JOURNAL OF VIROLOGY, Feb. 1970, p. 123-131
Vol.5, No. 2

Printed in U.S.A.

Proteins Specified by Herpes Simplex Virus

II. Viral Glycoproteins Associated with Cellular Membranes

PATRICIA G. SPEAR, JOHN M. KELLER, AND BERNARD ROIZMAN
Department of Microbiology, University of Chicago, Chicago, Illinois 60637

Received for publication 10 November 1969

Membranes prepared from HEp-2 cells infected with herpes simplex virus and free from soluble proteins, virus, ribosomes, and other cellular constituents were solubilized and subjected to electrophoresis on acrylamide gels. The electropherograms showed the following. (i) The synthesis of host proteins and glycoproteins ceases after infection. However, the spectrum of host proteins in membranes remains unaltered. (ii) Between 4 and 22 hr postinfection, at least four glycoproteins are synthesized and bound to the smooth cytoplasmic membranes. On electrophoresis, these glycoproteins form two major and two minor bands in the gel and migrate with proteins ranging from 50,000 to 100,000 daltons in molecular weight. (iii) The same glycoproteins are present in all membranes fractionated by density and in partially purified virus. The implications of the data are discussed.

The preceding paper of this series dealt with the proteins synthesized in HEp-2 cells infected with herpes simplex virus (29). In that paper, we showed that proteins are made in the cytoplasm and selectively migrate into the nucleus. The proteins synthesized after infection differ from those of uninfected cells. Most of the proteins made after 4 hr of infection are structural components of the virion. We have subsequently reported that some of the proteins made in infected cells bind to membranes (P. G. Spear, S. B. Spring, and B. Roizman, Bacterial. Proc., p. 170, 1969). In this paper, we describe in greater detail the nature and properties of the proteins and of the membranes to which these proteins are bound.

The circumstances which led us to examine the membranes of infected cells are of interest. Work in this and in other laboratories furnished ample evidence that the membranes of cells infected with herpesviruses must necessarily become modified after infection. The evidence comes from three series of observations. (i) The nucleocapsid acquires an inner envelope in the nucleus and at the same time becomes infectious (30; B. Roizman, S. B. Spring, and J. Schwartz, Fed. Proc., *in press*). However, the outer envelope is derived from a membrane of the cell. Although some virus strains obtain their envelopes from the inner lamella of the nuclear membrane (7, 17, 25, 26, 31), others appear to utilize all membranes of infected cells (10, 24, 27). These observations indicate that the virus specifies the site of envelopment, and, moreover, electron microscopic observations suggest that the membranes are modified at the time of envelopment. (ii) After envelopment, the virion is usually found in structures delineated by a membrane. Recent studies (23) have shown that these structures are probably modified endoplasmic reticulum and serve as channels or ducts for the egress of the virus from infected cells. (iii) Herpes simplex viruses have been known for many years to alter the "social behavior" of infected cells. These alterations are specified by the virus and range from rounding of cells with little or no clumping to formation of loose clumps, tight clumps, and, finally, fusion of cells (9, 20). Concurrent with the change in the social behavior, the membranes of infected cells become modified with respect to both structure and immunological specificity (19, 21). (iv) Histochemical studies on human amnion cells infected with herpes simplex virus (22) revealed the appearance of glycoprotein material in the nucleus and a proliferation of glycoprotein structures distributed around the nucleus and in the cytoplasm.

In this and subsequent papers of the series, we hope to elucidate the biochemical basis for the alteration of the membranes in herpesvirus-infected cells.

MATERIALS AND METHODS

Media and solutions. Minimal essential medium of Eagle (8) was obtained from Microbiological Associates, Inc., Bethesda, Md., and supplemented with 10% calf serum for the growth of cells. Complete mixture 199 and a special formulation lacking arginine, leucine, isoleucine, and valine were obtained

from Grand Island Biological Co., Grand Island, N.Y. Solutions of reagent-grade urea (8 M) were deionized on mixed-bed columns of an ion-exchange resin (Amberlite MB-1).

Chemicals. Special mixtures of leucine, isoleucine, and valine containing equal activities of either the ^3H or ^{14}C isotope were prepared for us by Schwarz Bio-Research, Inc., Orangeburg, N.Y. D-Glucosamine-6-^3H (>200 mc/mmole) was furnished by New England Nuclear Corp., Boston, Mass. Eastman Organic Chemicals, Rochester, N.Y., supplied the *N,N,N',N'*-tetramethylethylenediamine. Acrylamide and ethylene diacrylate were obtained from K & K Laboratories, Inc., Plainview, N.Y.

Cells. HEp-2 cells were obtained from Microbiological Associates, Inc.

Virus and infection of cells. The F strain of herpes simplex virus was isolated from a recurrent eruption on the face and was passaged in HEp-2 cells less than six times. The effects of the F strain on the social behavior of infected cells and the immunological properties of the virus were previously described (9). In all of the experiments described here, HEp-2 cells were infected by exposing monolayer cultures (approximately 4×10^7 cells) for 1 hr at 37 C to sufficient virus to yield effective multiplicities of approximately 20 (plaque-forming units) PFU/cell. The inoculum was then aspirated and replaced with 35 ml of mixture 199 supplemented with 1% calf serum. Subsequent manipulations of the infected cells are described in the following sections.

Labeling of cells with radioactive isotopes. Infected or uninfected cells were radioactively labeled with amino acids or glucosamine, or both, during the time intervals indicated below. The special 199 mixture, supplemented with one-tenth the usual concentrations of leucine, isoleucine, and valine, was used to incubate the cells with the ^3H or ^{14}C mixtures of leucine, isoleucine, and valine. Complete mixture 199 or 199 deficient in the three amino acids was used to label the cells with D-glucosamine-6-^3H.

Preparation of virus. Infected cells were washed two times with isotonic saline containing 10^{-3} M calcium acetate buffer, *p*H 7.0. The washes and the extracellular fluid were combined and centrifuged at $4,000 \times g$ for 10 min to remove cells and debris. The supernatant fluid was then layered on 3-ml cushions of 60% (w/w) sucrose in cellulose nitrate tubes [1 by 3.5 inches (2.54 by 8.9 cm)] and centrifuged at $81,500 \times g$ (average) and 4 C for 2 hr in the SW 27 rotor. From each tube, the visible band on top of the 60% sucrose cushion was aspirated with a syringe, diluted with 2 volumes of 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.0), and centrifuged at $55,000 \times g$ (average) and 4 C for 1 hr. The pellet containing virus was suspended in a small volume of water for solubilization.

Preparation of membrane fractions. Smooth membranes were purified from infected and uninfected cells by a procedure developed by Bosmann et al. (2) for the isolation of smooth endoplasmic reticulum and plasma membranes from HeLa cells. A flow diagram of the steps in the procedure is presented in Fig. 1. Briefly, 20- to 22-hr infected cells were washed twice with saline containing 10^{-3} M calcium acetate buffer (*p*H 7.0) and then suspended in approximately 10 volumes of 0.02 M Tris buffer (*p*H 7.0) containing 0.01 M ethylenediaminetetraacetic acid (EDTA). The cells were disrupted in a tight-fitting Dounce homogenizer; homogenization was monitored by phase microscopy to avoid excessive nuclear breakage. The homogenate was centrifuged at $4,000 \times g$ for 10 min to remove nuclei and debris. In some experiments, the pellet (P_1) was washed once with 5 volumes of buffer and the supernatant fluids (S_1 and S_2) were combined and made 45% (w/w) with respect to sucrose. About 10-ml samples of the $4,000 \times g$ supernatant fluid containing 45% (w/w) sucrose were placed in the bottom of cellulose nitrate tubes [1 by 3 inches (2.54 by 7.62 cm)] and overlaid with 6.5-ml amounts of 35 and 30% sucrose, 5 ml of 25% sucrose, and finally 2.5 ml of 0.05 M Tris buffer (*p*H 7.0). The discontinuous gradients were then centrifuged in the SW 25.1 rotor for approximately 20 hr at $64,000 \times g$ (average) and 4 C. After centrifugation, four visible bands and a pellet were obtained. The bands (fractions 1 to 4) were carefully aspirated with a syringe, and corresponding fractions from replicate gradients were pooled. Fractions 1 to 4 were diluted with approximately 4 volumes of 0.05 M Tris buffer and centrifuged at $55,000 \times g$ (average) and 4 C for 1 hr in the 30 rotor. The pellets (fraction 1-P to 4-P) were suspended in a small volume of 0.05 M Tris buffer. The pooled pellets (fraction 5) from the sucrose gradients were washed two times with 0.05 M Tris buffer and pelleted at $27,000 \times g$ for 10 min in a Sorvall centrifuge (fraction 5-P). As described below, purified smooth membranes were present in fraction 2-P and most of the infectious virus was found in fraction 4-P.

Acrylamide gel electrophoresis. Fractions obtained during the course of membrane purification were solubilized for gel electrophoresis as described by Summers et al. (34). Briefly, sodium dodecyl sulfate (SDS) was added to the cell fractions to yield a final concentration of 1 to 2%; the extracts were then acidified by the addition of a one-tenth volume of glacial acetic acid. Urea and β-mercaptoethanol were added to final concentrations of 0.5 M and 1%, respectively. After incubation at 60 C for 1 hr, the extracts were dialyzed for 15 to 20 hr against 0.01 M sodium phosphate buffer (*p*H 7.1) containing 0.1% SDS, 0.5 M urea, and 0.1% β-mercaptoethanol. The extracts were then dialyzed for an additional 3 to 4 hr against the same buffer containing 10% sucrose; if necessary, they were concentrated by vacuum dialysis. Acrylamide gel electrophoresis was performed in a continuous system essentially as described by Summers et al. (34), except that the gels were 0.6 by 20 cm and consisted of 5.7% (w/v) acrylamide (5.3% acrylamide plus 0.4% ethylene diacrylate), 0.1% *N, N, N', N'*, tetramethylethylenediamine, 0.1 M sodium phosphate buffer (*p*H 7.2), 0.5 M urea, 0.1% SDS, and 0.1% ammonium persulfate. Ethylene diacrylate was used as a cross-linking agent instead of the usual *N-N*-bismethylene acrylamide because gels polymerized with the former agent can be solubilized

Procedure for Purifying Cellular Membranes

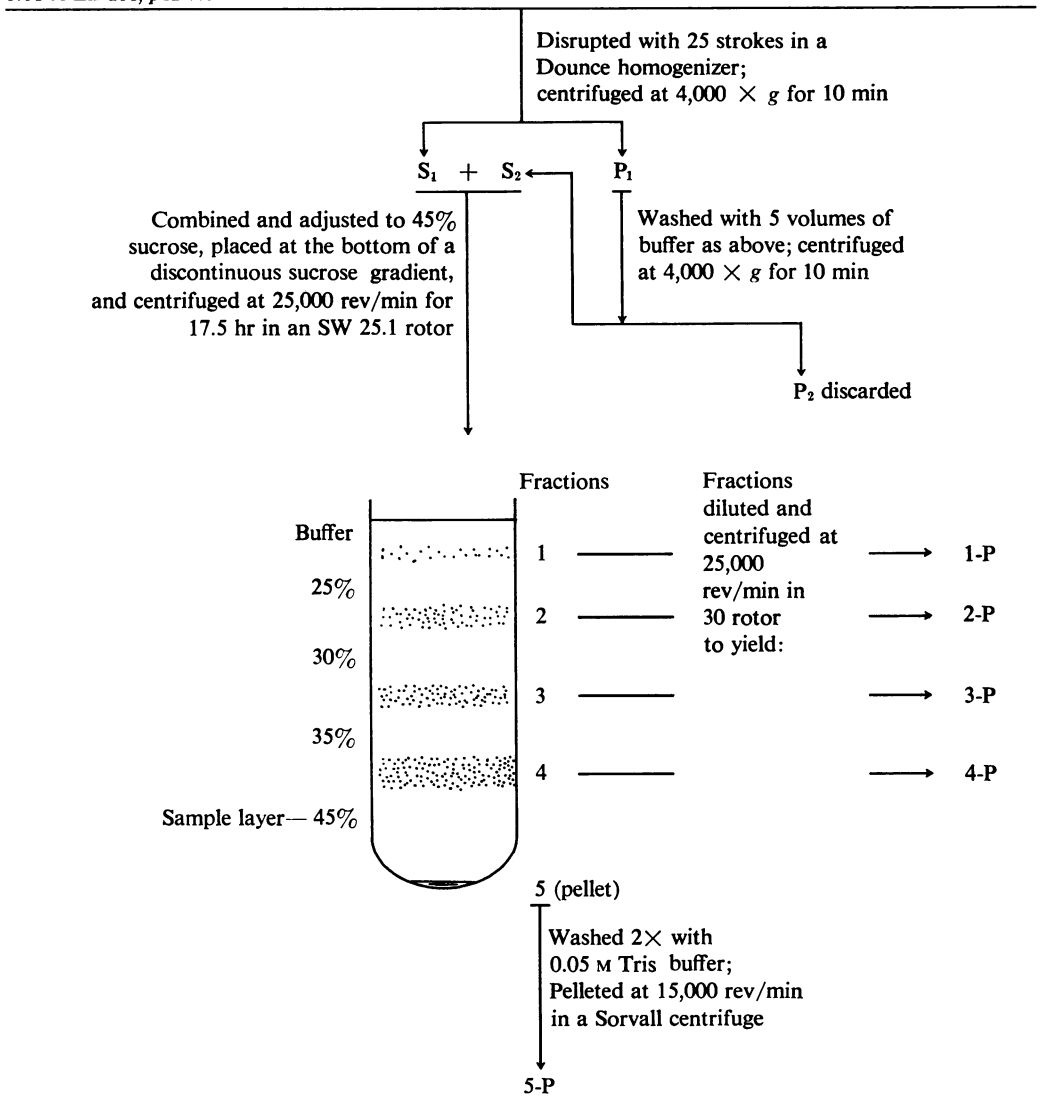

FIG. 1. *Flow diagram of the purification procedure used to obtain smooth membranes (2).*

at high *p*H (4), thereby facilitating the quantitation of radioactive proteins by liquid scintillation spectrometry. The protein samples (50 to 100 μliters), containing trace amounts of bromophenol blue, were layered on top of the gels and subjected to electrophoresis for about 15 hr at a regulated voltage of 3 v/cm. The upper and lower buffer chambers contained 0.1% SDS in 0.1 M sodium phosphate buffer, *p*H 7.2. The following procedure for quantitating radioactive proteins in acrylamide gels by using a nonaqueous liquid scintillation system has been described elsewhere (28). (i) The gels were sliced into 2-mm segments, and each segment was placed in a scintillation vial. (ii) A 2-ml amount of concentrated ammonium hydroxide was added to each vial, and the vials were agitated at room temperature until the segments were hydrolyzed. (iii) A strip of glass fiber paper (2 by 4 cm; Reeve Angel, grade GF81) was placed in each vial so that the paper touched the bottom and absorbed the liquid. (iv) The glass fiber wicks, still in vials, were heated under infrared lamps until they were completely dry. (v) Scintillation fluid [7.4 g of 2,5-diphenyloxazole plus 0.3 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene in 1 liter of toluene] was added to cover the wicks.

Electron microscopy of membrane fractions. Samples of the various fractions from infected or uninfected cells were diluted with water and centrifuged at 27,000 × *g* for 1 hr in the Sorvall centrifuge. The pellets were then suspended in 1.6% osmium tetroxide prepared in 0.067 M *s*-collidine buffer, *p*H 7.2. After 1 hr at 0 C, the membranes were removed from the osmium tetroxide solution by centrifugation. The pellets were then mixed with liquid Ionagar made up in 1% uranyl acetate; after the agar had solidified, the embedded pellets were cut into 2-mm cubes. The agar cubes were soaked in a 1% uranyl acetate solution overnight at 4 C, dehydrated in a graded series of ethyl alcohol, and embedded in Epon. Thin sections of the membranes were prepared by using a Porter-Blum MT-2 ultramicrotome and were examined after staining with uranyl acetate and lead citrate on an AEI EM-6B microscope.

RESULTS

Purity of membrane preparation. The fractions obtained during the course of membrane purification were very similar in composition, with respect to cellular constituents, to those obtained from HeLa cells by Bosmann et al. (2). Electron micrographs of thin sections of the various fractions from infected cells are shown in Fig. 2. Note that 4-P and 5-P contained considerable amounts of virus (see also Table 1). In addition, fractions 3-P, 4-P, and 5-P consisted of mixtures of various kinds of membranes contaminated with other cytoplasmic constituents. However, the fraction of greatest interest, designated 2-P, contained only smooth membranes. The degree of purity of this preparation is indicated by the following observations. (i) Electron microscopy of preparations from both infected and uninfected cells failed to reveal contamination with ribosomes or other cellular constituents. (ii) As shown in Table 1, less than 0.05% of the infectious virus put in the gradient was found in fraction 2-P. (iii) Soluble proteins (i.e., proteins not sedimentable at 64,000 × *g* for 1 hr) were not found in fraction 2 or 2-P (Table 1); these proteins remained in the sample layer (45% sucrose) during centrifugation.

Proteins incorporated into membranes of fraction 2-P after infection. To determine whether proteins synthesized after infection become bound to the smooth membranes of fraction 2-P, approximately 10^8 cells were incubated in medium containing ^{3}H-glucosamine (2 μc/ml) and ^{14}C-leucine, -isoleucine, and -valine (0.5 μc/ml) from 4 to 22 hr and from 6 to 22 hr postinfection, respectively. Radioactive glucosamine was used as well as amino acids, since many membrane-associated proteins have been shown to be glycoproteins. Membrane fraction 2-P was prepared, solubilized, and analyzed by gel electrophoresis. The profiles in Fig. 3 indicate that both glucosamine and amino acids become incorporated into proteins forming two major bands and possibly two minor bands in the gel. Preliminary results indicate that the ^{3}H-glucosamine is incorporated into the membranes as glucosamine and galactosamine. We conclude that three to four glycoproteins synthesized after infection become incorporated into smooth membranes of the cell.

Are host membrane proteins displaced? It was of interest to determine whether host proteins in the membranes are displaced by the glycoproteins synthesized after infection. In an attempt to provide information concerning this point, two experiments were done. In the first experiment, three replicate sets of cultures (approximately 10^8 cells in each set) were used. One set was incubated in medium containing ^{14}C-leucine, -isoleucine, and -valine (0.2 μc/ml) for 12 hr and then in medium containing nonradioactive amino acids for an additional 24 hr. The second set was incubated in medium containing the ^{14}C-amino acids and in nonradioactive medium as above, except that the cultures were infected after 3 hr in nonradioactive medium. The third set of cultures was infected and incubated with medium containing ^{3}H-leucine, -isoleucine, and -valine (5 μc/ml) from 8 to 21 hr postinfection. The cells in each set of cultures were then harvested, mixed with approximately 3 × 10^8 uninfected or infected unlabeled cells, and fractionated. Fraction 2-P from the ^{3}H-labeled cultures (labeled after infection) was then coelectrophoresed with each of the ^{14}C-labeled fractions. A comparison of the ^{14}C profiles in Fig. 4 reveals that, by this procedure, one cannot demonstrate displacement of host membrane proteins synthesized during a 12-hr interval before infection by proteins synthesized after infection. Since the host proteins in fraction 2-P are numerous and difficult to resolve, in the second experiment, we traced the fate of host glycoproteins only. In this experiment, two replicate cultures, each containing 3 × 10^7 cells, were incubated with ^{3}H-glucosamine (3 μc/ml) for 23 hr. One culture was then incubated in medium containing nonradioactive glucosamine (6 μg/ml) for an additional 23 hr; the other was incubated for 1 hr in the presence of nonradioactive glucosamine and then infected and incubated until 22 hr postinfection in the absence of glucosamine. A third culture was labeled with ^{3}H-glucosamine (3 μc/ml) from 4 to 22 hr postinfection. The electrophoretic profiles of proteins containing ^{3}H-glucosamine from fractions 2-P of the cultures labeled as described are presented in Fig. 5. Again, there was no evidence of a displacement of membrane-bound proteins synthesized before infection by the glycoproteins synthesized

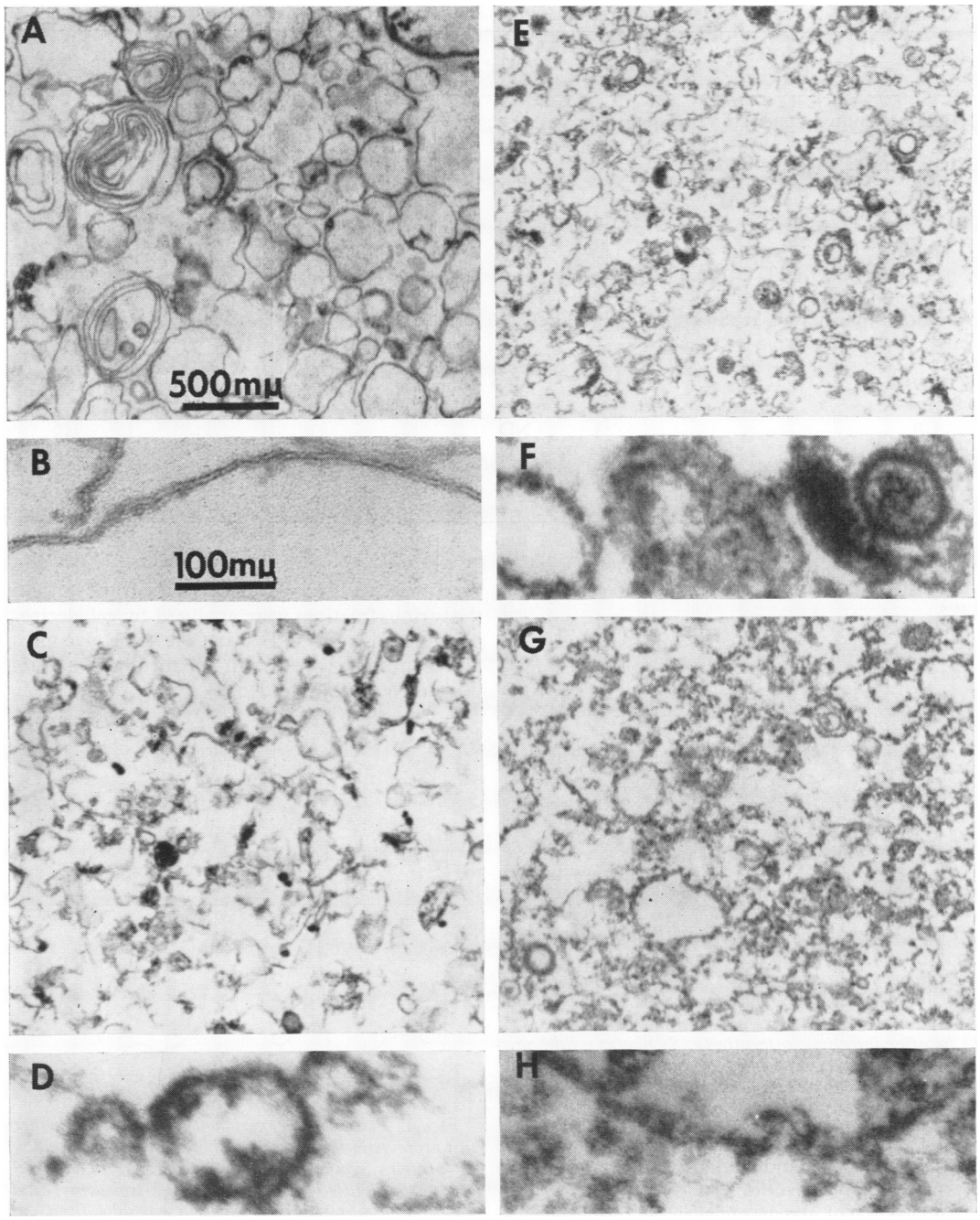

FIG. 2. *Electron micrographs of thin sections of material found in fractions 2-P through 5-P. A and B, fraction 2-P; C and D, fraction 3-P; E and F, fraction 4-P; G and H, fraction 5-P. A, C, E, and G, magnification as shown in A. B, D, F, and H, magnification as shown in B.*

after infection. It appears that the proteins made after infection are either added to preexisting membranes or become incorporated into newly synthesized membranes. Finally, these two experiments demonstrate, as we previously reported (P. G. Spear, S. B. Spring, and B. Roizman, Bacteriol. Proc., p. 170, 1969), that there is no resemblance between the profile of host proteins

TABLE 1. *Assays of infectious virus and protein during the course of membrane purification*

Fraction	PFU	Per cent of initial PFU	Protein (mg)	Per cent of initial protein
Cell homogenate plus washings (S_1 and S_2)	$1,090 \times 10^6$	100	85.4	100
Discontinuous sucrose gradient				
1	0.04×10^6	0.004	0.42	0.5
2	0.54×10^6	0.05	1.63	1.9
3	18.5×10^6	1.7	1.69	2.0
4	292×10^6	26.8	16.00	18.7
5 (pellet)	41×10^6	3.8	4.45	5.2
Remainder of gradient	153×10^6	14.0	49.80	58.3
Totals for sucrose gradient	505×10^6	46.4	74.0	86.6
Pellets of fractions from sucrose gradient				
1-P	ND[a]		0.30	0.4
2-P	ND		1.50	1.8
3-P	ND		1.38	1.6
4-P	ND		4.32	5.1
Washed pellet from sucrose gradient				
5-P	22×10^6	2.0	1.32	1.5

[a] Assay not done.

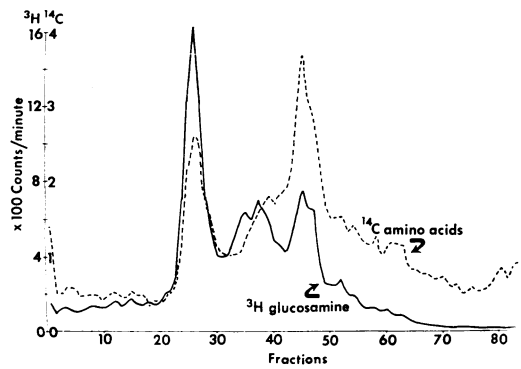

FIG. 3. *Electrophoretic profiles of the proteins synthesized after infection and incorporated into the smooth membranes of fraction 2-P. Cells were incubated in medium containing ^{3}H-glucosamine and ^{14}C-leucine, -isoleucine, and -valine from 4 to 22 hr and from 6 to 22 hr after infection, respectively. Symbols: solid line, ^{3}H-glucosamine; dashed line, ^{14}C-amino acids. In this and all of the following figures, migration of proteins is from left to right toward the anode.*

from fraction 2-P and that of the glycoproteins synthesized after infection.

Comparison of glycoproteins in the various bands obtained after flotation of membranes. To determine whether the glycoproteins found associated with the membranes of fraction 2-P are also associated with the other membranes from infected cells, the following experiment was done. A culture containing 3×10^7 cells was incubated from 4 to 22 hr postinfection in medium containing ^{3}H-glucosamine (3 μc/ml); the infected cells were then fractionated as described above, and the various fractions were analyzed by gel electrophoresis. Figure 6 shows that the same glycoprotein bands are present in all membranes fractionated according to density.

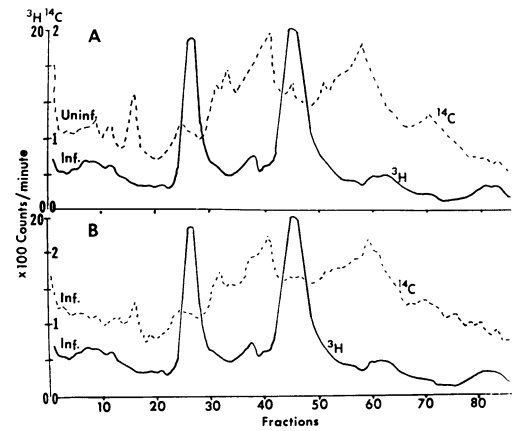

FIG. 4. *Pulse-chase experiment to determine whether host proteins in the smooth membranes are displaced by the proteins synthesized after infection. Fraction 2-P from cells labeled with ^{3}H-leucine, -isoleucine, and -valine from 8 to 21 hr postinfection was solubilized and coelectrophoresed with A, the proteins of fraction 2-P from uninfected cells labeled with the same ^{14}C-amino acids for 12 hr and then incubated with nonradioactive medium for an additional 24 hr, and B, the proteins of fraction 2-P from cells labeled as in A with ^{14}C-amino acids and then infected after 3 hr in nonradioactive medium. Symbols: solid line, ^{3}H-leucine, -isoleucine, and -valine; dashed line, ^{14}C-leucine, -isoleucine, and -valine.*

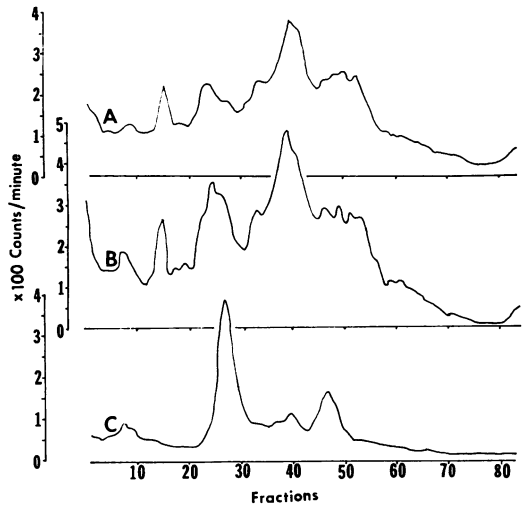

FIG. 5. *Pulse-chase experiment to determine whether host glycoproteins in the smooth membranes are displaced by glycoproteins synthesized after infection. The electrophoretic profiles are shown of ^{3}H-glucosamine incorporated into the proteins of fraction 2-P from cells treated as follows. (A) Uninfected cells were incubated with medium containing ^{3}H-glucosamine for 23 hr and with medium containing nonradioactive glucosamine for an additional 23 hr. (B) Cells were incubated with medium containing ^{3}H-glucosamine for 23 hr and then infected after incubation for 1 hr in medium containing nonradioactive glucosamine. (C) Cells were labeled with ^{3}H-glucosamine from 4 to 22 hr postinfection.*

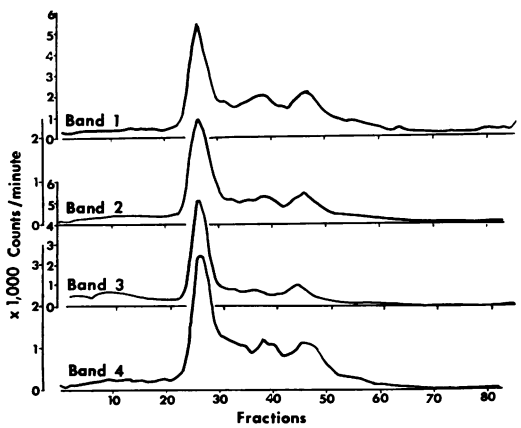

FIG. 6. *Electrophoretic profiles of glycoproteins in fractions 1-P to 4-P from cells incubated with medium containing ^{3}H-glucosamine from 4 to 22 hr postinfection.*

Comparison of membrane glycoproteins with structural proteins of the virus. To determine whether the glycoproteins present in the membranes of infected cells are also structural components of the virus, partially purified virus was prepared as described above. This virus was prepared from the tissue culture fluid and saline washings of the same infected cells which yielded the 2-P preparation and its electropherogram shown in Fig. 3. The choice of extracellular fluid as a source of virus for purification is based on electron microscopic observations (*unpublished data*) that it yields consistently cleaner preparations of virus than cell lysates. The electrophoretic profiles in Fig. 7 show the distribution of glucosamine and amino acids in the proteins of the solubilized virus preparation. A comparison of Fig. 3 and 7 shows that the glucosamine profiles are practically indistinguishable; the amino acid profiles differ in that several additional protein bands are present in virus. We conclude that, with respect to the F strain of herpes simplex (a type 1 virus), the electrophoretic profiles of the glycoproteins in the membranes and virion are identical. Glycoproteins similar in electrophoretic mobilities to those present in the F virus have also been found in partially purified virus prepared by Olshevsky and Becker (Virology, *in press*). The choice of the F strain for these studies may have been fortuitous, since studies of glycoproteins made in cells infected with several other variants (Keller, Spear, and Roizman, *manuscript in preparation*) have shown that purified virus contains glycoproteins in addition to those contained in the membranes.

DISCUSSION

Characteristics of the membranes in fraction 2-P. Biochemical data furnished by Bosmann et al (2) showed that the membranes recovered by their procedure, in a fraction equivalent to 2-P, are smooth endoplasmic reticulum. Some of the

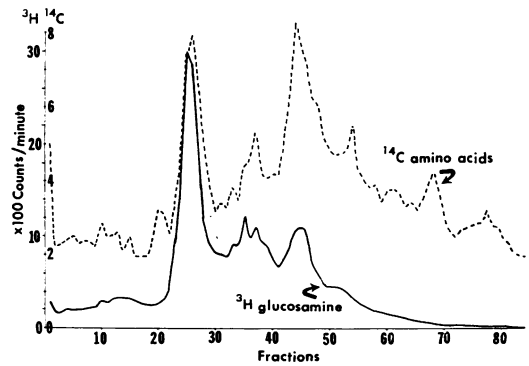

FIG. 7. *Electropherogram of proteins present in partially purified herpesvirus. The virus was prepared from the extracellular fluid of cells incubated in medium containing ^{3}H-glucosamine and ^{14}C-leucine, -isoleucine, and -valine from 4 to 22 hr and from 6 to 22 hr postinfection, respectively. Symbols: solid line, ^{3}H-glucosamine; dashed line, ^{14}C-leucine, -isoleucine, and -valine.*

characteristics of the membranes in our fraction 2-P, which was studied in greatest detail, are as follows. (i) The membranes are free from ribosomes, virus, and soluble proteins. (ii) Preliminary chemical analyses performed by E. D. Kieff indicate that they consist of 50% lipid and are free from sphingomyelins. (iii) The spectrum of host glycoproteins in the smooth membranes appears unaltered after infection. It should be pointed out that the absence of infectious virus in the 2-P membrane band is particularly significant with respect to the purity of the membrane preparation. Specifically, only enveloped or partially enveloped virus is infectious (30). The buoyant density of infectious virus is lower than that of unenveloped virus. However, even enveloped virus appears more dense than the membranes in band 2-P.

Nature of the proteins incorporated into the membranes. In this paper, we have demonstrated that three to four proteins are synthesized after infection and are subsequently incorporated into cellular membranes. The following points concerning these proteins should be noted. (i) The glycoproteins incorporated into the membranes migrate at the same rate as proteins ranging from 50,000 to 100,000 daltons in molecular weight (29). (ii) The same glycoproteins appear to be incorporated into all membrane fractions examined. (iii) In the previous paper in this series, we cited evidence that the proteins made 4 to 6 hr after infection are specified by the virus (29). To this list we may add two findings. First, the glycoproteins made after infection appear to be structural components of the herpesvirion. Second, recent studies (Keller, Spear, and Roizman, *manuscript in preparation*) show that the electrophoretic profiles of the glycoproteins synthesized in infected HEp-2 cells vary with the strain of herpesvirus used.

Significance of this work. Recent interest in structure and biosynthesis of membranes of infected cells stems from three considerations. First, in the course of infection, all viruses must necessarily pass through at least one cellular membrane when they enter and exit from the cell. The structure of cell membranes is a determinant of infection, and information concerning membranes is essential for the understanding of the mechanisms of entry and egress of the virus. Second, nearly all viruses studied to date have been shown to affect the biosynthesis and structure of membranes. The interactions of some viruses with cell membranes are highly specific; these are usually viruses containing lipid structural constituents. With the apparent exception of vaccinia (6), viruses containing lipids acquire an envelope from a membrane of the cell. In the case of some viruses, viral proteins associated with lipid and making up the envelope have been identified and characterized (1, 11, 13, 32, 33). It is thought that these viruses are enveloped by a membrane modified by prior insertion of viral proteins, but proof is lacking since purified membranes containing these proteins have not been furnished. Third, cells infected with some viruses survive infection but express new surface antigens and exhibit altered behavior with respect to interaction with other cells, the surfaces on which they grow, and transplantability into a nonimmune host. Some of the altered properties of the transformed cells are necessarily mediated by the surface of the cells. At least in the case of cells transformed by papovaviruses, the virus-induced surface antigens that have been identified have been shown to result from a reorganization of the plasma membrane which results in host antigens coming accessible at the surface (3, 12, 16, 18).

In relation to the basic problems cited above, the significance of our findings is as follows. (i) The isolation from infected cells of a membrane fraction containing only the glycoprotein constituents of the virion, coupled with the presence of the same glycoproteins in all membrane fractions, suggests very strongly that the glycoproteins bind to membranes and modify them before envelopment. The experimental data presented in this paper are in agreement with conclusions based on purely immunological grounds, namely, that the new antigens on the plasma membrane of herpesvirus-infected cells are identical to those on viral envelopes (19, 21). (ii) Several herpesviruses are associated with tumors in man and animals. In one, the Burkitt lymphoma of African children, it has been shown that tumor cells exhibit new surface antigens, that there is a good correlation between antibodies to surface antigens and to intracellular viral antigens (14, 15), and that regression of the tumor correlates with an increase in titers of antibody to surface antigens (5). The demonstration that herpes simplex virus specifies glycoproteins which bind to membranes gives credence to the hypothesis that the new surface antigens in these tumors are specified by the virus. (iii) To our knowledge, this is the first isolation of cellular membranes which contain viral glycoproteins and are free from all other protein constituents of the virus. Such a membrane preparation should be useful for the study of membrane structure. Studies of the properties of the glycoproteins and of the membranes to which they bind are continuing.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant CA 08494 from the National Cancer Institute, American Cancer Society grant E 314E, and National Science Foundation

grant GB 8242. P.G.S. is a Public Health Service postdoctoral trainee (TO 1 HD 00297-01). J.M.K. is a fellow of the Leukemia Society.

We acknowledge the advice of Jerome Schwartz and Robert Friis and the technical assistance of Patricia Wiedner in the preparation of the electron photomicrographs. A preliminary account of this paper was presented at the meetings of the American Society for Microbiology in May of 1969.

LITERATURE CITED

1. Bikel, I., and P. H. Duesberg. 1969. Proteins of Newcastle disease virus and of the viral nucleocapsid. J. Virol. 4:388–393.
2. Bosmann, H. B., A. Hagopian, and E. H. Eylar. 1968. Cellular membranes: the isolation and characterization of the plasma and smooth membranes of HeLa cells. Arch. Biochem. Biophys. 128:51–69.
3. Burger, M. M. 1969. A difference in the architecture of the surface membrane of normal and virally transformed cells. Proc. Nat. Acad. Sci. U.S.A. 62:994–1001.
4. Choules, G. L., and B. H. Zimm. 1965. An acrylamide gel soluble in scintillation fluid: its application to electrophoresis at neutral and low *p*H. Anal. Biochem. 13:336–344.
5. Clifford, P., S. Singh., J. Stjernsward, and G. Klein. 1967. Long-term survival of patients with Burkitt's lymphoma: an assessment of treatment and other factors which may relate to survival. Cancer Res. 27:2578–2615.
6. Dales, S., and E. H. Mosbach. 1968. Vaccinia as a model for membrane biogenesis. Virology 35:564–583.
7. Darlington, R. W., and L. H. Moss III. 1968. Herpesvirus envelopment. J. Virology 2:48–55.
8. Eagle, H. 1959. Amino acid metabolism in mammalian cell cultures. Science (Washington) 130:432–437.
9. 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.
10. Epstein, M. A. 1962. Observations on the mode of release of herpesvirus from infected HeLa cells. J. Cell Biol. 12:589–597.
11. Evans, M. J., and D. W. Kingsbury. 1969. Separation of Newcastle disease virus proteins by polyacrylamide gel electrophoresis. Virology 37:597–604.
12. Hakomori, S., C. Teather, and H. Andrews. 1968. Organizational difference of cell surface "hematoside" in normal and virally transformed cells. Biochem. Biophys. Res. Commun. 33:563–568.
13. Haslam, E. A., I. M. Cheyne, and D. O. White. 1969. The structural proteins of Newcastle disease virus. Virology 39:118–129.
14. Klein, G., G. Pearson, G. Henle, W. Henle, V. Diehl, and J. C. Niederman. 1968. Relation between Epstein-Barr viral and cell membrane immunofluorescence in Burkitt tumor cells. II. Comparison of cells and sera from patients with Burkitt's lymphoma and infectious mononucleosis. J. Exp. Med. 128:1021–1030.
15. Klein, G., G. Pearson, J. S. Nadkarni, J. J. Nadkarni, E. Klein, G. Henle, W. Henle, and P. Clifford. 1968. Relation between Epstein-Barr viral and cell membrane immunofluorescence of Burkitt tumor cells. I. Dependence of cell membrane immunofluorescence on presence of EB virus. J. Exp. Med. 128:1011–1020.
16. Meezan, E., H. C. Wu, P. H. Black, and P. W. Robbins. 1969. Comparative studies on the carbohydrate-containing membrane components of normal and virus-transformed mouse fibroblasts. II. Separation of glycoproteins and glycopeptides by sephadex chromatography. Biochemistry 8:2518–2524.
17. Morgan, C., S. A. Ellison, H. M. Rose, and D. H. Moore. 1954. Structure and development of viruses as observed in the electron microscope. I. Herpes simplex virus. J. Exp. Med. 100:195–202.
18. Pollack, R. E., and M. M. Burger. 1969. Surface-specific characteristics of a contact-inhibited cell line containing the SV40 viral genome. Proc. Nat. Acad. Sci. U.S.A. 62:1074–1076.
19. Roane, P. R., Jr., and B. Roizman. 1964. Studies of the determinant antigens of viable cells. II. Demonstration of altered antigenic reactivity of HEp-2 cells infected with herpes simplex virus. Virology 22:1–8.
20. Roizman, B. 1962. Polykaryocytosis. Cold Spring Harbor Symp. Quant. Biol. 27:327–342.
21. Roizman, B., and S. B. Spring. 1967. Alteration in immunologic specificity of cells infected with cytolytic viruses, p. 85–96. *In* J. J. Trentin (ed), Cross-reacting antigens and neoantigens, Williams & Wilkins Co., Baltimore.
22. Rosan, R. C., A. J. Nahmias, S. Kibrick, and J. A. Kerrigan. 1964. Studies in glycoprotein production: infection of primary cultures of human amnion with herpes simplex virus. Exp. Cell Res. 36:611–624.
23. Schwartz, J., and B. Roizman. 1969. Concerning the egress of herpes simplex virus from infected cells: electron and light microscope observations. Virology 38:42–49.
24. Schwartz, J., and B. Roizman. 1969. Similarities and differences in the development of laboratory strains and freshly isolated strains of herpes simplex virus in HEp-2 cells. Electron miscopic studies. J. Virol. 4:879–889.
25. Shipkey, F. H., R. B. Erlandsen, R. B. Bailey, V. J. Babcock, and C. M. Southam. 1967. Virus biographies. II. Growth of herpes simplex virus in tissue culture. Exp. Mol. Pathol. 6:39–67.
26. Siegert, R., and D. Falke. 1966. Elektronen mikroskopishe Untersuchungen uber die Entruicklung des Herpes Virus hominus in Kulturzellen. Arch. Ges. Virusforsch. 19:230–246.
27. Siminoff, P., and M. G. Menefee. 1966. Normal and 5-bromodeoxyuridine-inhibited development of herpes simplex virus. An electron microscope study. Exp. Cell Res. 44:241–255.
28. Spear, P. G., and B. Roizman. 1968. An improved procedure for H^3 and C^{14} counting in acrylamide gels with a nonaqueous scintillation system. Anal. Biochem. 26:197–200.
29. 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.
30. Spring, S. B., and B. Roizman. 1968. Herpes simplex virus products in productive and abortive infection. III. Differentiation of infectious virus derived from nucleus and cytoplasm with respect to stability and size. J. Virol. 2:979–985.
31. Spring, S. B., B. Roizman, and J. Schwartz. 1968. Herpes simplex virus products in productive and abortive infection. II. Electron microscopic and immunological evidence for failure of virus envelopment as a cause of abortive infection. J. Virol. 2:384–392.
32. Strauss, J. H., Jr., B. W. Burge, and J. E. Darnell. 1969. Sindbis virus infection of chick and hamster cells; synthesis of virus-specific proteins. Virology 37:367–376.
33. Strauss, J. H., Jr., B. W. Burge, and E. R. Pfefferkorn. 1968. Identification of membrane protein and "core" protein of Sindbis virus. Proc. Nat. Acad. Sci. U.S.A. 50:533–537.
34. Summers, D. F., J. V. Maizel, Jr., and J. E. Darnell, Jr. 1965. Evidence for virus-specific noncapsid proteins in poliovirus-infected HeLa cells. Proc. Nat. Acad. Sci. U.S.A. 54:505–513.