than those indicated by "h" in Fig. 4 (and the components travelling at the dye front in Fig. 4). Thus, incorporation into ICP 34, 35, and 42, 43 was quantitatively comparable to that observed over the same region of the uninfected cell.

This type of comparison may be termed the criterion of induction; it provides some evidence for virus specificity of ICP other than those comigrating with prominent components of the uninfected cells. These conclusions are reinforced by the results of analyses of the kinetics of synthesis of ICP considered later in the text.

Precipitation of ICP by antiserum specific for virus antigens. In this experiment, infected cells were labeled with ^{14}C-amino acids from 4.25 to 6.25 h after infection with HSV-1(F). A sample of the infected cell homogenate was sedimented (35,000 rpm, 1 h, in a Beckman SW40 rotor), and the supernatant fluid, which contained 35% of the acid-insoluble radioactivity of the total cell homogenate, was mixed with a sufficient volume of a hyperimmune rabbit serum specific for herpes simplex virus antigens to give maximal precipitation of labeled components (R. W. Honess and D. H. Watson, J. Gen. Virol., in press). The resulting immune precipitate contained 25 to 27% of the radioactivity present in the supernatant fluid. Samples of the total infected cell homogenate, the supernatant fluid, and the immune precipitate were then subjected to electrophoresis on 8.5% polyacrylamide gel cylinders. Autoradiograms of these gels are shown in Fig. 5. The samples of the total infected cell homogenate and the supernatant fraction were derived from a comparable number of cells; the sample of the immune precipitate was obtained from a volume of supernatant fluid six to eight times greater than

TABLE 1. *Enumeration, classification and evidence for virus specificity of HSV-1 (F) ICP*

1	2[a]	3	4[b]	5[c]	6[d]		7[e]		8[f]
Infected cell polypeptide (ICP)	Polypeptide molecular weight ($\times 10^{-3}$)	Relationship to VP	Classification of ICP and stability to prolonged chase	Specific stimulation and kinetics of synthesis	Precipitation by antiserum		F-ICP different from G-ICP	F-ICP different from mP-ICP	Criteria for virus specificity satisfied (columns)
					Available for precipitation	Precipitated			
1-2	275	VP 1-2	S	+A	0			F > mP	5, 7
3	260	VP 3	S	+	0				5
4	184	VP 4	S	+C	0			mP > F	5, 7
5	155	VP 5	S	+A	+	+	+		5, 6, 7
6	146	VP 6	S	+C	+	+	+		5, 6, 7
7	140		NS	+	+	+			5, 6
8	135	VP 6A	S	+C	+	+			5, 6
9	130	VP 7	U	+B	+	+			5, 6
10	126	VP 8, 8A	S	+B	+	+	+	F > mP	5, 6, 7
11	120	broad virion	U	+	+	+	+		5, 6, 7
12	117	glycoprotein	U	+B	+	+			5, 6
13		bands)	U	+	0				5
14	112	VP 9	U	+	0				5
15			S	+	0				5
16			NS	+	+	+	+		5, 6, 7
17	103		NS(D)	+A	0				5
18	98	VP 10	S	+E	0		+		5, 7
19	93	VP 11	S	+C	+	+	+		5, 6, 7
20	87	VP 12	S	+C	0		+	mP > F	5, 7
21	78	VP 14	S	+A	+	+	+	mP > F	5, 6, 7
22	76		NS	+	+	−		mP > F	5, 7
23	74		NS(D)	+F	+	−		mP > F	5, 7
24	72		NS(I)	+	+	−		mF > F	5, 7
25	71	VP 15	S	+C	+	−			5
26	65	VP 16	S	+B	+	+		F > mP	5, 6, 7
27	63		NS	+F	+	+	+	F > mP	5, 6, 7
28	61	VP 17	S	+F	+	−	+		5, 7
29	59		U	+F	+	+	+		5, 6, 7
30	57	VP 18	S	+	+	−			5
31	53	VP 19	S	+A	+	−	+	F > mP	5, 7
32	50	VP 20	S	+		−			5
33	48		NS	±	0				0
34	44	VP 21	S	−E	0				0
35	43		NS	−E	+	−			0
36	42		NS	+D	+	+			5, 6
37	40		NS	+F	+	+			5, 6
38	37		NS	+D	+	+			5, 6
39	36	VP 22	S	+D	+	+			5, 6
40	33	VP 23	S	+F	+	+	+		5, 6, 7
41	32		NS	+F	+	−	+		5, 7
42	31		U	−E	0			mP > F	7
43	30		U	−E	Trace	−		mP > F	7
44	27		U	+	0		+		5, 7
45	25	VP 24	S	+	0		+		5, 7
46	23		U	+	0				5
47			NS	+					5
48			NS	+				mP > F	5, 7
49			NS	+					5

[a] Molecular weights of ICP obtained by interpolation in previously determined values for virion polypeptides of Spear and Roizman (24).

[b] S, structural; NS, nonstructural; U, unassigned. See text for criteria applied in the classification. In addition to the 24 polypeptides described by Spear and Roizman (24), these analyses took cognizance of two additional polypeptides found in purified virions (Heine, Honess, and Roizman, manuscript in preparation).

TABLE 1.—*Continued*

These were VP 6A, a minor virion polypeptide which co-electrophoresed with ICP 8, and a glycoprotein designated VP 8A. Due to poor resolution of VP 7, 8, and 8A, the mobility analogue of VP 8A among the ICP is uncertain and all of the ICP included within this mobility range are therefore classified as S (ICP 10 which co-electrophoreses with VP 8) or as U. (D), Components declining or disappearing on prolonged chase. (I), Components increasing or appearing on prolonged chase.

[c] +, Amino acid incorporation into components migrating in these regions was higher in infected than in uninfected cells. −, Amino acid incorporation into components migrating in these regions in infected cells was comparable to, or lower than, that observed in the same regions of uninfected cells. See Fig. 3 and related text. Letters A to F refer to the patterns of synthesis as shown in Fig. 11. Absence of a letter designation indicates that the pattern of synthesis is uncertain.

[d] In the left column ("Available for precipitation"), 0 and + indicate, respectively, undetectable amounts and the presence of a particular ICP in the supernatant fluid. In the right column, + indicates precipitated and − indicates not precipitated, by antiserum. See Fig. 5 and related text.

[e] In the comparison of HSV-1(F) and HSV-2(G) (left column), + indicates any HSV-1(F) ICP for which there was no exact counterpart in mobility or amount in HSV-2(G)-infected cells. Comparisons of HSV-1(F) and HSV-1(mP) (right column) indicate significant quantitative differences between ICP with identical mobilities produced in cells infected with the two viruses, i.e., F > mP refers to a polypeptide produced in larger amounts in F than in mP-infected cells.

[f] Numbers refer to the columns summarizing those criteria for virus specificity which were satisfied by a particular ICP of HSV-1(F). For example, ICP 5 satisfied the criterion of induction (column 5), possessed viral antigenic specificity (column 6), and its mobility was affected by virus type-specific variation (column 7). Symbol 0 indicates that none of these criteria were fulfilled.

that subjected to electrophoresis on the companion gel.

By inspection of Fig. 5 it may be seen that certain polypeptides of the homogenate partitioned selectively with the pellet after sedimentation and were in relatively low concentration (ICP 9 to 12, 20, 21, and 31) or were not detected (ICP 44 and ICP 45) in the supernatant fluid. Of the polypeptides present in the supernatant fluid, some, i.e., ICP 5, 6, 7, 8, 9 to 12, 19, 26, 32, 36, 38, and 39 were precipitated efficiently, whereas others, i.e., ICP 16, 21, 28, 29, 37, and 40 were precipitated less efficiently, and some, ICP 22 to 24 and 41, were not precipitated at all.

Although precipitation by such antisera is additive evidence for virus specificity, the failure to precipitate a particular component is not evidence that this component is host specified.

Virus type and intratype (strain) specific differences in ICP. In these experiments replicate monolayers of HEp-2 cells were exposed to 20 PFU of either HSV-1(F), HSV-1(mP), or HSV-2(G) per cell and labeled with ^{14}C-amino acids from 3.5 to 8.7 h postinfection. The cells were then harvested and solubilized, and the proteins were subjected to electrophoresis in parallel. The results of autoradiographic analyses (not shown) may be summarized briefly as follows. (i) ICP synthesized in cells infected with HSV-1(F) and HSV-1(mP) were largely congruent in both number and electrophoretic mobility but differed in the relative amounts of a small number of polypeptides which were made within the same time interval. Thus, significantly more of ICP 1-2, 10, 26, 27, and 31 were present in HSV-1(F)-infected cells. Conversely, HSV-1(mP)-infected cells accumulated more of ICP 4, 21, 22, 23, 24, 42, 43, and 48 than did HSV-1(F)-infected cells. (ii) Although the general appearance of profiles of proteins synthesized in HSV-1(F)- and HSV-2(G)-infected cells were similar, there were differences both in mobility and molar concentrations of polypeptides migrating in corresponding regions of the two electrophoretic separations. For our present purpose of establishing the virus specificity of the ICP in HSV-1(F)-infected cells, it is sufficient to indicate those HSV-1(F) ICP for which an exact counterpart, either in mobility or molar concentration, was absent from lysates of HSV-2(G)-infected cells. Thus, HSV-2(G)-infected cells lacked exact counterparts to HSV-1(F), ICP 5, 6, 10, 11, 16, 18, 19, 21, 27, 28, 29, 40, 41, 44, and 45.

The evidence for virus specificity of HSV-1(F) ICP which we have adduced in the preceding section is summarized in columns 5, 6, 7, and 8 of Table 1.

Evidence that HSV-1(F) ICP are the primary products of translation: comparison of HSV-1(F) ICP labeled during short and long pulses and after a pulse chase. In these experiments replicate cultures of cells were exposed to ^{14}C-amino acids at 4.5 and 6.5 h postinfection for periods of 10, 30, and 60 min. Some of the additional cultures labeled for 10 min were incubated for an additional 40 min in maintenance medium without labeled precursors. The proteins of the harvested, solubilized cells were then subjected to electrophoresis in parallel on an 8.5% acrylamide gel slab. Comparisons of the autoradiograms (not shown)

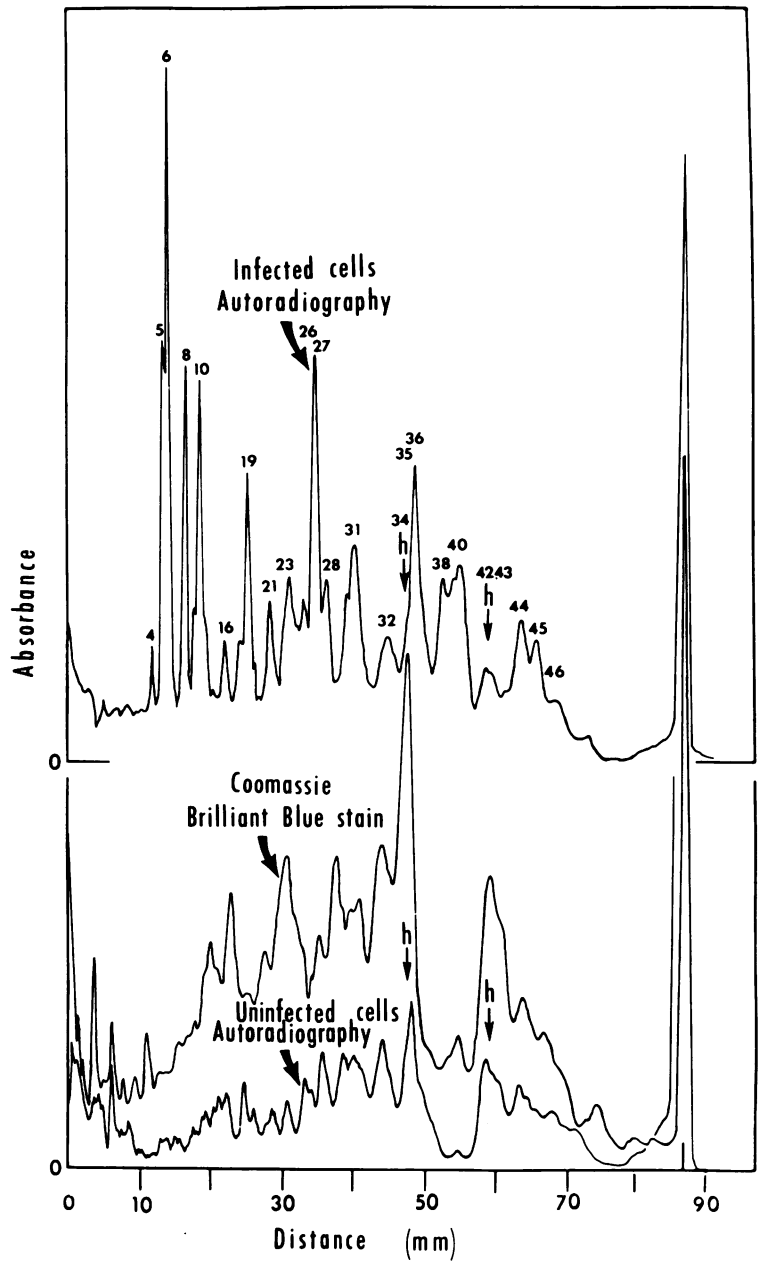

FIG. 4. *Absorbance profiles of autoradiographic images of electrophoretically separated proteins from infected (top tracing) or uninfected cells (bottom tracing) pulse labeled from 4.5 to 5.0 h after infection or mock infection. The profile of the total electrophoretically separated infected cell proteins determined by scanning the Coomassie brilliant blue stained gel is also shown. The proteins were separated on 8.5% polyacrylamide gels. Numbers on upper profile are of selected ICP to facilitate comparison with profiles. The letter "h" identifies proteins in the infected cell which were synthesized at rates comparable to proteins of similar mobility in uninfected cells.*

revealed no significant differences in the mobility or relative quantity of polypeptides accumulating in the cells during 10- to 60-min pulses administered at a given time postinfection. Neither were there any significant differences between the autoradiograms of a 10-min pulse

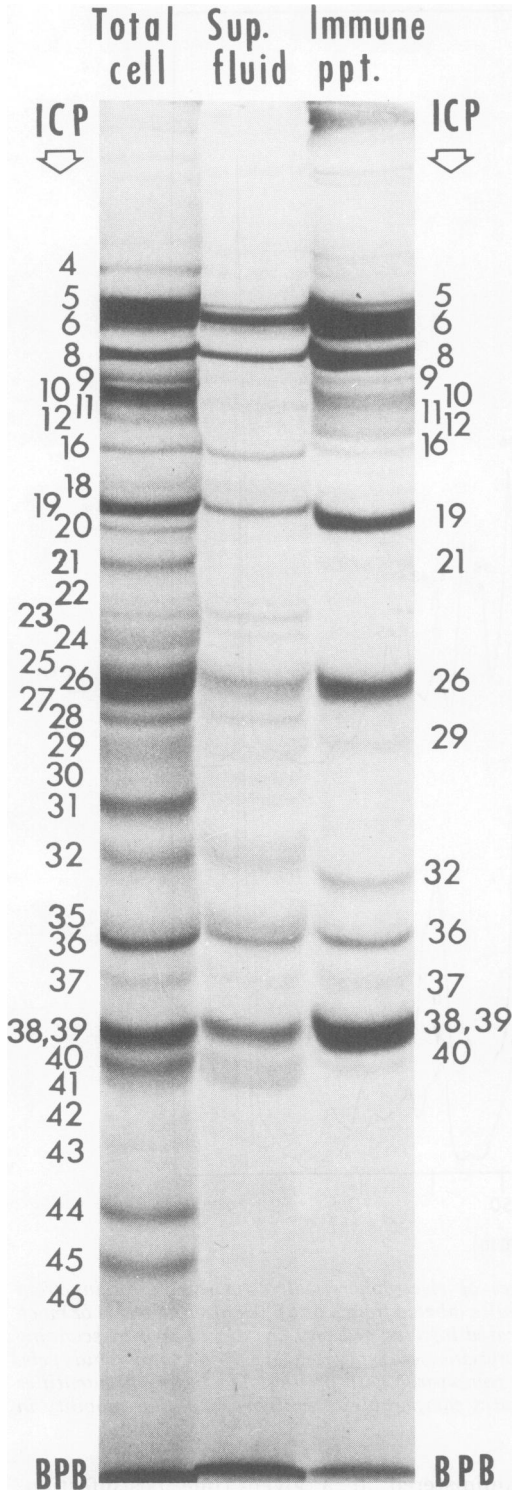

FIG. 5. *Autoradiograms of 8.5% polyacrylamide gels containing electrophoretically separated (i) total infected cell lysate labeled with ^{14}C-amino acids from 4.25 to 6.5 h postinfection (total cell), (ii) the supernatant fluid obtained after high-speed sedimentation of the lysate (sup. fluid), and (iii) an immune precipitate formed by the addition of antiserum specific for virus antigens (immune ppt), to the supernatant fluid. Numbers to the left of the three gels refer to ICP in the total cell lysate, those to the right indicate the ICP precipitated by the virus-specific antiserum.*

and a 10-min pulse followed by a 40-min chase. As discussed below and shown in Fig. 6, similar pulse and pulse-chase experiments clearly demonstrated rapid posttranslational processing of polypeptides in poliovirus-infected HEp-2 cells. Moreover, a prolonged chase resulted in relatively minor changes in the profile of HSV-1(F) ICP (compare the 5.0- to 5.25-h pulse with the 5.0- to 5.5-h pulse chased to 9.0 h; Fig. 9).

Failure to modify the pattern of synthesis of HSV-1(F) ICP by exposure to specific inhibitors of trypsin (TLCK) and chymotrypsin (TPCK) proteases. Chloromethyl ketone derivatives inhibiting proteolysis by trypsin and chymotrypsin have been shown to prevent the rapid posttranslational cleavages of poliovirus proteins in a number of cell lines (11, 26) and to cause the accumulation of "polyprotein" precursors. The effect of these inhibitors on the synthesis of HSV-1(F) ICP was therefore examined in conjunction with experiments on poliovirus-infected HEp-2 cells as a positive control system.

Replicate HEp-2 cell cultures were infected at high multiplicity either with HSV-1(F) or poliovirus type 1, labeled with ^{14}C-amino acids for a 15-min interval beginning at 5.0 h postinfection, either in the absence of inhibitors or in the presence of 10^{-4} M TPCK or of TLCK, and thereafter removed and processed for electrophoresis. An additional culture of cells infected with poliovirus was pulse labeled for a similar 15-min period in the absence of inhibitors and thereafter incubated for 40 min with medium lacking labeled precursors. At the end of this chase period these cells were also processed for electrophoresis. All samples were subjected to electrophoresis on the same (9.0% acrylamide) gel slab. The autoradiograms of these samples (Fig. 6) showed the following. (i) Control experiments with poliovirus-infected cells readily demonstrated rapid posttranslational cleavages which were effectively blocked by inhibitors of protease activity. Thus, comparisons of lysates from cells given a pulse and pulse chase (Fig. 6) show that, in the absence of inhibitors, radioactivity rapidly disappeared from bands NCVP 0 and NCVP 1½ and appeared in bands VP 0, VP 1 (VP 2), and VP 3. In the presence of 10^{-4} M

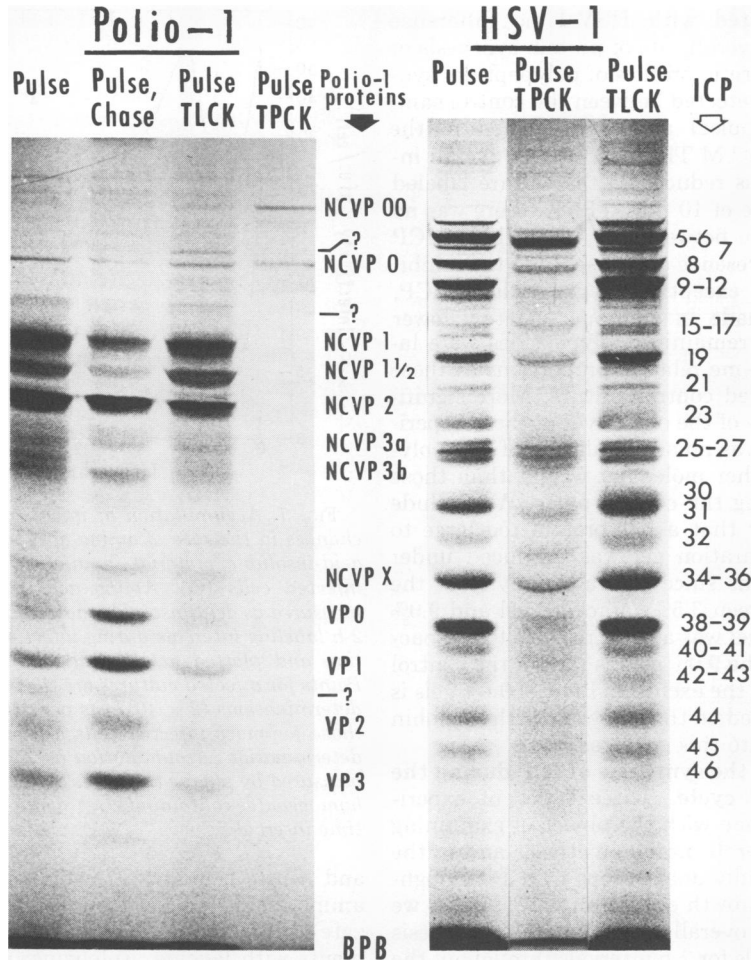

FIG. 6. *Autoradiogram of a 9.0% polyacrylamide slab gel containing electrophoretically separated polypeptides synthesized in HSV-1(F) and poliovirus type 1-infected HEp-2 cells labeled with ^{14}C-amino acids in the presence and absence of specific inhibitors of trypsin (TLCK) and chymotrypsin (TPCK) proteases. Left, proteins synthesized in poliovirus-infected cells, pulsed for 15 min in the absence of inhibitors (Pulse), pulsed for 15 min and chased for 30 min in the absence of inhibitors (Pulse, Chase), and pulsed for 15 min in the presence of 10^{-4} M TLCK (Pulse TLCK) and TPCK (Pulse TPCK). Samples on the right are of HSV-1(F) ICP synthesized in the absence (Pulse) or presence of inhibitors (TPCK, TLCK). The poliovirus type 1 proteins are designated according to Jacobson, Asso, and Baltimore (9). The numbers to the right of HSV-1(F) samples refer to selected HSV-1(F) ICP. The original autoradiogram was cut for the preparation of this figure, and the exposure interval employed for the printing of the autoradiograms of HSV-1(F)—Pulse TPCK sample was longer than that employed for other samples. The molecular weight estimates of poliovirus proteins agreed well with those of virion proteins subjected to electrophoresis in peripheral chambers of the same gel slab but removed to simplify this figure.*

TLCK, the poliovirus protein profile was modified in a rather minor way (compare sample TLCK with the control pulse), leading to detection of a novel polypeptide slightly larger than NCVP 0 with a molecular weight of approximately 175,000 and to a slight reduction in label in the region of bands NCVP 3a and NCVP 3b in comparison to the untreated control. However, 10^{-4} M TPCK drastically reduced incorporation into total protein, almost abolished the appearance of secondary products, and led to the accumulation of polypeptides of molecular weight 235,000 (NCVP 00?) and 160,000 (NCVP 0). In general our results concur with those of Summers et al. (26).

(ii) In contrast, at 5.0 h postinfection, no rapid posttranslational cleavages were detected in herpesvirus-infected HEp-2 cells. Thus, in

samples infected with HSV-1 no difference either in the overall rate of protein synthesis or the electrophoretic profiles of polypeptides synthesized was detected between the control sample (Fig. 6, pulse) and that labeled in the presence of 10^{-4} M TLCK. Although overall incorporation was reduced in the culture labeled in the presence of 10^{-4} M TPCK, there was no clear difference between the mobilities of ICP made in the presence and absence of the inhibitor. With the exception of glycosylated ICP, which were made in disproportionately lower amounts, the remaining polypeptides were labeled in the same relative proportions as those in the untreated control culture. More significantly, in view of the objectives of these experiments, there was no accumulation of any polypeptide of higher molecular weight than those detected during the control pulse. We exclude the possibility that a polyprotein too large to enter the separation gel was produced under these conditions since the radioactivity at the interface between 3.5% "stacker" gel and 9.0% "separation" gel was as low, relative to radioactivity within the 9.0% gel, as that of the control gel. Moreover, the exclusion limit of 9.0% gels is >335,000, based on the entry of the thyroglobin polypeptide into this gel strength.

Changes in the synthesis of ICP during the virus growth cycle. Three series of experiments were done with the object of examining changes in overall protein synthesis and in the synthesis of individual groups of ICP throughout the virus growth cycle. In the first series we measured the overall rate of protein synthesis by labeling cells for 2-h intervals throughout the growth cycle of the virus. The incorporation of ^{14}C-amino acids into acid-insoluble material per 2×10^6 cells per 2 h in one such experiment is shown in Fig. 7, along with the titers of the infectious virus accumulated intracellularly throughout the same experiment. The overall rate of synthesis first declined rather rapidly, reaching a minimum at 2 to 3 h postinfection, it then recovered to a maximum rate comparable to that of mock-infected control cultures at 5 to 6 h, and thereafter declined in a nonuniform manner.

In the preceding experiments and in those which follow, ICP were labeled with leucine, isoleucine, and valine. Quantitative estimations of the relative rate or amount of synthesis of ICP could therefore be in error if their content of these three amino acids was not uniform. To examine this possibility, in the second series of experiments two sets of replicate cultures of cells infected with HSV-1(F) were labeled with mixtures of ^{14}C-leucine, isoleucine, and valine

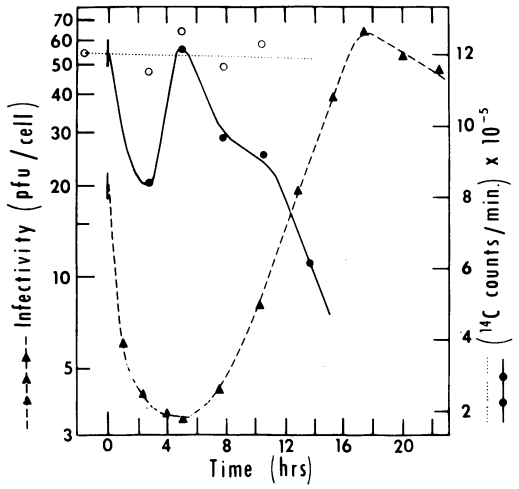

FIG. 7. *Accumulation of infectious virus (▲) and changes in the rate of amino acid incorporation into acid-insoluble material in infected (●) and mock-infected cells (○). Amino acid incorporation was measured as acid-insoluble radioactivity at the end of 2-h labeling intervals during infection or mock infection and plotted at the midpoint of the interval. Points for infected cultures are the mean of duplicate determinations (2×10^6 cells per determination) and those for mock-infected cells are results of a single determination. Accumulation of infectious virus was measured by plaque titrations of duplicate samples of homogenates containing 2×10^6 infected cells for each time interval.*

and with a mixture of thirteen ^{14}C-labeled amino acids (reconstituted protein hydrolyzate), respectively. Labeling medium in experiments with leucine, isoleucine, and valine was as described in Materials and Methods; the medium used for labeling with the ^{14}C-protein hydrolysate was EMEM with one-tenth the normal concentrations of amino acids supplemented with 1% dialyzed calf serum. Cultures were labeled for 15-min intervals beginning at 7.25 and at 22.25 h postinfection. The labeled samples were subjected to electrophoresis in parallel on a polyacrylamide gel slab. Comparison of absorbance tracings of the derived autoradiograms of the 7.25-h (Fig. 8) and of the 22.25-h (not shown) labeling intervals indicated that, although the population of polypeptides synthesized at the two time periods was markedly different, at neither time was there any significant difference between estimates of the relative amounts of ICP synthesized based on incorporation of leucine, isoleucine, and valine or of the mixture of thirteen labeled amino acids.

It should be emphasized that these findings do not indicate that the amino acid composi-

tions of ICP are uniform. In the same experiment there were significant differences between the profiles of ICP labeled with the thirteen amino acids and those labeled with single amino acids (^{35}S-methionine or ^{14}C-tyrosine). However, in the context of this paper, the pertinent observation is that HSV-1(F) ICP do not differ significantly in the sum of their contents of the three amino acids which we routinely employed as labeled precursors.

In the third series of experiments, replicate cultures of infected cells were labeled from 2.5 to 2.9, 3.75 to 4.0, 5.0 to 5.25, and 11.0 to 11.5 h postinfection, respectively. These times were chosen on the basis of Fig. 7 and other temporal features of the virus growth cycle. The interval from 2.5 to 2.9 h coincided with the end of the initial decline in protein synthesis and before viral DNA synthesis is normally detected (14, 16). The next two intervals were just before (3.75 to 4.0 h) and at (5.0 to 5.25 h) the time of maximal overall protein synthesis and high rates of viral DNA synthesis. The last interval (11.0 to 11.5 h) was at a time at which rates of protein and viral DNA syntheses were declining and the intracellular rate of accumulation of infectious virus was near maximal.

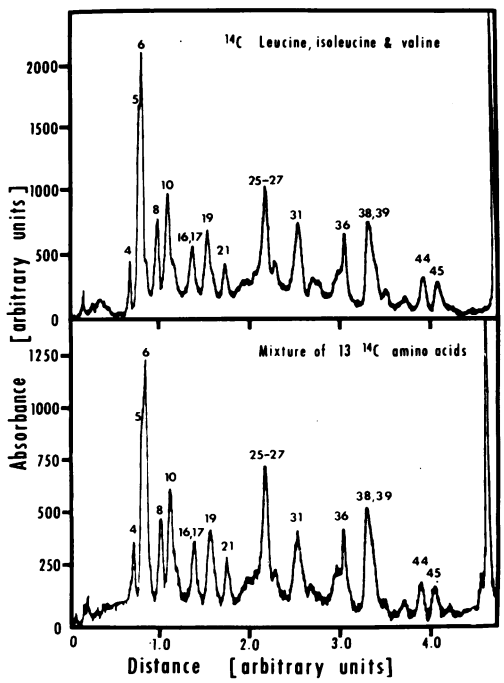

FIG. 8. *Absorbance tracings of the autoradiographic images of polyacrylamide gel slabs containing electrophoretically separated polypeptides from HSV-1(F)-infected cells labeled with ^{14}C-leucine, isoleucine, and valine (top panel) and with a mixture of 13 ^{14}C-amino acids (bottom panel) for a 15-min interval from 7.25 h post-infection.*

Figure 9 shows an autoradiogram of a polyacrylamide gel slab on which these samples were subjected to electrophoresis. To facilitate the presentation and interpretation of the data available from this figure it is convenient to express the amount of each ICP synthesized during the various intervals as a "relative molar amount synthesized," or a "relative molar rate of synthesis" (RM). This datum was obtained as follows: the autoradiograms of the gel slab (Fig. 9) were scanned with the aid of a Gilford spectrophotometer connected to a GA 16/45 computer. The latter displayed the absorbance tracing, the absorbance of each band (A_1, . . ., A_n) and of the sum of the bands (A_1 . . . A_n). Since electrophoresis on more than one gel strength was necessary to resolve certain of the 49 ICP, the absorbance of those bands known to contain more than one polypeptide was divided by the number of known components, on the formal assumption that the band was composed of equal masses of its known constituents. The relative molar amount, RM, of protein i synthesized was then obtained from the relation:

$$RM_i = 10^7 \cdot K_t \cdot \frac{A_i}{M_i \cdot (\Sigma A_i \ldots A_n)}$$

where K_t is the overall rate of protein synthesis at the time of the determination expressed as a fraction of the highest observed rate, A_i and M_i are the absorbance of band i and its molecular weight, respectively, and 10^7 is a constant.

The RM profiles of the polypeptides detected in the autoradiograms of Fig. 9 are shown in Fig. 10.

Figure 11 shows the manner in which the RM values of synthesis of polypeptides vary throughout infection.

The results presented in Fig. 9 to 11 may be summarized as follows. (i) During any one pulse-labeling interval, the RM for most S, NS, and U polypeptides were within an eightfold range; S and NS polypeptides were distributed throughout this range. The most significant exceptions were the RM values of the S polypeptides, ICP 1-2 and ICP 31, which differed by 130-fold during the 11.0- to 11.5-h labeling interval (Fig. 10) and formed the extremes of the observed range of values.

(ii) The variation in the relative proportions of S, NS, and U classes of polypeptides synthesized throughout the interval of the growth cycle analyzed in this study was less than the variation between polypeptides within the S grouping. Thus, of the total amount of protein

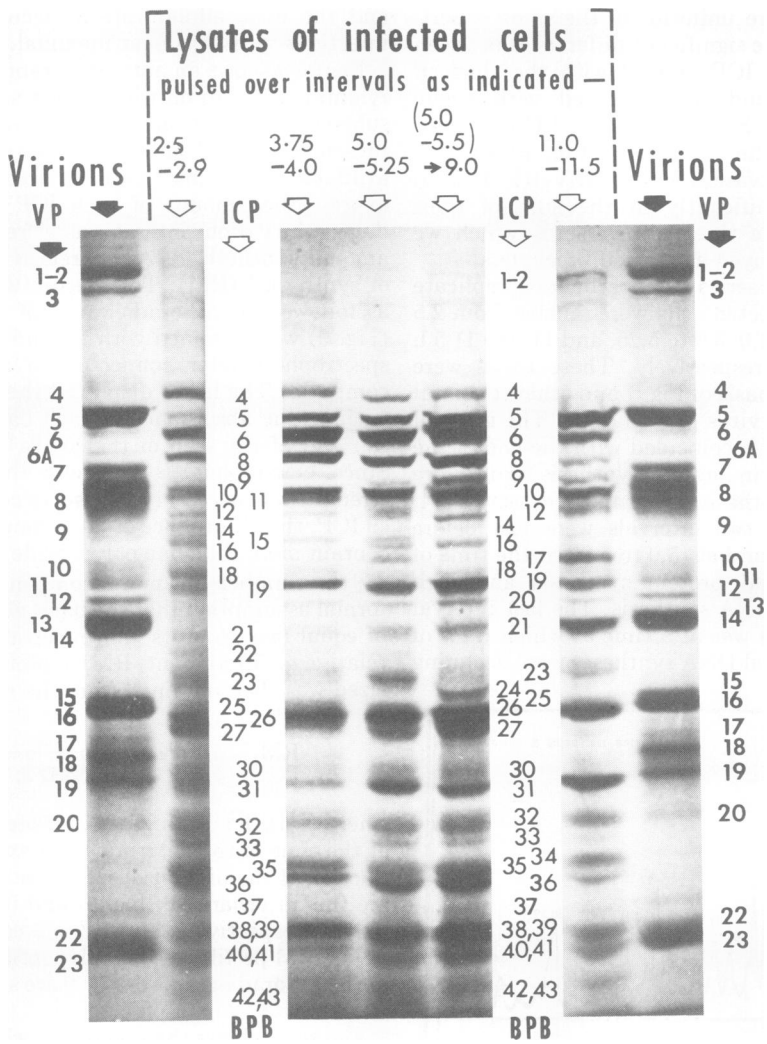

FIG. 9. *Autoradiogram of a part of a 7.75% polyacrylamide gel slab containing electrophoretically separated proteins pulse labeled in HSV-1(F)-infected cells during intervals shown on top of each sample. The legend on top of the third gel from the right indicates that cells were labeled from 5.0 to 5.5 h and then incubated in medium lacking labeled precursors until 9 h postinfection. Peripheral samples are virion proteins, identified by large numbers to the extreme right and left of the figure. The autoradiogram was cut in the preparation of this figure to permit convenient location of numbering for the ICP (smaller numbers between sample positions 2 and 3 and 5 and 6 of this figure).*

synthesized, the S polypeptide fraction (expressed in moles per cent) increased only two-fold from early (2.5 to 2.9 h) to late (11.0 to 11.5 h) times in infection, whereas during the same interval the molar ratios of S polypeptide ICP 5 to that of S polypeptide ICP 4 increased more than 30-fold.

(iii) Analysis of the kinetics of synthesis of ICP (Fig. 11) showed six patterns of synthesis, designated by the letters A to F. The segregation of ICP among these six patterns was independent of the maximum rate of synthesis of the polypeptides. However, the distribution of S, NS, and U polypeptides among the six groups was nonrandom. All but one of the polypeptides presently included in group A are S. The exception, ICP 17, may be a long-lived precursor since it disappeared on prolonged chase (compare 5.0- to 5.25-h pulse and 5.0- to 5.5-h pulse chased to 9.0 h shown in Fig. 9). Group C contained S polypeptides only. The S ICP included in the analysis presented in Fig. 11 had an aggregate molecular weight of 2.3×10^6, and this S protein molecular weight was

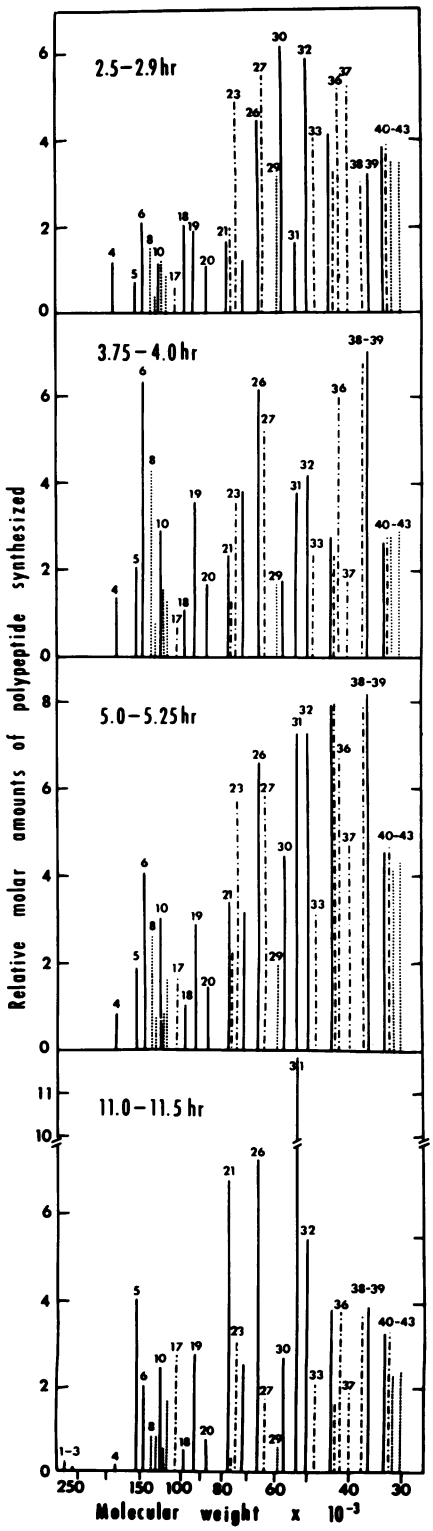

FIG. 10. *Relative molar amounts of infected cell polypeptides synthesized during short labeling intervals computed from data shown in Fig. 9 by the procedure described in the text. The relative molar amounts of polypeptides (RM, see text for method of computation) classified as S, U, and NS are represented by "solid" lines, "dotted" lines, and "dashed" lines, respectively. The polypeptides are identified by ICP number (above bars) and the molecular weight estimate (abscissa).*

distributed in groups A, C, B, F, E, and D in descending order in amounts of 47, 31, 8.1, 8.0, 4.2, and 1.6%, respectively.

(iv) The rates of synthesis of polypeptides comprising groups A, B, C, and D show either a gradual increase (A), or an initial increase followed by a leveling off (group B) or a decline (groups C and D). As we noted earlier in the text, the initial stimulation in their rate of synthesis is additional evidence that these polypeptides are virus specific. However, polypeptides of group E could be "very early" virus-specific products or host polypeptides with gradually diminishing rates of synthesis. It is noteworthy that several polypeptides which do not fulfill the general criterion of induction, and which are not precipitated by the antiserum (e.g., ICP 34, 35 and ICP 42, 43; see Fig. 4 and Table 1, columns 5 and 6), fall into group E.

Patterns F of Fig. 11 were probably consequences of superimpositions of the patterns of synthesis of two or more polypeptides, of which one or more belonged to class E and the others to class A, B, C, or D. This artifactual pattern was observed for polypeptides migrating in gels of a particular strength in multiple, incompletely resolved bands. In the electropherogram illustrating this analysis (Fig. 9, 7.75% gel) the groups of ICP 34 to 36 and 40 to 43 may be cited as examples of poorly resolved species which, in the absence of other data, would be included in category F. Analyses of the same and similar samples on other gel strengths (7 and 9%) permitted classification of ICP 34, 35, 42, and 43 as group E polypeptides (Table 1, column 5).

DISCUSSION

Autoradiographic analyses of electrophoretically separated polypeptides in polyacrylamide gels revealed that HEp-2 cells infected with HSV-1(F) make at least 49 polypeptides, numbered in order of decreasing size from 1 to 49. Some evidence for virus specificity is available for 47 of these polypeptides and consists of one or more of the following: (i) synthesis of the polypeptide is stimulated after infection, (ii) the amount and in some instances the electrophoretic mobility are determined by the virus in that these parameters vary depending on the virus type and intratype variant infecting the

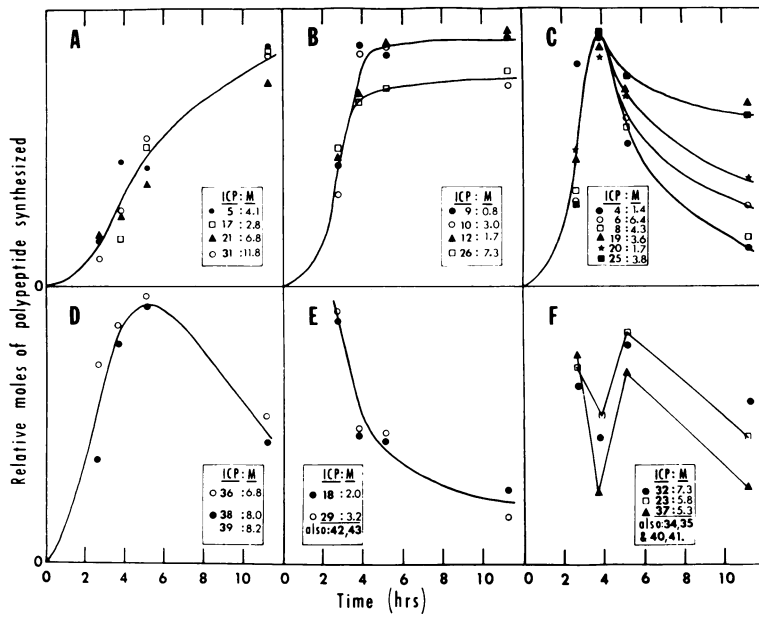

FIG. 11. *Patterns of synthesis of ICP in HSV-1(F)-infected cells. The ICP were grouped by the pattern of synthesis designated A to F. Since the RM for the ICP comprising each group are not the same, the plots were normalized. However, the maximum RM observed during the reproductive cycle is shown under column M in the boxed legend next to the number and symbolic representation of the ICP.*

cell, and (iii) the polypeptides are precipitable by anti-HSV-1 antisera of known specificity. The remaining two polypeptides (ICP 34 and 35) are probably host polypeptides, a conclusion reinforced by the observation that these polypeptides comigrate with major proteins of the uninfected cell.

These 47 virus-specific polypeptides have an aggregate molecular weight corresponding to that of about 41,000 amino acids. Assuming that the distribution of "sense" information in the HSV-1 DNA is asymmetric as suggested by analyses of transcription (6), HSV DNA can specify the sequence of a maximum of 55,000 amino acids. On the basis of these calculations the 47 polypeptides account for about 75% of the maximum information content of HSV-1 DNA. Of the 27 known virion polypeptides, 24 "analogues," i.e., polypeptides with identical electrophoretic mobilities, were provisionally identified among the 47 ICP. Of the remainder, 15 did not correspond to any known virion polypeptide and 9 remained unclassified, although it is likely that most are nonstructural. Polypeptides specified by the remaining 25% of HSV genetic information are either made in amounts too small to be detected or are obscured by major polypeptides. Although the resolution and identification of polypeptides is incomplete and their classification is provisional pending further studies, several aspects of the synthesis of ICP are apparent from results of this paper and merit discussion.

Posttranslational modification of viral polypeptides. At least three kinds of posttranslational modifications may alter the mobility of virus polypeptides, i.e., (i) rapid posttranslational cleavages such as those seen in picornavirus-infected cells (9, 25, 26), (ii) slow posttranslational cleavages determined by intracellular translocation or occurring during assembly of the virion, and (iii) conjugation or addition of prosthetic groups, e.g., glycosylation, phosphorylation, amidation, acetylation, methylation, etc. Studies presented in this paper have excluded rapid posttranslational cleavages of the type seen in picornavirus-infected cells. However, a prolonged chase revealed modifications in the mobility of some polypeptides, notably of ICP 17 and of a component of ICP 23 (Fig. 9). The magnitude of these changes suggest that ICP 17 is cleaved to as yet unknown products. The nature of the conversion of a component of ICP 23 (to ICP 24) is uncertain. In general, the operation of slow modifications requires further study in view of the considerable number of HSV polypeptides which are substrates for glycosylation (18, 22) and phosphorylation, and particularly since we have not identified protein 22A of B-capsids, the putative precursor to VP

22 (8; W. Gibson and B. Roizman, J. Virol., submitted for publication) in our present analysis.

Kinetics of synthesis of structural and nonstructural polypeptides. Analysis of the kinetics of synthesis of ICP revealed six basic patterns designated A through F. Of these six, F is probably artifactual, a consequence of multiple comigrating polypeptides synthesized according to pattern E and B, C or D. Pattern E could describe equally well host proteins with progressively declining rates of synthesis as well as virus proteins synthesized very early in infection. Most of virus-specific polypeptides follow patterns A through D, but not in a random fashion. Thus, structural proteins and their likely precursors almost all follow patterns A and C, whereas NS polypeptides clearly identified by two or more criteria as virus-specific follow patterns B, D, and E. Several comments should be made regarding patterns of synthesis of these polypeptides and their function. Briefly, (i) it is convenient to regard S polypeptides comprising group C and the virus-specific NS polypeptides comprising groups E as early. Conversely, the S polypeptides comprising group A are late, whereas the NS polypeptides of groups B and D might be designated stable and unstable, reflecting their rates of synthesis late in infection. (ii) Comparison of the rates of synthesis of the S polypeptides at various time intervals with the amounts of these polypeptides in the virion show that they are not synthesized in proportion to their incorporation into virions. As seen from data presented in Fig. 9, the probability of incorporation is not uniform and appears to be much higher for members of group A (late) polypeptides than for group C (early) polypeptides. The interpretation of this observation is presently uncertain; we may speculate that early S polypeptides may have functions unrelated to their role in the virion in addition to having aggregation rate constants markedly different from those of group A polypeptides.

Temporal and abundance control of viral protein synthesis. In parallel with studies on the control of transcription (6, 7, 21), we are concerned with two aspects of viral protein synthesis, i.e., temporal control of protein synthesis and control of viral protein abundance.

The existence of a temporal control of protein synthesis is apparent from analyses of the kinetics of synthesis of viral proteins summarized in Fig. 11. As discussed earlier in the text, the distribution of S, NS, and U polypeptides among the different kinetic classes is nonrandom, with NS proteins following patterns B, D, and E, and S proteins largely following patterns A and C. Because S polypeptides are synthesized both early (pattern C) and late (pattern A), there is no clear temporal differentiation between S and NS proteins. The incorporation of amino acids into S polypeptides early is therefore appreciable; S polypeptides incorporated 53, 67, and 72% of total amino acids during the labeling intervals beginning at 2.5, 3.75, and 11.0 h postinfection, respectively. We conclude from the foregoing analyses that (i) temporal controls of HSV-1 gene expression do exist, (ii) these controls define the synthesis of at least five groups of proteins, and (iii) there is no simple differentiation of proteins into early nonstructural and late structural. Structural proteins are members of both early and late groups and form a substantial fraction of total proteins made throughout infection.

The search for abundance control of protein synthesis arises from the observation that viral transcripts form two classes differing in their accumulated mean molar abundance. The abundant class is transcribed from 14 to 16% and 19 to 22% of virus DNA at 2 and 8 h postinfection, respectively, and is from 140- to at least 15-fold more abundant than the scarce transcripts which arise from about 30% of the DNA both early (2 h) and late (8 h) in infection. Abundant and scarce transcripts arising from a total of 43% of virus DNA were found on polyribosomes at 8 h postinfection, and it was found that abundant transcripts were adenylated whereas scarce transcripts were not (21). These observations raise two questions: (i) is there a control of protein abundance and (ii) is protein abundance determined solely by the transcript concentration or by the operation of translational controls.

It seems clear that a protein abundance control does exist since proteins are not synthesized in equimolar amounts. This is evident from a cursory examination of Fig. 10 and from the fact that the molar ratio of protein 31 to proteins 1 to 2 is 130 at 11 h postinfection, even though both proteins follow the same general pattern of synthesis.

The answer to the second question is at present uncertain for two reasons. First, it is difficult to compare the results of analyses of transcriptional and translational programs. The first type of analysis is fundamentally more sensitive in that the hybridization of scarce transcripts is not obscured by that of abundant transcripts in the way that trace amounts of one protein may be obscured by proteins in the same size range present in much larger amounts. However, the abundant class of transcript has not been

analyzed for homogeneity in the same way that the proteins were analyzed for their relative molar rates of synthesis and, moreover, little is known of the amounts of abundant and scarce transcripts in the cell or on polyribosomes throughout the reproductive cycle. The second reason for uncertainty arises from the fact that we accounted for only some 75% of the potential information content of HSV-DNA. The nature of the products specified by the remaining 25% is not known. Although it seems certain that we have detected the protein products of some of both scarce and abundant transcripts, the protein products specified by about 25% of transcribed virus information remain to be detected. Nevertheless, even though it is impossible on the basis of present data to determine whether the range in rates of protein synthesis at different times corresponds in some direct fashion to the ratio of abundant and scarce RNA, the data clearly indicate that structural and nonstructural proteins do not differ substantially in their relative molar rates of synthesis (Fig. 10) and it seems that the hypothesis that abundant and scarce transcripts specify structural and nonstructural polypeptides, respectively (6), is untenable in that simple form. The question as to what is specified by abundant and scarce RNA species remains unresolved. Regardless of the assigned function of abundant and scarce transcripts, the available data are not sufficient to determine whether the quantity of gene product is solely determined by RNA transcript abundance or by controls expressed during translation. The answer to these questions should emerge from analyses of the transcription and translation of messenger RNAs for known proteins.

ACKNOWLEDGMENTS

We thank Chi Shi Chen and Brad Cox for assistance with the computer analyses.

These studies were supported by Public Health Service grant CA 08494 from the National Cancer Institute, and grants from the American Cancer Society (VC 103H) and the National Science Foundation (GB 27356).

R.W.H. is a postdoctoral fellow of the Jane Coffin Childs Memorial Fund for Cancer Research.

LITERATURE CITED

1. Courtney, R. J., R. M. McCombs, and M. Benyesh-Melnick. 1971. Antigens specified by herpesviruses. II. Effect of arginine deprivation on the synthesis of cytoplasmic and nuclear proteins. Virology **43**:356-365.
2. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404-427.
3. Dimmock, N. J., and D. H. Watson. 1969. Proteins specified by influenza virus in infected cells: analysis by polyacrylamide gel electrophoresis of antigens not present in the virus particle. J. Gen. Virol. **5**:499-509.
4. Ejercito, P. M., E. D. 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.
5. Fairbanks, G., Jr., C. Levinthal, and R. H. Reeder. 1965. Analysis of C^{14}-labeled proteins by disc electrophoresis. Biochem. Biophys. Res. Commun. **20**:393-399.
6. Frenkel, N., and B. Roizman. 1972. Ribonucleic acid synthesis in cells infected with herpes simplex virus: controls of transcription and of RNA abundance. Proc. Nat. Acad. Sci. U.S.A. **69**:2654-2658.
7. Frenkel, N., S. Silverstein, E. Cassai, and B. Roizman. 1973. RNA synthesis in cells infected with herpes simplex virus. VII. Control of transcription and of transcript abundancies of unique and common sequences of herpes simplex virus 1 and 2. J. Virol. **11**:886-892.
8. Gibson, W., and B. Roizman. 1972. Proteins specified by herpes simplex virus. VIII. Characterization and composition of multiple capsid forms of subtypes 1 and 2. J. Virol. **10**:1044-1052.
9. Jacobson, M. F., J. Asso, and D. Baltimore. 1970. Further evidence on the formation of poliovirus proteins. J. Mol. Biol. **49**:657-669.
10. Kaplan, A. S., H. Shimono, and T. Ben-Porat. 1970. Synthesis of proteins in cells infected with herpesvirus. III. Relative amino acid content of various proteins formed after infection. Virology **40**:90-101.
11. Korant, B. D. 1972. Cleavage of viral precursor proteins in vivo and in vitro. J. Virol. **10**:751-759.
12. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-684.
13. Ornstein, L. 1964. Disc electrophoresis. I. Background and theory. Ann. N.Y. Acad. Sci. **121**:321-349.
14. Roizman, B. 1969. Herpesviruses—a biochemical definition of the group, p. 1-79. *In* B. Benacerraf et al. (ed.), Current topics in microbiology and immunology, vol. 49. Springer-Verlag, Heidelberg.
15. Roizman, B., and P. R. Roane, Jr. 1961. A physical difference between two strains of herpes simplex virus apparent on sedimentation in caesium chloride. Virology **15**:75-79.
16. Roizman, B., and P. R. Roane, Jr. 1964. The multiplication of herpes simplex virus. II. The relation between protein synthesis and duplication of viral DNA in infected HEp-2 cells. Virology **22**:262-269.
17. Roizman, B., and P. G. Spear. 1968. Preparation of herpes simplex virus of high titer. J. Virol. **2**:83-84.
18. Savage, T., B. Roizman, and J. W. Heine. 1972. Immunological specificity of the glycoproteins of herpes simplex virus subtypes 1 and 2. J. Gen. Virol. **17**:31-48.
19. Shapiro, A. L., E. Viñuela, and J. V. Maizel, Jr. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. **23**:815-820.
20. Shaw, E. 1967. Site-specific reagents for chymotrypsin and trypsin, p. 677-686. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 11. Academic Press Inc., New York.
21. Silverstein, S., S. L. Bachenheimer, N. Frenkel, and B. Roizman. 1973. Relationship between post-transcriptional adenylation of herpes virus RNA and messenger RNA abundance. Proc. Nat. Acad. Sci. U.S.A. **70**:2101-2104.
22. Spear, P. G., J. M. Keller, and B. Roizman. 1970.

Proteins specified by herpes simplex virus. II. Viral glycoproteins associated with cellular membranes. J. Virol. **5**:123-131.

23. Spear, P. G., and B. Roizman. 1968. The proteins specified by herpes simplex virus. I. Time of synthesis, transfer into nuclei, and properties of proteins made in productively infected cells. Virology **36**:545-555.
24. Spear, P. G., and B. Roizman. 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. J. Virol. **9**:143-159.
25. Summers, D. F., and J. V. Maizel. 1968. Evidence for large precursor proteins in poliovirus synthesis. Proc. Nat. Acad. Sci. U.S.A. **59**:966.
26. Summers, D. F., E. N. Shaw, M. L. Stewart, and J. V. Maizel, Jr. 1972. Inhibition of cleavage of large poliovirus-specific precursor proteins in infected HeLa cells by inhibitors of proteolytic enzymes. J. Virol. **10**:880-884.
27. Watson, D. H., W. I. H. Shedden, A. Elliot, T. Tetsuka, P. Wildy, D. Bourgaux-Ramoisy, and E. Gold. 1966. Virus specific antigens in mammalian cells infected with herpes simplex virus. Immunology **11**:399-408.