BIKEN JOURNAL Vol. 21, 77-94, 1978

STUDIES ON THE POLYPEPTIDES OF POXVIRUS
II. COMPARISON OF VIRUS-INDUCED POLYPEPTIDES IN CELLS INFECTED WITH VACCINIA, COWPOX AND SHOPE FIBROMA VIRUSES

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(Received April 26, 1978)

SUMMARY Virus-induced polypeptides in cells infected with vaccinia, cowpox and Shope fibroma viruses were examined by SDS-polyacrylamide gel electrophoresis followed by autoradiography.

At least 42 vaccinia virus-induced polypeptides were identified among the polypeptides of cells pulse-labeled with [^{35}S]-methionine and/or of fractionated cells labeled with [^{14}C]-leucine for 24 hr. They consisted of 15 polypeptides (early polypeptides) which were synthesized even in the presence of cytosine-1-β-D-arabinofuranosyl-HCl, and 27 polypeptides (late polypeptides) which were synthesized only in the absence of cytosine-1-β-D-arabinofuranosyl-HCl.

By the same procedure at least 40 cowpox virus-induced polypeptides (14 early polypeptides and 26 late polypeptides) and at least 31 Shope fibroma virus-induced polypeptides (13 early polypeptides and 18 late polypeptides) were identified.

Comparative studies of virus-induced polypeptides on the basis of migration in SDS-polyacrylamide gel electrophoresis revealed that 11 polypeptides were early polypeptides common to both vaccinia and cowpox viruses; 21 were late polypeptides common to both vaccinia and cowpox viruses; 4 were early polypeptides common to both vaccinia and Shope fibroma viruses; 7 were late polypeptides common to both vaccinia and Shope fibroma viruses; 5 were early polypeptides common to both cowpox and Shope fibroma viruses; 9 were late polypeptides common to both cowpox and Shope fibroma viruses; 4 were early polypeptides common to all three viruses; and 7 were late polypeptides common to all three viruses.

INTRODUCTION

Numerous investigations have shown that vaccinia virus-induced proteins in infected cells can be divided into "early" and "late" classes, the former being synthesized before, and the latter after, virus DNA synthesis (Joklik, 1968; Moss and Salzman, 1968; Esteban and Metz, 1973). Several virus-induced enzymes and some structural proteins of

virus particles were included in the "early" class (McAuslan, 1963; Jungwirth and Joklik, 1965; McAuslan and Kates, 1967; Holowczak and Joklik, 1967). Most of structural proteins of virus particles are synthesized after the onset of virus DNA synthesis (Holowczak and Joklik, 1967; Salzman and Sebring, 1967). Pennington (1974) pulse-labeled BSC_1 cells infected with vaccinia virus (Evans vaccine strain) with [^{14}C]-protein hydrolysate or [^{35}S]-methionine and examined the polypeptides by discontinuous polyacrylamide gel electrophoresis followed by autoradiography. He reported about 80 virus-induced polypeptides in schematic form, 30 appearing before and 50 after the onset of virus DNA synthesis.

There have been few papers on virus-induced polypeptides of poxviruses other than vaccinia virus. Previously we reported that the structural polypeptides of Shope fibroma virus differed considerably from those of vaccinia or cowpox virus (Ikuta et al., 1978).

This paper reports studies on the polypeptides induced by vaccinia, cowpox and Shope fibroma viruses in infected RK_{13} cells. The polypeptides were examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography, and their mobilities on SDS-PAGE were compared.

MATERIALS AND METHODS

1. *Cell culture*

Monolayer cultures of RK_{13} cells purchased from Flow Laboratories were grown in Eagle's minimum essential medium (MEM) (Eagle, 1959) supplemented with 10% calf serum in petri dishes of 90 mm diameter.

2. *Viruses*

IHD-W, IHD-J and Lister strains of vaccinia virus, cowpox virus (LB red strain) and Shope fibroma virus (OA strain) were used in these studies. Stock viruses for inoculation were used after purification and titration as described previously (Ikuta et al., 1978).

3. *Radioactive labeling of infected or mock-infected cells*

Monolayer cells were infected with each virus at moi 10 or mock-infected as described previously (Ikuta et al., 1978). After adsorption for 1 hr at 37 C in the presence or absence of cytosine-1-β-D-arabinofuranosyl-HCl (Ara C) (50 μg/ml), infected or mock-infected cells were washed three times with phosphate-buffered saline, pH 7.4 (PBS), and then incubated in medium containing 1 μCi/ml of [^{14}C]-leucine (297.0 mCi/mmole) or 2 μCi/ml of [^{35}S]-methionine (442.1 Ci/mmole) for appropriate periods in the presence or absence of Ara C (50 μg/ml). Precise details of the radioactive labeling are described in the legends to the figures.

After labeling, the cells designated as "whole cells" were washed with PBS, and frozen at -20 C until use as a sample for fractionation or SDS-PAGE.

Radioisotopes were purchased from the New England Nuclear.

4. *Fractionation of cells*

Infected or mock-infected cells labeled with [^{14}C]-leucine ("whole cells"), were solubilized in 4 ml of TD buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium phosphate buffer, pH 7.2) per petri dish, and sonicated for 3 min in an ice bath. The preparation was stood for 1 hr on ice, and then centrifuged in a Beckman SW50.1 rotor at 30,000 rpm for 2 hr at 4 C to separate the supernatant ("sup fraction") and the pellet ("ppt fraction").

5. *SDS-PAGE*

SDS-PAGE was conducted by the method of Maizel (1971). The gel concentration was 13% and the ratio of acrylamide to bisacrylamide was 30 to 0.8.

Samples to be analyzed were concentrated by the cold 10% trichloroacetic acid (TCA) precipitation procedure and washed twice with cold 5% TCA and twice with cold acetone. The concentrated samples were dissolved in "sample buffer", and subjected to SDS-PAGE. The constituents of "sample buffer" and the procedures of SDS-PAGE have been described previously (Ikuta et al., 1978).

RESULTS

1. *Identification of vaccinia virus-induced polypeptides*

1) Analysis of "whole cells" pulse-labeled with [^{35}S]-methionine

Vaccinia virus-induced early (VE) and late (VL) polypeptides were identified using Ara C as an inhibitor of virus DNA synthesis. VE polypeptides are vaccinia virus-induced polypeptides synthesized even in the presence of Ara C, and VL polypeptides are vaccinia virus-induced polypeptides synthesized only in the absence of Ara C.

Monolayer cells of RK_{13} were infected with the IHD-J strain of vaccinia virus or mock-infected, and labeled with [^{35}S]-methionine for 1 hr at various times postinfection in the presence or absence of Ara C (see legend to Fig. 1). Polypeptides in labeled cells were analyzed by SDS-PAGE and autoradiography. Figure 1 shows the profile of polypeptides synthesized in infected and mock-infected cells. On polypeptide analysis by this method, the polypeptide bands in infected cells may overlap the polypeptide bands which can be observed even in mock-infected cells. In such cases, it is impossible to determine whether the polypeptide is a virus-induced polypeptide or not. In this work all bands showing increase in intensity at various times postinfection were tentatively classified as virus-induced polypeptide bands. Based on this criterion, at least 8 polypeptide bands were identified as VE polypeptides detected even in the presence of Ara C, and at least 20 polypeptide bands were identified as VL polypeptides detected only in the absence of Ara C. The molecular weights (MW) of these 8 VE and 20 VL polypeptides, calculated by comparison of their mobilities with those of standard proteins, as described in the legend to Fig. 1, ranged from about 140,000 to about 30,000, and from about 100,000 to about 14,000, respectively, and the polypeptides were provisionally named VE140K to VE30K and VL100K to VL14K, respectively. These VE and VL polypeptides are summarized in Table 1.

2) Analysis of fractionated cells

For more precise studies of VE and VL polypeptides and for detection of other polypeptides, infected or mock-infected cells which had been labeled with [^{14}C]-leucine from 1 to 25 hr postinfection in the presence or absence of Ara C, were fractionated as described in Materials and Methods. Since fractionated samples contain fewer protein species than "whole cells", minor virus-induced polypeptide bands can be more clearly distinguished from the bands of host cell protein by SDS-PAGE. About 70% of the TCA-insoluble radioactivity of "whole cells" was consistently found in the "sup fraction".

Figure 2 shows the autoradiograms obtained after SDS-PAGE of cells infected with the IHD-W, IHD-J and Lister strains of vaccinia virus or mock-infected in the presence of Ara C. Six VE polypeptides were identified in "whole cells", 9 VE polypeptides in the "sup fraction" and 13 VE polypeptides in the "ppt fraction". The MW of these VE polypeptides, calculated as described above, ranged from about 190,000 to about 17,500, and the polypeptides were provisionally named VE190K to VE17.5K polypeptide. These VE polypeptides are summarized in Table 1. Six VE polypeptides detected in "whole cells" were also detected either in the "sup fraction" or "ppt fraction". Two VE polypeptides (VE94K and VE28K) were detected only in the "sup fraction", 6 VE polypeptides (VE190K, VE110K, VE49K, VE37K, VE30K and VE17.5K) only in the "ppt fraction", and the other VE polypeptides in both fractions. The 7 VE polypeptides (VE190K, VE88K, VE60K, VE58K, VE49K, VE28K and VE17.5K) which were not detected in "whole cells" pulse-labeled with [^{35}S]-methionine, were newly identified in "whole cells" and/or fractionated cells labeled with [^{14}C]-leucine. Thus 15 VE polypeptides were identified in Figs. 1 and 2.

No difference was found in the numbers of VE polypeptides in cells infected with the three

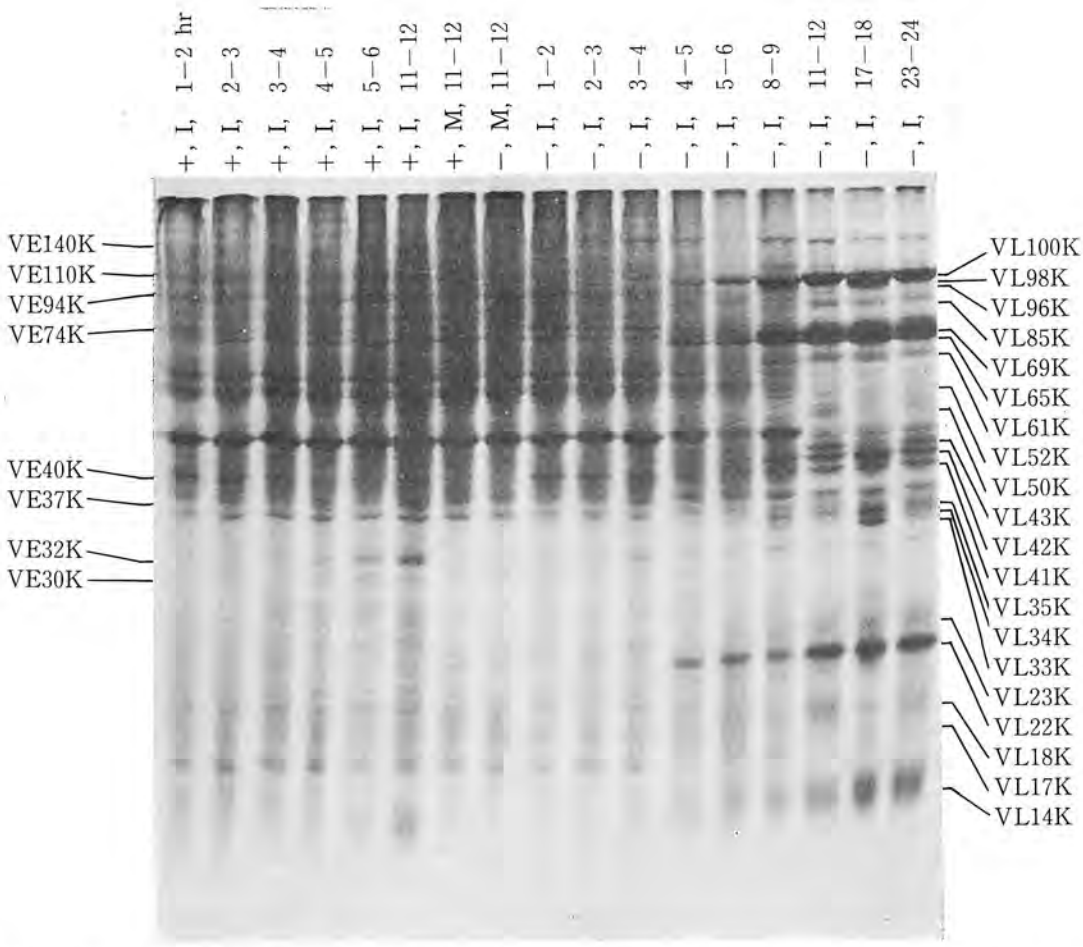

FIGURE 1. Time course of vaccinia virus-induced polypeptide synthesis in infected RK_{13} cells. RK_{13} cells were infected (I) with the IHD-J strain of vaccinia virus at moi 10 or mock-infected (M) in the presence (+) or absence (−) of Ara C (50 μg/ml). Infected and mock-infected cells were incubated with MEM supplemented with 2% calf serum in the presence or absence of Ara C (50 μg/ml) until pulse-labeling, and then labeled with 2 μCi/ml of [^{35}S]-methionine in methionine-free MEM supplemented with 2% dialyzed calf serum (4 ml per dish of 90 mm diameter) for 1 hr at the times indicated in the figure (hours postinfection) in the presence or absence of Ara C (50 μg/ml). Samples were processed as described in Materials and Methods and analyzed by SDS-PAGE, followed by autoradiography. In this and all succeeding electropherograms, the bottom of the figure corresponds to the anodal end of the gel. The MW of polypeptides was calculated by comparison of their mobilities with those of standard proteins [BDH MW marker mixtures (product No. 44223 2U, MW range 14,300—71,500, and 44230 2R, MW range 53,000—265,000) purchased from BDH Chemicals Ltd., England; myosin, MW 215,000; bovine serum albumin, MW 67,000; ovalbumin, MW 43,500; and cytochrome c, MW 12,400].

TABLE 1. *Vaccinia virus-induced early and late polypeptides*

Virus-induced early polypeptides			Virus-induced late polypeptides		
[^{35}S]-Met[a] "Whole"[c]	[^{14}C]-Leu[b] "Sup"[d]	[^{14}C]-Leu[b] "Ppt"[e]	[^{35}S]-Met[a] "Whole"[c]	[^{14}C]-Leu[b] "Sup"[d]	[^{14}C]-Leu[b] "Ppt"[e]
					VL250K
					VL230K
		VE190K			
VE140K	VE140K	VE140K			
VE110K		VE110K			
			VL100K	VL100K	VL100K
			VL98K	VL98K	VL98K
			VL96K		VL96K
VE94K	VE94K				
	VE88K	VE88K			
			VL85K		VL85K
				VL80K	VL80K
VE74K	VE74K	VE74K			
			VL69K	VL69K	VL69K
			VL65K	VL65K	VL65K
			VL61K		VL61K
	VE60K	VE60K			
	VE58K	VE58K			
			VL52K		
			VL50K	VL50K	VL50K
		VE49K			
			VL43K	VL43K	VL43K
			VL42K	VL42K	VL42K
			VL41K	VL41K	VL41K
				VL40.5K	
VE40K	VE40K	VE40K			
VE37K		VE37K			
			VL35K		VL35K
			VL34K		VL34K
			VL33K		
VE32K	VE32K	VE32K			
VE30K		VE30K			
	VE28K				
				VL25K	
			VL23K	VL23K	VL23K
			VL22K	VL22K	
					VL20K
					VL19K
			VL18K	VL18K	VL18K
		VE17.5K			
			VL17K		VL17K
			VL14K	VL14K	VL14K

[a] Polypeptide identified in the cells labeled with [^{35}S]-methionine.
[b] Polypeptide identified in the cells labeled with [^{14}C]-leucine.
[c] Polypeptide identified in "whole cells".
[d] Polypeptide identified in "sup fraction".
[e] Polypeptide identified in "ppt fraction".

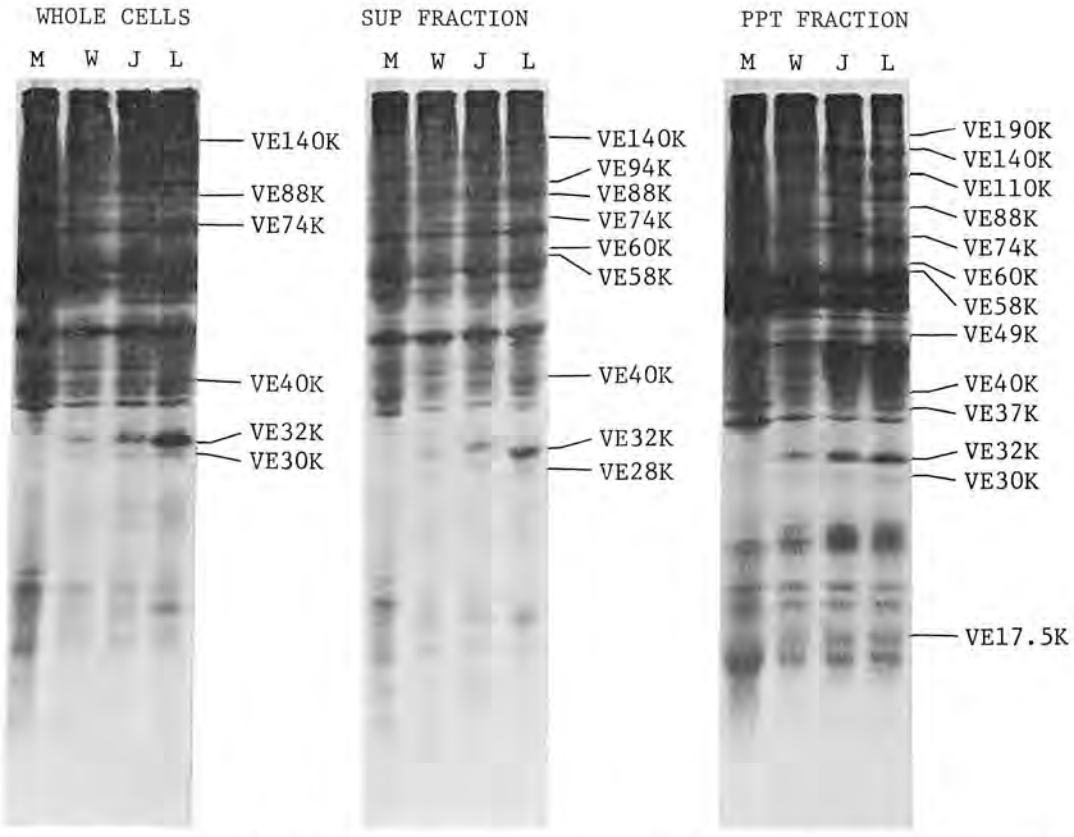

FIGURE 2. Distribution of vaccinia virus-induced early polypeptides in fractions of infected RK_{13} cells. RK_{13} cells were infected with the IHD-W, IHD-J and Lister strains of vaccinia virus (W, J and L, respectively) at moi 10 or mock-infected (M) in the presence of Ara C (50 μg/ml) and then labeled with 1 μCi/ml of [^{14}C]-leucine in MEM containing one-tenth the normal concentration of leucine and 2% dialyzed calf serum from 1 to 25 hr postinfection in the presence of Ara C (50 μg/ml). These labeled cells, "whole cells", were fractionated as described in detail in Materials and Methods ("sup fraction" and "ppt fraction"). The resulting samples were analyzed by SDS-PAGE followed by autoradiography. The MW of polypeptides was calculated as described in the legend to Fig. 1.

strains of vaccinia virus (IHD-W, IHD-J and Lister).

Figure 3 shows the results obtained by SDS-PAGE on cells infected with the IHD-W, IHD-J and Lister strains of vaccinia virus or mock-infected in the absence of Ara C. Fifteen, 15 and 22 VL polypeptides were identified in "whole cells", "sup fraction" and "ppt fraction", respectively. The MW of these VL polypeptides was calculated as described above, and the polypeptides were provisionally designated as VL250K to VL14K polypeptide. These VL polypeptides are summarized in Table 1. The VL polypeptides detected in "whole cells" were all found in the "sup fraction" or "ppt fraction". Three VL polypeptides (VL40.5K, VL25K and VL22K) were detected only in the "sup fraction", 10 VL polypeptides (VL250K, VL230K, VL96K, VL85K, VL61K, VL35K, VL34K,

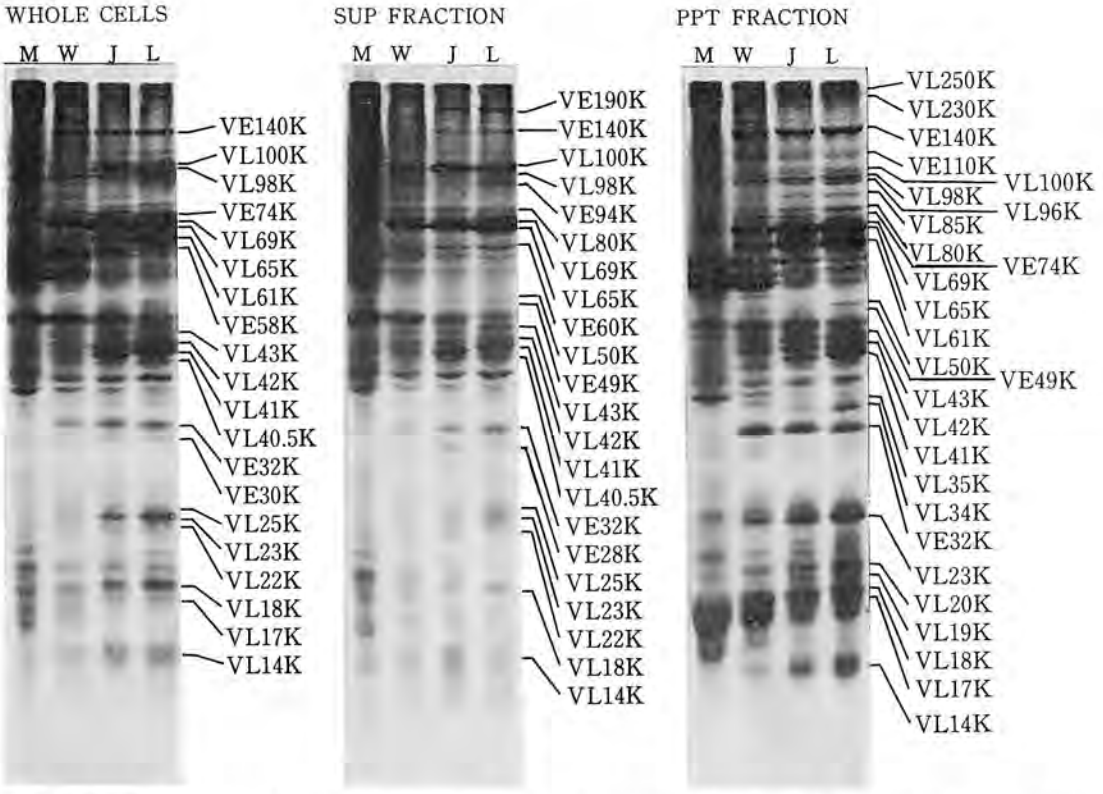

FIGURE 3. Distribution of vaccinia virus-induced late polypeptides in fractions of infected RK_{13} cells. RK_{13} cells were infected with the IHD-W, IHD-J and Lister strains of vaccinia virus (W, J and L, respectively) at moi 10 or mock-infected (M) in the absence of Ara C and then labeled as described in the legend to Fig. 2, except for the absence of Ara C. The labeled cells, "whole cells", were fractionated as described in the legend to Fig. 2 ("sup fraction" and "ppt fraction"). The resulting samples were analyzed by SDS-PAGE followed by autoradiography. The MW of polypeptides was calculated as described in the legend to Fig. 1.

VL20K, VL19K and VL17K) only in the "ppt fraction", and the other VL polypeptides were found in both fractions. The VL polypeptides newly identified in "whole cells" and/or fractionated cells labeled with [^{14}C]-leucine were 7 species of polypeptides (VL-250K, VL230K, VL80K, VL40.5K, VL25K, VL20K and VL19K). Thus 27 species of VL polypeptide were identified in Figs. 1 and 3.

The numbers of VL polypeptides in cells infected with the three strains of vaccinia virus (IHD-W, IHD-J and Lister) were almost the same. However, VL41K polypeptide was not detected in cells infected with the IHD-W strain of vaccinia virus, which has been shown not to produce hemagglutinin (HA) (Kaku and Kamahora, 1964; Ichihashi and Matsumoto, 1969).

The VE and VL polypeptides shown in Figs. 1, 2 and 3 are summarized in Table 1. Seven VE and 7 VL polypeptides were detected only in the cells labeled with [^{14}C]-leucine as described above, 2 VL polypeptides (VL52K and VL33K) only in cells labeled with [^{35}S]-methionine, and the other VE and

VL polypeptides in both cells labeled with [^{14}C]-leucine and those labeled with [^{35}S]-methionine.

2. *Identification of cowpox virus-induced polypeptides*

1) Analysis of " whole cells " pulse-labeled with [^{35}S]-methionine

Cowpox virus-induced early (CE) and late (CL) polypeptides were identified using Ara C, as in studies with vaccinia virus.

Monolayer cells of RK_{13} infected with cowpox virus or mock-infected were labeled with [^{35}S]-methionine for 1 hr at various times postinfection in the presence or absence of Ara C (see legend to Fig. 4) and subjected to SDS-PAGE and the results are shown in Fig. 4. At least 6 CE polypeptides and at least 18 CL polypeptides were separated from infected cells. The MW of these 6 CE and 18 CL polypeptides, calculated as described above, ranged from about 140,000 to about 30,000 and from about 150,000 to about 14,000, respectively, and the polypeptides were provisionally named CE140K to CE30K and CL150K to CL14K polypeptides, respectively. These CE and CL polypeptides are summarized in Table 2.

2) Analysis of fractionated cells

Monolayer cells of RK_{13} were infected with cowpox virus or mock-infected, labeled with [^{14}C]-leucine from 1 to 25 hr postinfection, and then fractionated as described above. About 70% of the TCA-insoluble radioactivity of " whole cells " was consistently found in the " sup fraction ". The " sup fraction " and " ppt fraction " were subjected to SDS-PAGE, and the results are shown in Fig. 5. At least 10 CE and 14 CL polypeptides were found in the " sup fraction " of infected cells, and at least 7 CE and 16 CL polypeptides were found in the " ppt fraction ". The MW of these cowpox virus-induced polypeptides were calculated as described above, and the polypeptides in the " sup fraction " were provisionally named CE94K to CE25K and CL100K to CL14K, while those in the " ppt fraction " were named CE140K to CE17.5K and CL250K to CL14K. These CE and CL polypeptides are summarized in Table 2. Six CE polypeptides (CE94K, CE88K, CE43K, CE35K, CE30K and CE25K) and 6 CL polypeptides (CL80K, CL50K, CL40.5K, CL40K, CL22K and CL17K) were detected only in the " sup fraction ", 3 CE polypeptides (CE140K, CE110K and CE17.5K) and 8 CL polypeptides (CL250K, CL230K, CL150K, CL98K, CL61K, CL42K, CL20K and CL19K) only in the " ppt fraction ", and the other CE and CL polypeptides were found in both fractions. The newly identified virus-induced early and late polypeptides, which were not detected in " whole cells " pulse-labeled with [^{35}S]-methionine, were 8 CE polypeptides (CE110K, CE94K, CE88K, CE60K, CE58K, CE35K, CE25K and CE17.5K) and 8 CL polypeptides (CL250K, CL230K, CL98K, CL80K, CL42K, CL40.5K, CL20K and CL19K).

Thus 14 species of CE polypeptides and 26 species of CL polypeptides were identified from the results of Figs. 4 and 5. Eight CE and 8 CL polypeptides were detected only in the cells labeled with [^{14}C]-leucine as described above, CE37K and 4 CL polypeptides (CL54K, CL52K, CL34K and CL28K) only in the cells labeled with [^{35}S]-methionine, and the other CE and CL polypeptides in both cells labeled with [^{14}C]-leucine and those labeled with [^{35}S]-methionine.

3. *Identification of Shope fibroma virus-induced polypeptides*

1) Analysis of " whole cells " pulse-labeled with [^{35}S]-methionine

Shope fibroma virus-induced early (SE) and late (SL) polypeptides were identified in the same way using Ara C.

Monolayer cells of RK_{13} infected with Shope fibroma virus or mock-infected were labeled with [^{35}S]-methionine for 1 hr at various times postinfection in the presence or absence of Ara C (see legend to Fig. 6), and subjected to SDS-PAGE and the results are shown in Fig. 6. At least 6 SE and at least

17 SL polypeptides were found in infected cells. The MW of these 6 SE and 17 SL polypeptides, calculated as described above, ranged from about 90,000 to about 24,000 and from about 150,000 to about 14,000 and the polypeptides were provisionally named SE90K to SE24K and SL150K to SL14K, respectively. These SE and SL polypeptides are summarized in Table 3.

2) Analysis of fractionated cells

Monolayer cells of RK_{13} were infected with Shope fibroma virus or mock-infected,

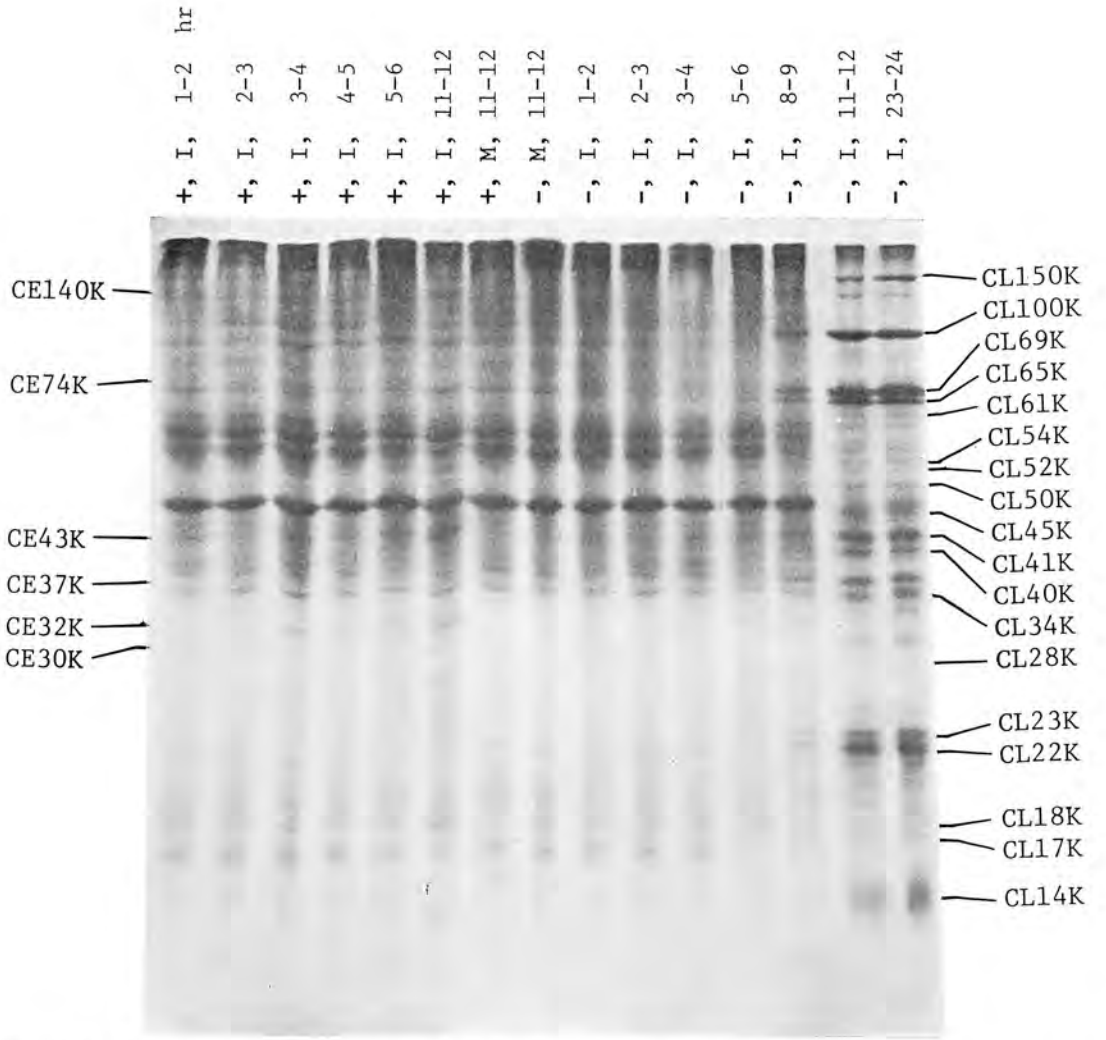

FIGURE 4. Time course of cowpox virus-induced polypeptide synthesis in infected cells. RK_{13} cells were infected (I) at moi 10 or mock-infected (M) in the presence (+) or absence (−) of Ara C (50 μg/ml). Infected and mock-infected cells were labeled with [^{35}S]-methionine, and analyzed by SDS-PAGE, as described in the legend to Fig. 1. The MW of polypeptides was calculated as described in the legend to Fig. 1.

TABLE 2. *Cowpox virus-induced early and late polypeptides*

Virus-induced early polypeptides			Virus-induced late polypeptides		
[^{35}S]-Met[a] "Whole"[c]	[^{14}C]-Leu[b] "Sup"[d]	[^{14}C]-Leu[b] "Ppt"[e]	[^{35}S]-Met[a] "Whole"[c]	[^{14}C]-Leu[b] "Sup"[d]	[^{14}C]-Leu[b] "Ppt"[e]
					CL250K
					CL230K
			CL150K		CL150K
CE140K		CE140K			
		CE110K			
			CL100K	CL100K	CL100K
					CL98K
	CE94K				
	CE88K				
				CL80K	
CE74K	CE74K	CE74K			
			CL69K	CL69K	CL69K
			CL65K	CL65K	CL65K
			CL61K		CL61K
	CE60K	CE60K			
	CE58K	CE58K			
			CL54K		
			CL52K		
			CL50K	CL50K	
			CL45K	CL45K	CL45K
CE43K	CE43K				
					CL42K
			CL41K	CL41K	CL41K
				CL40.5K	
			CL40K	CL40K	
CE37K					
	CE35K				
			CL34K		
CE32K	CE32K	CE32K			
CE30K	CE30K				
			CL28K		
	CE25K				
			CL23K	CL23K	CL23K
			CL22K	CL22K	
					CL20K
					CL19K
			CL18K	CL18K	CL18K
		CE17.5K			
			CL17K	CL17K	
			CL14K	CL14K	CL14K

See Table 1 for details.

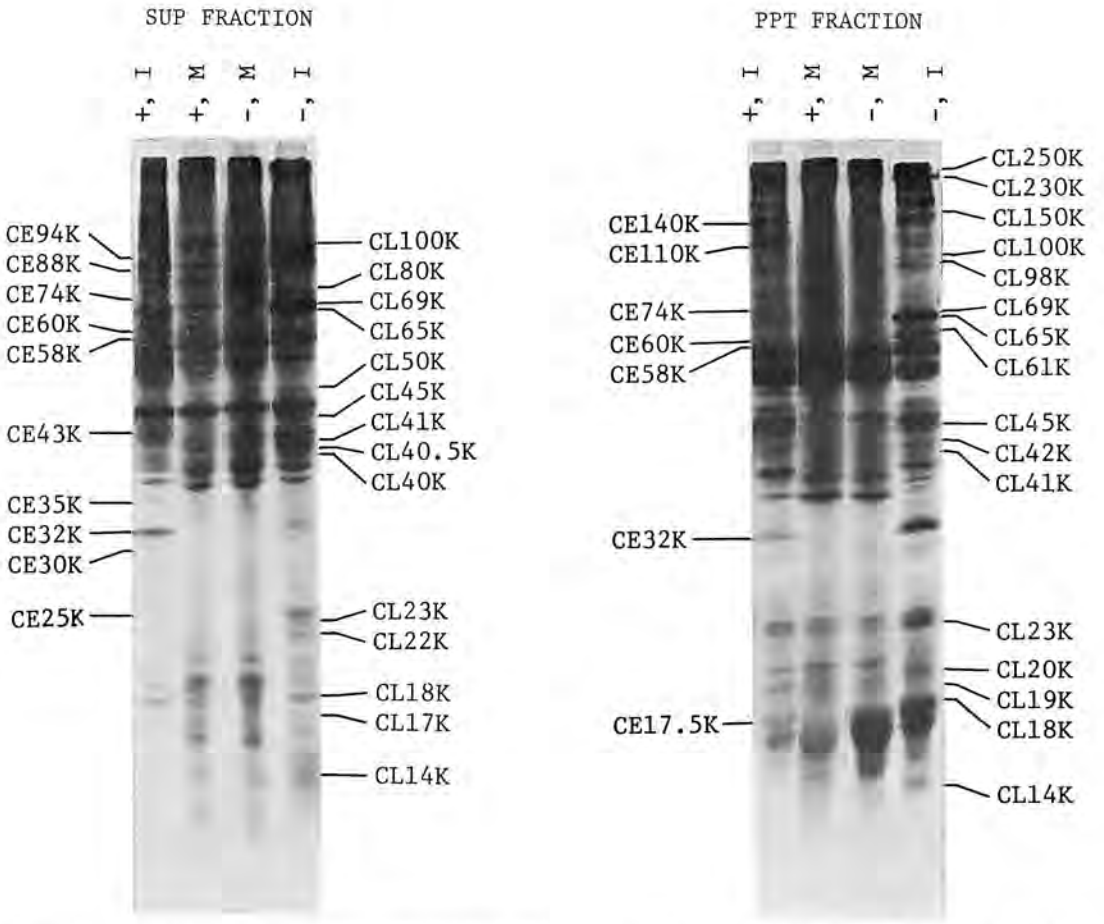

FIGURE 5. Distribution of cowpox virus-induced polypeptides in fractions of infected RK_{13} cells. RK_{13} cells were infected (I) at moi 10 or mock-infected (M) in the presence (+) or absence (−) of Ara C (50 μg/ml), labeled with [^{14}C]-leucine, and then fractionated, as described in the legend to Fig. 2. Fractionated samples, "sup fraction" and "ppt fraction", were analyzed by SDS-PAGE. The figure shows the autoradiogram of the gel. The MW of polypeptides was calculated as described in the legend to Fig. 1.

labeled with [^{14}C]-leucine from 1 to 25 hr postinfection, and then fractionated as described above. About 70% of the TCA-insoluble radioactivity of "whole cells" was consistently found in the "sup fraction". The fractionated samples were subjected to SDS-PAGE and the results are shown in Fig. 7. At least 8 SE and 5 SL polypeptides were found in the "sup fraction". At least 7 SE and 10 SL polypeptides were found in the "ppt fraction". The MW of these Shope fibroma virus-induced polypeptides were calculated as described above, and the polypeptides in the "sup fraction" were provisionally named SE90K to SE24K and SL105K to SL23K, while these in the "ppt fraction" were named SE140K to SE17.5K and SL250K to SL14K. These SE and SL polypeptides are summarized in Table 3. Six SE polypeptides (SE90K, SE88K, SE67K, SE62K, SE46K and

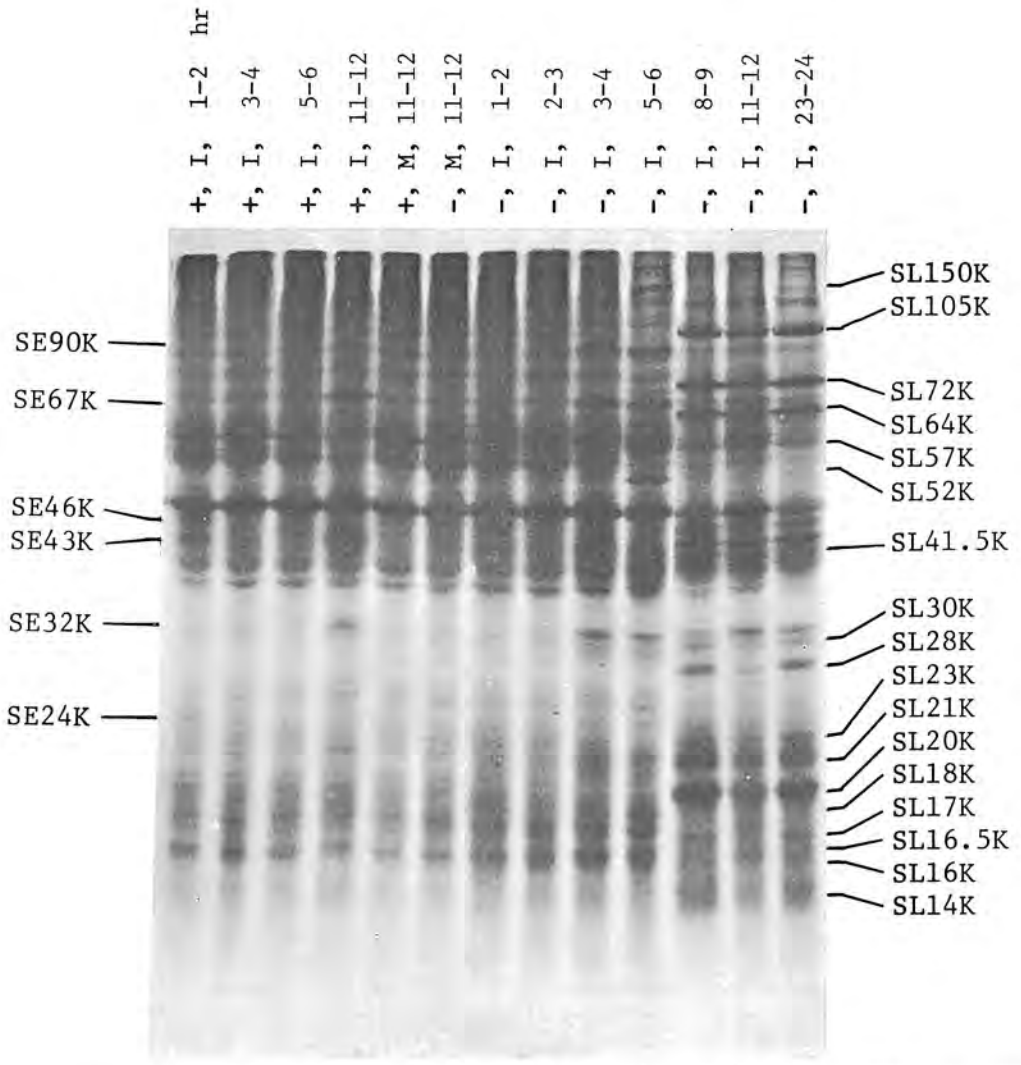

FIGURE 6. Time course of Shope fibroma virus-induced polypeptide synthesis in infected RK_{13} cells. RK_{13} cells were infected (I) at moi 10 or mock-infected (M) in the presence (+) or absence (−) of Ara C (50 μg/ml). Infected and mock-infected cells were labeled with [^{35}S]-methionine, and analyzed by SDS-PAGE, as described in the legend to Fig. 1. The MW of polypeptides was calculated as described in the legend to Fig. 1.

SE24K) and SL23K polypeptide were detected only in the " sup fraction ", 5 SE polypeptides (SE140K, SE120K, SE48K, SE-40.5K and SE17.5K) and 6 SL polypeptides (SL250K, SL72K, SL28K, SL20K, SL18K and SL14K) only in the " ppt fraction ", and the other SE and SL polypeptides were found in both fractions. The newly identified virus-induced early and late polypeptides in the fractionated cells labeled with [^{14}C]-leucine, which were not detected in " whole cells " pulse-labeled with [^{35}S]-methionine, were SE-

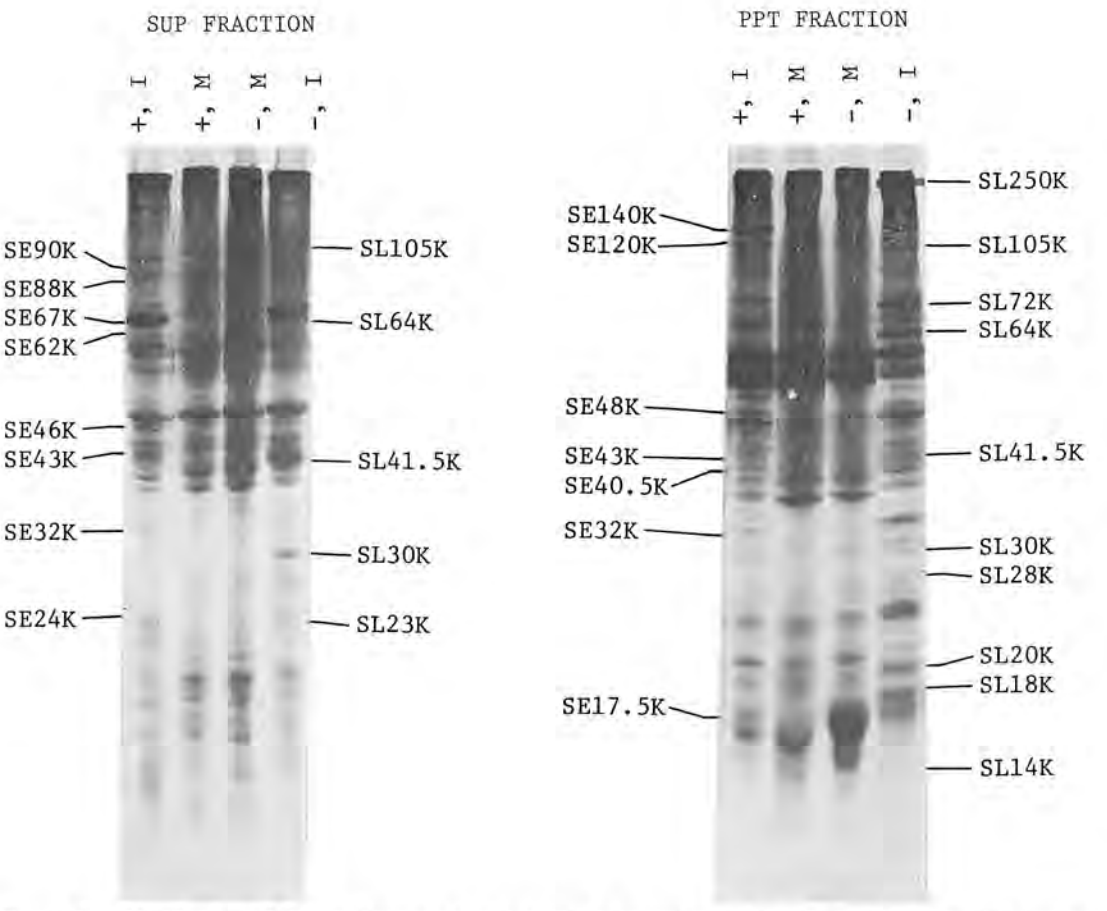

FIGURE 7. Distribution of Shope fibroma virus-induced polypeptides in fractions of infected RK_{13} cells. RK_{13} cells were infected (I) at moi 10 or mock-infected (M) in the presence (+) or absence (−) of Ara C (50 μg/ml), labeled with [^{14}C]-leucine, and then fractionated, as described in the legend to Fig. 2. Fractionated samples, "sup fraction" and "ppt fraction", were analyzed with SDS-PAGE. The figure shows the autoradiogram of the gel. The MW of polypeptides was calculated as described in the legend to Fig. 1.

140K, SE120K, SE88K, SE62K, SE48K, SE40.5K, SE17.5K and SL250K polypeptides.

Thus 13 and 18 species of polypeptides were identified from the results of Figs. 6 and 7. Seven SE and 1 SL polypeptide were detected only in the cells labeled with [^{14}C]-leucine, 7 SL polypeptides (SL150K, SL57K, SL52K, SL21K, SL17K, SL16.5K and SL-16K) only in the cells labeled with [^{35}S]-methionine, and the other SE and SL polypeptides in both cells labeled [^{14}C]-leucine and those labeled with [^{35}S]-methionine.

4. *Comparative studies of virus-induced polypeptides in vaccinia, cowpox and Shope fibroma viruses*

The early and late polypeptides induced by vaccinia, cowpox and Shope fibroma viruses were identified in studies on cells pulse-labeled with [^{35}S]-methionine and/or on fractionated cells labeled with [^{14}C]-leucine by SDS-PAGE and autoradiography, and are summarized in

Table 4.

It is uncertain whether the virus-induced polypeptides detected only in cells labeled with [^{35}S]-methionine were detected in this way because the pulse-labeling is more sensitive than longer labeling or because these polypeptides had a high content of methionine. Similarly, it is unknown whether the virus-induced polypeptides detected only in the cells labeled with [^{14}C]-leucine were detected because cell fractions rather than whole cells were used, or because the polypeptides are

TABLE 3. *Shope fibroma virus-induced early and late polypeptides*

Virus-induced early polypeptides			Virus-induced late polypeptides		
[^{35}S]-Met[a] "Whole"[c]	[^{14}C]-Leu[b] "Sup"[d]	[^{14}C]-Leu[b] "Ppt"[e]	[^{35}S]-Met[a] "Whole"[c]	[^{14}C]-Leu[b] "Sup"[d]	[^{14}C]-Leu[b] "Ppt"[e]
					S L250K
			S L150K		
		S E140K			
		S E120K			
			S L105K	S L105K	S L105K
S E90K	S E90K				
	S E88K				
			S L72K		S L72K
S E67K	S E67K				
			S L64K	S L64K	S L64K
	S E62K				
			S L57K		
			S L52K		
		S E48K			
S E46K	S E46K				
S E43K	S E43K	S E43K			
			S L41.5K	S L41.5K	S L41.5K
		S E40.5K			
S E32K	S E32K	S E32K			
			S L30K	S L30K	S L30K
			S L28K		S L28K
S E24K	S E24K				
			S L23K	S L23K	
			S L21K		
			S L20K		S L20K
			S L18K		S L18K
		S E17.5K			
			S L17K		
			S L16.5K		
			S L16K		
			S L14K		S L14K

See Table 1 for details.

TABLE 4. *Virus-induced early and late polypeptides of vaccinia, cowpox and Shope fibroma viruses*

Vaccinia		Cowpox		Shope fibroma	
Early	Late	Early	Late	Early	Late
	V L250K[bc]		C L250K[bc]		S L250K[bc]
	V L230K[bc]		C L230K[bc]		
V E190K[b]					
			C L150K[c]		S L150K[ac]
V E140K		C E140K		S E140K[bc]	
				S E120K[b]	
V E110K		C E110K[b]			
					S L105K[c]
	V L100K[c]		C L100K[c]		
	V L98K[bc]		C L98K[bc]		
	V L96K[c]				
V E94K		C E94K[b]			
				S E90K[c]	
V E88K[b]		C E88K[b]		S E88K[b]	
	V L85K[c]				
	V L80K[bc]		C L80K[bc]		
V E74K[c]		C E74K			
					S L72K[c]
	V L69K[c]		C L69K[c]		
				S E67K	
	V L65K[c]		C L65K[c]		
					S L64K[c]
				S E62K[b]	
	V L61K[c]		C L61K[c]		
V E60K[b]		C E60K[b]			
V E58K[b]		C E58K[b]			
					S L57K[ac]
			C L54K[a]		
	V L52K[ac]		C L52K[ac]		S L52K[ac]
	V L50K[c]		C L50K[c]		
V E49K[b]					
				S E48K[bc]	
				S E46K[c]	
			C L45K[c]		
	V L43K[c]	C E43K[c]		S E43K[c]	
	V L42K[c]		C L42K[bc]		
					S L41.5K[c]
	V L41K[c]		C L41K[c]		
	V L40.5K[b]		C L40.5K[b]	S E40.5K[bc]	
V E40K			C L40K[c]		

Continued . . .

TABLE 4. Continued.

Vaccinia		Cowpox		Shope fibroma	
Early	Late	Early	Late	Early	Late
VE37K		CE37K[a]			
	VL35K[c]	CE35K[bc]			
	VL34K[c]		CL34K[ac]		
	VL33K[ac]				
VE32K		CE32K		SE32K	
VE30K		CE30K			SL30K[c]
VE28K[b]			CL28K[a]		SL28K[c]
	VL25K[bc]	CE25K[bc]			
				SE24K	
	VL23K[c]		CL23K[c]		SL23K[c]
	VL22K[c]		CL22K[c]		
					SL21K[ac]
	VL20K[bc]		CL20K[bc]		SL20K[c]
	VL19K[bc]		CL19K[bc]		
	VL18K[c]		CL18K[c]		SL18K[c]
VE17.5K[b]		CE17.5K[b]		SE17.5K[b]	
	VL17K[c]		CL17K[c]		SL17K[ac]
					SL16.5K[a]
					SL16K[ac]
	VL14K[c]		CL14K[c]		SL14K[c]

[a] Virus-induced polypeptide found only in cells pulse-labeled with [^{35}S]-methionine.
[b] Virus-induced polypeptide found only in fractionated cells labeled with [^{14}C]-leucine.
[c] Virus-induced polypeptide corresponding in MW to the virion structural polypeptide identified previously (Ikuta *et al.*, 1978).

leucine-rich and methionine-poor.

Attempts were made to compare the mobilities of virus-induced polypeptides on SDS-PAGE. Eleven of 15 VE polypeptides (73%) and 21 of 27 VL polypeptides (78%) corresponded to 11 of 14 CE polypeptides (79%) and 21 of 26 CL polypeptides (81%), respectively. Thus there is a considerable similarity between the polypeptides induced by vaccinia and cowpox viruses.

On the other hand, only 4 of 15 VE polypeptides (27%) and 7 of 27 VL polypeptides (26%) corresponded to 4 of 13 SE polypeptides (31%) and 7 of 18 SL polypeptides (39%), respectively. Five of 14 CE polypeptides (36%) and 9 of 26 CL polypeptides (35%) corresponded to 5 of 13 SE polypeptides (38%) and 9 of 18 SL polypeptides (50%), respectively. These results show that Shope fibroma virus-induced polypeptides differed considerably from cowpox virus-induced polypeptides, and more especially from vaccinia virus-induced polypeptides. Four polypeptides (140K, 88K, 32K and 17.5K) common to VE and SE polypeptides, and 7 polypeptides (250K, 52K, 23K, 20K, 18K, 17K and 14K) common to VL and SL polypeptides, were also found in CE or CL polypeptides, respectively. These polypeptides may be induced by all poxviruses used in this study. The intensities of the bands thus compared were not necessarily the same.

We previously identified 35 VV, 36 CV and 37 SV polypeptides in vaccinia, cowpox and Shope fibroma virions (Ikuta et al., 1978). The mobilities of these structural polypeptides of virions were compared with those of virus-induced polypeptides separated in this work by SDS-PAGE. The results are shown in Table 4. Twenty-seven of 42 vaccinia virus-induced polypeptides (1 VE and 26 VL polypeptides) corresponded in MW to 27 of 35 structural polypeptides of vaccinia virions. Twenty-six of 40 cowpox virus-induced polypeptides (3 CE and 23 CL polypeptides) corresponded in MW to 26 of 36 structural polypeptides of cowpox virions. Twenty-three of 31 Shope fibroma virus-induced polypeptides (6 SE and 17 SL polypeptides) corresponded in MW to 23 of 37 structural polypeptides of Shope fibroma virions. VL-100K and CL100K polypeptides were the main late polypeptides while VV100K and CV100K polypeptides were minor polypeptides.

DISCUSSION

Pennington (1974) analyzed the polypeptides of BSC_1 cells infected with vaccinia virus or mock-infected by SDS-PAGE, and reported about 80 virus-induced polypeptides in schematic form. In this study we identified vaccinia, cowpox and Shope fibroma virus-induced early and late polypeptides in infected RK_{13} cells, and provisionally named them VE and VL, CE and CL, and SE and SL, respectively. We compared the mobilities of these virus-induced polypeptides on SDS-PAGE.

Virus-induced polypeptides were separated by SDS-PAGE from cells pulse-labeled with [^{35}S]-methionine and/or from fractionated cells labeled with [^{14}C]-leucine. As summarized in Table 4, at least 15 species of VE, 27 species of VL, 14 species of CE, 26 species of CL, 13 species of SE and 18 species of SL polypeptides were found. The SL polypeptides were fewer in number than the VL and CL polypeptides, possibly due to the slower growth of Shope fibroma virus.

We also analyzed the polypeptides of RK_{13} cells infected with the DIs strain of vaccinia virus, a mutant strain isolated by Tagaya et al. (1961) which produces "surface (S) antigen" but does not induce viral DNA or late protein synthesis in infected RK_{13} cells. However, using the same method we could not detect any virus-induced polypeptides (data not shown).

This work shows that most virus-induced late polypeptides in cells infected with vaccinia, cowpox or Shope fibroma virus correspond in MW to the virion structural polypeptides identified previously (Ikuta et al., 1978), as observed with vaccinia virus by other workers (Holowczak and Joklik, 1967; Salzman and Sebring, 1967). Several virus-induced early polypeptides also corresponded in MW to virion structural polypeptides identified previously (Ikuta et al., 1978). These results are consistent with the results of recent studies employing tryptic peptide analysis, which confirmed that some "early" polypeptides are structural components of vaccinia virions (Moss and Rosenblum, 1973). Eight VV, 10 CV and 14 SV polypeptides among structural polypeptides of virions identified previously (Ikuta et al., 1978) could not be detected among the virus-induced polypeptides identified in this work, probably because they were minor components of virions, or because they could not be separated from host cell proteins.

We suggested previously that VV41K polypeptide detected in vaccinia virions might be a component of HA (Ikuta et al., 1978). In this work also, VL41K polypeptide was not detected in cells infected with the IHD-W strain of vaccinia virus, although it was detected in cells infected with the IHD-J and Lister strains of vaccinia virus. Since the IHD-W strain of vaccinia virus does not produce HA (Kaku and Kamahora, 1964; Ichihashi and Matsumoto, 1969), these results support the idea that VL41K polypeptide is a component of HA.

Thirty-two of 40 cowpox virus-induced

polypeptides (80%) corresponded in MW to 32 of 42 vaccinia virus-induced polypeptides (76%). On the other hand, 11 of 42 vaccinia virus-induced polypeptides (26%) and 14 of 40 cowpox virus-induced polypeptides (35%) corresponded in MW to 11, and 14 of 31 Shope fibroma virus-induced polypeptides (35% and 45%), respectively. Orthopoxvirus (vaccinia and cowpox viruses) and Leporipoxvirus (Shope fibroma virus) share common antigens, usually called "nucleoprotein (NP) antigen", but their "heat labile and stable complex (LS) antigen" do not cross-react (Woodroofe and Fenner, 1962).

We plan to identify HA, NP, LS and S antigens on the basis of the virus-induced polypeptides in cells infected with vaccinia, cowpox and Shope fibroma viruses reported in this paper.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Y. Sakaue of this Institute, for critically reading the manuscript. This work was partly supported by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

Eagle, H. 1959. Amino acid metabolism in mammalian cell cultures. Science 130: 432–437.

Esteban, M., and D. H. Metz. 1973. Early virus protein synthesis in vaccinia virus-infected cells. J. Gen. Virol. 19: 201–216.

Holowczak, J. A., and W. K. Joklik. 1967. Studies on the structural proteins of vaccinia virus. II. Kinetics of the synthesis of individual groups of structural proteins. Virology 33: 726–739.

Ichihashi, Y., and S. Matsumoto. 1969. Genetic study of pox group viruses. Virus 19: 155–163.

Ikuta, K., H. Miyamoto, and S. Kato. 1978. Studies on the polypeptides of poxvirus. I. Comparison of structural polypeptides in vaccinia, cowpox and Shope fibroma viruses. Biken J. 21: 51–61.

Joklik, W. K. 1968. The poxviruses. Ann. Rev. Microbiol. 22: 359–390.

Jungwirth, C., and W. K. Joklik. 1965. Studies on "early" enzymes in HeLa cells infected with vaccinia virus. Virology 27: 80–93.

Kaku, H., and J. Kamahora. 1964. Giant cell formation in L cells infected with active vaccinia virus. Biken's J. 6: 299–315.

Maizel, J. V. 1971. Polyacrylamide gel electrophoresis of viral proteins. p. 179–246. *In* K. Maramorosch and H. Koprowski [ed.] Methods in Virology. vol. 5. Academic Press, New York.

McAuslan, B. R. 1963. Control of induced thymidine kinase activity in the poxvirus-infected cell. Virology 20: 162–168.

McAuslan, B. R., and J. R. Kates. 1967. Regulation of virus-induced deoxyribonucleases. Proc. Natl. Acad. Sci. USA 55: 1581–1587.

Moss, B., and E. N. Rosenblum. 1973. Protein cleavage and poxvirus morphogenesis: tryptic peptide analysis of core precursors accumulated by blocking assembly with rifampicin. J. Mol. Biol. 81: 267–269.

Moss, B., and N. P. Salzman. 1968. Sequential protein synthesis following vaccinia virus infection. J. Virol. 2: 1016–1027.

Pennington, T. H. 1974. Vaccinia virus polypeptide synthesis: sequential appearance and stability of pre- and post-replicative polypeptides. J. Gen. Virol. 25: 433–444.

Salzman, N. P., and E. D. Sebring. 1967. Sequential formation of vaccinia virus proteins and viral deoxyribonucleic acid replication. J. Virol. 1: 16–23.

Tagaya, I., T. Kitamura, and Y. Sano. 1961. A new mutant of dermovaccine virus. Nature (London) 192: 381–382.

Woodroofe, G. M., and F. Fenner. 1962. Serological relationships within the poxvirus group: an antigen common to all members of the group. Virology 16: 334–341.