JOURNAL OF VIROLOGY, May 1969, p. 490-497
Vol. 3, No. 5

Changes in Nuclear Basic Proteins During Pseudorabies Virus Infection

J. G. STEVENS, G. J. KADO-BOLL AND C. B. HAVEN

Department of Medical Microbiology and Immunology, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024

Received for publication 6 January 1969

As a preliminary study to investigation of the possible role played by basic proteins in the genetic regulation of virus-infected cells, acid-extractable proteins synthesized during pseudorabies virus infection were investigated. The synthesis of histones was found to decrease in a gradual manner, and arrest was complete by 6 hr after infection. Five virus-induced acid-extractable proteins appeared in nuclei of infected cells after 4 hr of infection. Four of these proteins were virus structural proteins; one was not. All these proteins contained tryptophan and, therefore, were not "classic" histones.

In a number of systems, replication and transcription of the host-cell chromosome is specifically inhibited by virus infection (11). In some instances, at least, the synthesis of protein is necessary to achieve these effects. Whether the proteins synthesized are themselves bound to the chromosome and function as inhibitors or whether they act in an indirect manner is not clear, since, with the possible exception of the adenovirus fiber and hexon proteins (10), an inhibitor has never been isolated and characterized.

The following results of others led us to begin a systematic investigation of basic proteins as potential candidates for direct regulatory molecules in cells infected with pseudorabies virus. First, several lines of evidence indicate that histones act directly as regulatory molecules in mammalian cells (2). Second, in pseudorabies virus-infected rabbit kidney cells, it is known that host-cell deoxyribonucleic acid (DNA) synthesis is completely inhibited by 7.5 hr after infection in the same nuclei in which viral DNA synthesis is proceeding at a rapid rate (9). This selective inhibitory process (which does not involve degradation of cellular DNA into acid-soluble material) requires protein synthesis (1). Lastly, replication of the related herpes simplex virus is profoundly affected by the presence or absence of the basic amino acids arginine, lysine, and histidine. Deletion of arginine or histidine drastically inhibits viral replication, whereas the absence of lysine seems to slightly potentiate virus yields. Effects noted with single deletions of these amino acids are greater than those noted for any of the 10 other amino acids in Eagle's basal medium (17).

The purpose of this initial report is to characterize some aspects of the synthesis of intranuclear basic proteins after pseudorabies virus infection. It will be shown that the synthesis of histones is completely inhibited by 6 hr after infection and that five virus-induced proteins which can be extracted with acid from nuclei of virus-infected cells appear. Data concerning the relationship of these five proteins to viral structural proteins and to histones will be presented.

MATERIALS AND METHODS

Cells. The RK_{13} rabbit kidney cell line was generously supplied by Nathlie Schmidt, California Department of Public Health, Berkeley. Cells were routinely grown in monolayer in Eagle's Minimal Essential Media (MEM) (5), supplemented with 10% hypogammaglobulinemic calf serum in a humidified atmosphere of 5% CO_2 and air. Stock cultures were treated periodically with kanamycin (Bristol Laboratories, Syracuse, N.Y.) and tylocin tartrate (Grand Island Biological Co., Grand Island, N.Y.) to control possible pleuropneumonia-like organism (PPLO) contamination. Cultures prepared by standard methods failed to detect PPLO in this cell line at any time.

Virus. Pseudorabies virus was kindly supplied by Albert Kaplan, Albert Einstein Medical Center, Philadelphia. It was subsequently cloned three times on RK_{13} cells in this laboratory. Virus stocks were prepared by infecting RK_{13} cells at a low-adsorbed multiplicity (about 1 plaque-forming unit (PFU)/10 cells), after which the cells were maintained in MEM plus 5% fetal calf serum until cytopathic effects were

complete (usually 36 hr). At this time, the cultures were frozen and thawed once (from −70 to 37 C), debris was removed by centrifugation at 1,000 × *g* for 15 min, and the supernatant fluid was stored at −70 C. Titers of virus stocks prepared by this method were usually about 10^8 PFU/ml. Titrations were performed on RK_{13} cell monolayers in 2-oz French square bottles by adsorbing 0.2 ml of the virus dilution at 37 C for 1 hr. The overlay consisted of MEM containing 0.225% $NaHCO_3$, 5% hypogammaglobulinemic calf serum, and 0.6% Difco Special Agar (Noble). After 24 to 36 hr of incubation at 37 C, the overlay was poured off, the monolayer was stained with crystal violet, and plaques were enumerated.

Media. Cells were grown in MEM plus 10% hypogammaglobulinemic calf serum and virus was replicated in MEM containing 5% fetal calf serum, since titers of virus in the hypogammaglobulinemic serum were consistently lower than in fetal calf serum. As will be noted later, MEM deficient in selected amino acids was used when the corresponding radioactive amino acids were used in labeling experiments.

Reagents and chemicals. L-Arginine-^{3}H (specific activity 300 to 1000 mc/mmole), L-arginine-^{14}C (specific activity 150 to 240 mc/mmole), L-lysine-^{3}H (specific activity 300 to 500 mc/mmole), and reconstituted ^{14}C- and ^{3}H-protein hydrolysates were purchased from Schwarz Bio Research Inc., Van Nuys, Calif. DL-Tryptophan (methylene-^{14}C, 52 mc/mmole) was purchased from Amersham Searle, Des Plaines, Ill. All sera were obtained from Hyland Laboratories, Los Angeles, Calif.

Preparation of radioactively-labeled intranuclear basic proteins. Cells which had been labeled for the appropriate length of time were removed from the glass with a rubber policeman and centrifuged at 750 × *g* for 5 min. The resulting pellet was washed once with phosphate-buffered saline (PBS), and nuclei were prepared by a modification of the technique described by Penman (13). Preliminary experiments showed that RK_{13} cells could not be efficiently broken by Dounce homogenization after incubation in reticulocyte standard buffer. However, if the cells were held for 10 min at 4 C in the Tween 40–deoxycholate mixture used by Penman and then homogenized in a tight-fitting Dounce homogenizer, all cells were broken, and the nuclei of uninfected cells were preserved. Nuclei were centrifuged from this solution, washed once with PBS, and held at 2 C for 30 min in 0.25 N HCl to extract basic proteins. Usually, cells were briefly sonically treated at the outset of this incubation to resuspend and break nuclei. The suspension was then centrifuged at 100,000 × *g* for 60 min, dialyzed against 0.001 M tris(hydroxymethyl)aminomethane (Tris) buffer at *p*H 8.0, and made successfully 2.5% with respect to sodium dodecyl sulfate (SDS), 0.7 M with respect to urea, and 1.5 M with respect to 2-mercaptoethanol. The mixture was held at 37 C for 1 hr and dialyzed for at least 14 hr against 0.01 M phosphate buffer (*p*H 7.1) containing 0.1% SDS, 0.5 M urea, and 0.1% 2-mercaptoethanol (8). Finally, the sample was concentrated to the desired volume by placing the dialysis bag in dry Sephadex G-10.

Gel electrophoresis was performed on 18-cm polyacrylamide gels containing 7.5% acrylamide, 0.73% *N*,*N*-bis methylene acrylamide, 0.1% SDS, 1.0 M urea, and 0.1 M phosphate buffer (*p*H 7.2). Electrophoresis was for about 25 hr at room temperature and 2.5 v/cm. After electrophoresis, gels were fractionated into liquid scintillation vials using the Maizel autogeldivider (Savant Instruments, Inc., Hicksville, N.Y.). About 100 1-ml samples were obtained from each gel. Bray's (3) solution (10 ml) was added to each vial and the radioactive disintegrations measured in a Mark I scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill). In some experiments, nuclei were not isolated, but whole cells were extracted with 0.25 N HCl.

Preparation and purification of radioactively labeled virus. Radioactively labeled virus was prepared by first infecting monolayers in 16-oz prescription bottles with an adsorbed multiplicity of about 1 PFU/10 cells. After adsorption, the inoculum was replaced with 10 ml of MEM containing 20% of the usual concentration of arginine, 2% dialyzed fetal calf serum, and 1 μc of ^{14}C-arginine per ml. When cytopathic effects were complete (after about 36 hr), the samples were subjected to Dounce homogenization to break cells and nuclei and then to low-speed centrifugation (1,000 × *g*, for 15 min). Potassium citrate was added to a concentration of 0.3 M, and the samples were centrifuged at 12,000 × *g* for 10 min. The supernatant fluid was layered over 7 ml of 20% sucrose in water (w/w) containing 0.15 M potassium citrate. This in turn was layered over 3 ml of a 65% (w/w) solution of sucrose in D_2O. The sample was centrifuged to equilibrium at 81,500 × *g* in the SW-27 rotor in a centrifuge (Spinco L-2-65B) at 4 C for 24 hr. At this time, four bands were found in the tube, three in the steep gradient formed by diffusion between the 65% sucrose and 20% sucrose, and one extending to the top of the tube. Band 1 was sharp, was placed far down in D_2O sucrose solution, and revealed few discernible virus particles when examined by the negative-staining technique and electron microscopy. Band 2 was also sharp and consisted mainly of "naked" particles or particles with partial membranes. Band 3 was more diffuse and, although strict quantitative experiments were not performed, was found to contain both more particles and a greater percentage of particles with membranes than band 2. Band 4 was extremely diffuse, extended to the top of the tube, and was composed of cellular debris. Since band 3 contained the greatest number of morphologically complete particles, in all experiments it was used as the source of virus for further purification, mainly to remove extraneous membranous material. After overnight dialysis against 0.01 M Tris buffer (*p*H 7.3), 0.1 M NaCl, and 0.001 M ethylenediaminetetraacetic acid, band 3 was sonically treated for 15 sec and layered over 7 ml of 20% sucrose in water containing the same buffer and other chemicals. This had previously been layered over a cushion of 3 ml of 65% sucrose in D_2O (w/w). This preparation was centrifuged for 3 hr at 81,500 × *g* and 4 C in the SW-27 rotor. The virus particles collected on the cushion, and a band of debris remained at the top of the 20% sucrose. The band containing virus particles

was again dialyzed to remove sucrose and sonicated for 15 sec and its purity was assessed by performing a velocity sedimentation in a 5 to 50% (w/w) sucrose gradient for 2.5 hr at 52,200 × *g* and 4 C in the SW-27 rotor. Radioactivity and infectivity move together indicating that the preparation is homogenous (Fig. 1). The width of the band is probably a function of the variation in size of the envelopes on this virus. The virus band taken from the cushion was dialyzed against 0.001 M Tris (*p*H 8.0) to remove sucrose, then treated with sucrose, then treated with SDS, urea, and 2-mercaptoethanol and was electrophoresed in the same fashion as were the acid-extractable proteins described earlier.

Two statements concerning the virus preparation should be made here. First, we have been unable to completely free the virus preparation from nonvirion-associated membranes without also stripping the virus particles of membranes. Thus, the preparation cannot be considered to be highly purified. However, we consider the purification to be adequate since (i) the preparation is homogenous upon velocity sedimentation and (ii) in the preparation of all radioactive, purified virus, cells were infected with a low multiplicity (1 PFU/10 cells), and the radioactive amino acids were added immediately after adsorption. Thus, radioactive host-cell proteins were made in 90% of the cells during the first cycle of virus replication and could be expected to contribute to an impure preparation. However, as will be shown, only selected proteins associated with virus infection appeared in the purified virus preparation. This argues strongly against significant contamination of the virus preparation with nonstructural proteins.

Secondly, although all purified virus preparations analyzed here were obtained from band 3 and further purified as indicated above, we have recently found that virus obtained from band 2 presents profiles which are at least qualitatively identical to those from band 3 after polyacrylamide-gel electrophoresis.

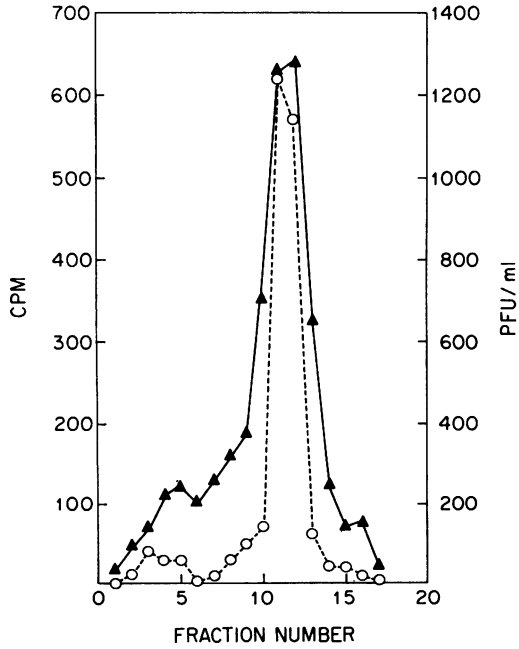

FIG. 1. *Velocity sedimentation of purified pseudorabies virus in a 5 to 50% sucrose density gradient. Centrifugation was for 2.5 hr at 20,000 rev/min in the SW-27 rotor of a Spinco ultracentrifuge at 4 C. Symbols: ▲, radioactivity (counts/min of ^{14}C-arginine per milliliter); ○, infectivity (PFU/ml).*

RESULTS

Differential arginine-histidine-lysine requirement for pseudorabies virus. First, it was essential to see if pseudorabies virus possessed the same differential arginine-histidine-lysine requirement which Tankersley (17) had previously shown for herpes simplex virus. To investigate this, RK_{13} cells in 2-oz French square bottles were synchronously infected with virus which previously had been passed through a Sephadex G-10 column to remove amino acids. At the end of a 1-hr adsorption period, the cells were washed three times with Hanks' balanced salt solution and complete MEM or MEM lacking arginine, histidine, or lysine was readded. At the end of 12 hr [the duration of the one-step growth cycles in this system (Stevens, *unpublished observations*)], the cultures were frozen and thawed once (from −70 to 37 C) and titrated. Pseudorabies virus requires arginine and histidine, but not lysine, for replication (Table 1). Whereas there was greater than a 100-fold drop in the titer of virus replicated in MEM without arginine and histidine, that replicated in MEM without lysine demonstrated a titer equivalent to that in complete MEM.

Acid-extractable, arginine-containing proteins in virus-infected RK_{13} cell nuclei. Using radioactive arginine as the label for proteins, we next looked at the synthesis of proteins extractable with acid from virus-infected and noninfected RK_{13} cell nuclei. Initially, proteins made in the period from 4 to 8 hr after infection were examined. This was done since Hamada and Kaplan (7) had shown earlier that both "early" and "late" viral-induced proteins were made in

TABLE 1. *Pseudorabies virus replication in the presence and absence of basic amino acids*

Medium	Virus titer (PFU/ml)	
	Expt 1	Expt 2
MEM	6×10^6	1.5×10^7
MEM without arginine	1.3×10^4	1×10^5
MEM without histidine	6×10^4	3.5×10^4
MEM without lysine	6.5×10^6	4×10^6

significant amounts in this time period. Monolayers of about 10^7 cells were synchronously infected with virus or sham-infected with MEM and 5% fetal calf serum. At the end of a 1-hr adsorption period, the inoculum was replaced with MEM and 5% fetal calf serum. After a 4-hr incubation at 37 C, the medium was changed to MEM with 20% of the usual concentration of arginine. In control cultures, this was supplemented with 1 μc of ^{14}C-arginine per ml, whereas infected cultures received 5 μc of ^{3}H-arginine per ml. After an additional 4 hr at 37 C, the monolayers were processed as described in Materials and Methods, the samples were pooled and coelectrophoresed on a polyacrylamide gel. As a histone marker, 100 μg of purified calf thymus histones (a gift from Douglas Fambrough, California Institute of Technology) was run on a companion gel. These proteins had previously been treated with SDS, urea, and 2-mercaptoethanol in a fashion identical to the treatment of nuclear extracts. After electrophoresis, this gel was stained with amido schwarz and destained with acetic acid and ethyl alcohol (6). The results of such an experiment are shown in Fig. 2. The positions of stained bands from the gel containing the purified histone preparations are marked under the radioactive peaks. The following features of the electropherogram are significant. In the noninfected sample, several

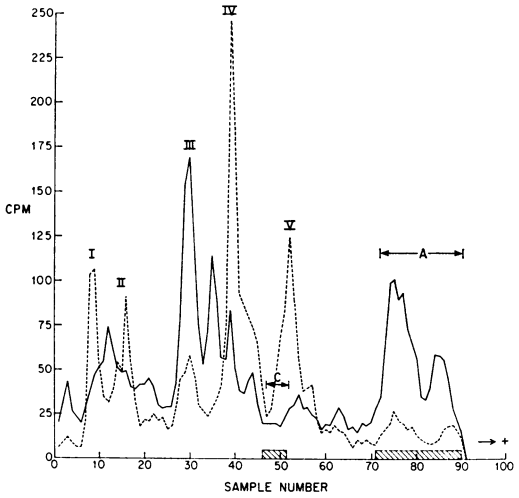

FIG. 2. *Electropherogram of radioactive proteins extracted with 0.25 N HCl from nuclei of pseudorabies virus infected and noninfected RK_{13} cells. The labeling period was from 4 to 8 hr after infection. ^{14}C-arginine control is represented by a solid line, virus-infected ^{3}H-arginine by a broken line; peaks I through V represent virus-induced proteins, and peaks A and C represent histones. Experimental details for this and subsequent figures are given in the text.*

radioactive peaks are evident. The bands composed of histones are marked A and C after the convention of Robbins and Borun (14) who analyzed histones from HeLa cells with similar methods. According to them, the arginine-rich, slightly arginine-rich, and slightly lysine-rich histones run together in the A group, whereas the very lysine-rich histone comprises the C peak. By radioactive measurements, the lysine-rich histone is not identifiable in this experiment. Further evidence that these radioactive bands are histones and that the lysine-rich histone can be identified in these cells is presented below. As shown by others (14), nonhistone proteins are also extracted with 0.25 N HCl. We do not know the nature of these molecules, although it has been suggested that at least some of them are ribosomal proteins (2). In the acid extract from virus-infected nuclei, five major virus-induced peaks (tentatively designated I to V) with minor components are evident. Although all the minor components were consistently found, they will not be emphasized here. Some preliminary statements concerning peaks II through IV can be made. Peak II in most extracts consists of 2 components. This will become more evident in later figures. Peak III electrophoreses with the main arginine-containing peak in uninfected cells and, from this experiment, may be postulated merely to represent incomplete inhibition of the synthesis of this protein in infected cells. However, subsequent experiments will show that this is a viral structural protein. Peak IV is the major peak as determined by the magnitude of arginine incorporation and also possesses a minor component on the right side. The significance of the two minor peaks in uninfected cells which coelectrophorese with IV is unclear. Peak V cannot be differentiated from the lysine-rich histone in this experiment. Finally, it appears that histone synthesis (of the A group at least) is inhibited by virus infection. This will be treated in greater detail later.

The remainder of this communication will be devoted to experiments showing that (i) histone synthesis is completely inhibited by pseudorabies virus infection and that this inhibition is achieved in a gradual manner between 0 and 6 hr after infection, (ii) none of the proteins I through V is selectively induced early in infection, (iii) proteins I through IV are viral structural proteins, but V most probably is not, and (iv) proteins I through V all contain tryptophan and, from this standpoint at least, are not "classic" histones.

Histone synthesis in pseudorabies virus-infected cells. The previous experiment suggested that histone synthesis was partially inhibited by 4 hr

after infection, but for several reasons the result was equivocal; for example, it might be argued that histones were selectively lost from the damaged isolated infected nuclei or were not transported to nuclei of virus-infected cells. In addition, proof that protein V was not histone C was lacking. To obviate these and other less important objections, infected cells were labeled with ^{3}H-lysine (5 μc/ml) and ^{14}C-tryptophan (1 μc/ml) in tryptophan- and lysine-free MEM between 6 and 7 hr after infection. Acid-soluble proteins were extracted from isolated nuclei obtained from control cells and from whole infected cells. These were then treated with chemicals and electrophoresed as before. In addition, a gel with "marker" histones was again tested. Figure 3 shows the result of such a double-label experiment in which the histones can be positively identified in preparations made from control cells. Clearly, the classification of A and C as histones is justified, since they electrophorese with the purified marker histones and contain significant amounts of lysine and only background amounts of tryptophan. In the virus-infected sample (Fig. 4) there are no lysine-rich, tryptophan-poor peaks corresponding to the histone fractions; in fact, there is no incorporation of lysine at all. This fact is consistent with the results previously found (Table 1) for pseudorabies virus, which does not require lysine for replication. From these experiments, we conclude that the synthesis of histones is completely inhibited in pseudorabies virus-infected RK_{13} cells by 6 hr after infection.

Because the previous experiment gave no indication of the kinetics of inhibition, we investigated them by "prelabeling" cells for 12 hr with 1 μc of ^{3}H-lysine per ml of MEM containing 20% of the normal amount of lysine and 2% dialyzed fetal calf serum. Virus was adsorbed, and complete medium was readded to all cultures. At various times (at 0, 2, 4, or 6 hr), this medium was removed from individual monolayers and replaced with lysine-free medium containing 1 μc of ^{14}C-lysine per ml. Monolayers containing about 10^{7} cells were used for each 2-hr labeling period. Thus, all cells were labeled with ^{3}H-lysine before infection, and individual monolayers in the group were labeled during 2-hr intervals through 8-hr after infection; prelabeling served as an internal control. At the end of each labeling period, cells were processed as usual for extraction of basic proteins, which were subsequently analyzed on polyacrylamide gels. Electropherograms were prepared, and the areas under histone peaks were integrated and compared. The ratio of ^{14}C to ^{3}H was adjusted to 1 in the 0 to 2 hr (base line) sample, and ratios of isotopes in other intervals were corrected by the same factor. Ratios of ^{14}C to ^{3}H were then calculated for each successive sample. The ratios fell to 0.43 in the 2 to 4-, 0.23 in 4 to 6-, and <0.04 in the 6 to 8-hr labeling periods, indicating that there was a rather smooth "shut off" in histone synthesis after infection. In addition, inspection of the curves showed that no histone fraction was inhibited to a degree greater than any other.

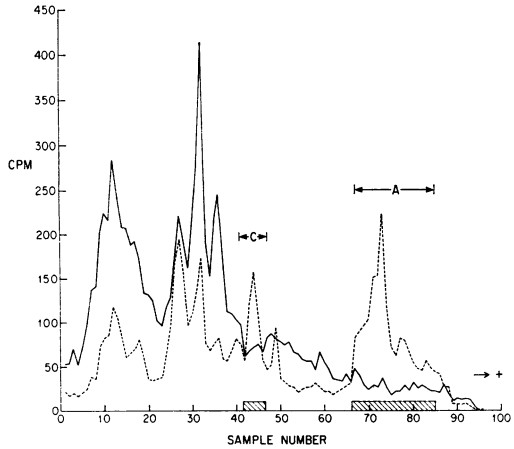

FIG. 3. *Electropherogram of radioactive proteins extracted with 0.25 N HCl from nuclei of RK_{13} cells labeled with ^{14}C-tryptophan (solid line) and ^{3}H-lysine (broken line). Peaks A and C represent histones.*

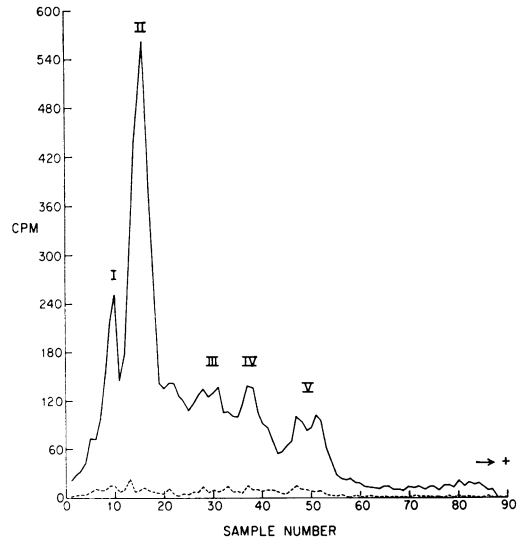

FIG. 4. *Electropherogram of radioactive proteins extracted with 0.25 N HCl from pseudorabies virus-infected RK_{13} cells. The labeling period was from 6 to 7 hr after infection. ^{14}C-tryptophan (solid line); ^{3}H-lysine (broken line). Peaks I through V represent virus-induced proteins.*

Characterization of acid-soluble proteins induced by pseudorabies virus infection. As a first step in characterization, it was desirable to see whether any of the virus-induced proteins (Fig. 2) were selectively induced early in infection. Cells were infected and labeled as in the experiment depicted in Fig. 2 except that the labeling period was between 0 and 4 hr after adsorption. Apparently, none of the peaks is significantly labeled in this time interval (Fig. 5). From this it is concluded that none of the proteins is selectively induced early in the infectious cycle. Additional experiments to determine when the individual peaks first appear are now in progress.

Next, the relationship of these proteins to viral structural proteins was investigated. Here ^{14}C-labeled virus purified as described in Materials and Methods was treated with SDS, urea, and 2-mercaptoethanol just as for the acid-extractable proteins and run with acid-extracted, ^{3}H-arginine-labeled marker proteins from the nuclei of infected cells labeled from 4 to 7 hr after infection. The results of a typical experiment are presented in Fig. 6. Identity cannot be unequivocally established by this type of analysis, since the basis of separation of peptides is molecular weight only (15). However, from the nature of the experiment, we can at least tentatively conclude that, with the exception of V, all of the major acid-extractable, arginine-containing peptides are virus structural proteins.

Since only one amino acid was used as the label here, it is possible that more general labeling of the proteins would allow us to better assess whether peak V was present in the virion. Therefore, reconstituted protein hydrolysate labeled with ^{3}H or ^{14}C was used as the label in similar experiments. When proteins labeled with these materials were analyzed on gels, the labeling patterns were similar to those with only arginine. In addition, it appeared that peptide V was relatively rich in arginine, since this peak was diminished in electropherograms prepared from proteins labeled with reconstituted protein hydrolysate (Stevens, *unpublished observations*). Thus, the presence or absence of peptide V in the virion could not be unequivocally established. However, the results of these experiments suggest strongly that peptide V is not a viral structural protein.

Since the principal structural proteins in the virion could be extracted with acid from infected nuclei, the selectivity of the extraction procedure was questioned. Nuclei isolated from infected cells labeled from 4 to 5.5 hr after infection with ^{14}C- or ^{3}H-arginine were extracted with 0.25 N HCl (^{3}H-arginine) or PBS (^{14}C-arginine). These extracts were analyzed as usual on polyacrylamide gels. Results (Fig. 7) show that the acid-extraction procedure is only partially selective as compared to extraction at neutral *p*H (peak V and major portions of II and IV are insoluble in PBS, the others are soluble to various degrees). Thus, some of the acid-extractable proteins behave as ordinary proteins, which are soluble

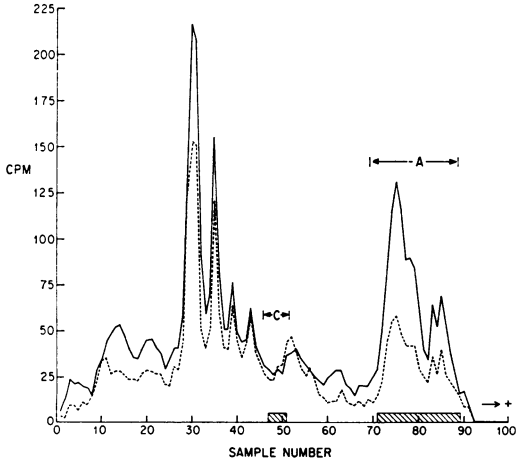

FIG. 5. *Electropherogram of radioactive proteins extracted with 0.25 N HCl from nuclei of pseudorabies virus-infected and noninfected RK_{13} cells. The labeling period was from 0 to 4 hr after infection. Control, ^{14}C-arginine (solid line); virus-infected, ^{3}H-arginine (broken line). Peaks A and C represent histones.*

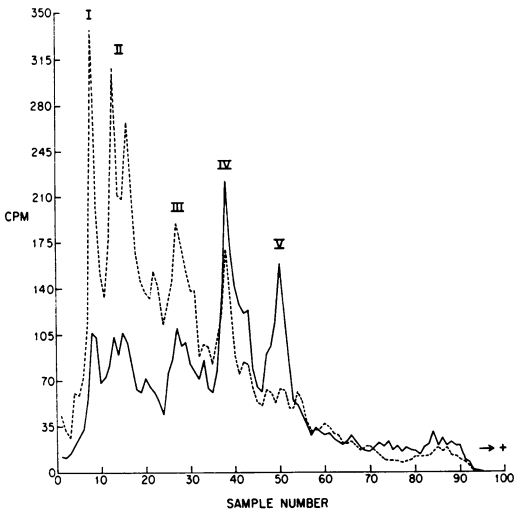

FIG. 6. *Comparison of pseudorabies virus radioactive structural proteins with proteins extractable with 0.25 N HCl from nuclei of virus-infected RK_{13} cells. Acid-extracted proteins, ^{3}H-arginine (solid line); virus structural proteins, ^{14}C-arginine (broken line). Peaks I through V, virus-induced proteins.*

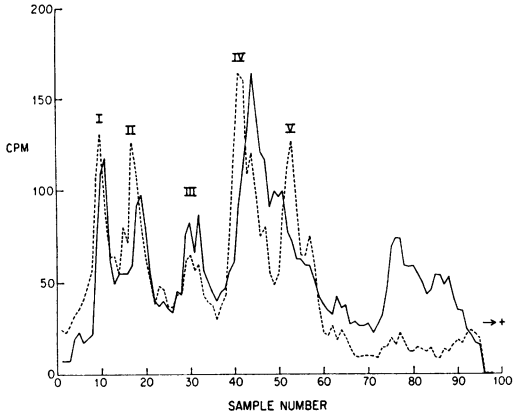

FIG. 7. *Electropherogram of radioactive proteins extracted with 0.25 N HCl or PBS from nuclei of pseudorabies virus-infected cells labeled with radioactive arginine between 4 and 5.5 hr after infection. ^{14}C-labeled 0.25 N HCl extract (solid line); ^{3}H-labeled PBS extract (broken line). Peaks I through V represent virus-induced proteins.*

under neutral conditions, and probably do not have an isoelectric point at basic *p*H.

Finally, although the results presented in Fig. 4 indicate that proteins I through V contained tryptophan and, therefore, did not fit the usual definition for histone (14), a tryptophan-arginine double-labeling experiment was performed on virus-infected cells to establish this unequivocally. Virus-infected cells were labeled between 5 and 7 hr after infection in MEM without arginine and tryptophan. This was supplemented with 5 μc of ^{3}H-arginine and 5 μc of ^{14}C-tryptophan per ml. Acid-soluble proteins were extracted and run on polyacrylamide gels. All major peaks do contain tryptophan (Fig. 8).

DISCUSSION

The significant results of this initial investigation may be summarized as follows. (i) The synthesis of histones in RK_{13} cells is completely inhibited by 6 hr after infection with pseudorabies virus. (ii) Five major virus-induced acid-extractable proteins appear in nuclei of virus-infected cells. Four are virus structural proteins; one most probably is not.

Histone synthesis. If histones are involved in the specific repression of host-cell DNA synthesis which occurs, one might expect them, either in mass or in selected cases, to be either induced to higher levels or inhibited after virus infection. The data (Fig. 4 and 5) show unequivocally that synthesis of all the "classic" histones is completely inhibited by 6 hr after infection, a conclusion reached independently by Shimono and Kaplan (16).

In addition, the pulse-labeling studies indicate that this inhibition is gradual and general for all histone classes. It has been observed by Kaplan and Ben-Porat (9) that the inhibition of host cell DNA synthesis was complete soon after this time. A possible relationship between the inhibition of host-cell DNA and histone synthesis in these experiments is suggested by the work of Robbins and Borun (14), who showed that synthesis of these macromolecules is specifically coordinated in normal HeLa cells. At present, we have no evidence for or against a specific interconnection in this virus-infected system. Note, however, that if there is a coordinate control of DNA and histone synthesis in the present system, it is specifically related to host-cell DNA synthesis, since viral DNA synthesis is proceeding at a time when histone synthesis is completely inhibited.

Synthesis of other acid-extractable proteins. Finding five virus-induced acid-extractable proteins in the nuclei of pseudorabies virus-infected cells led to a preliminary characterization of them. As with histones, they are extracted from cell nuclei with acid, and, since peptides electrophorese mainly on the basis of size under these conditions (15), it may be concluded that protein V is in the same general size range as the lysine-rich histone. However, all of these peptides differ from the "classic" histones in that they contain tryptophan (Fig. 8). Whether, like histones, any of them is specifically complexed to DNA (viral, cellular, or both) is currently under investigation.

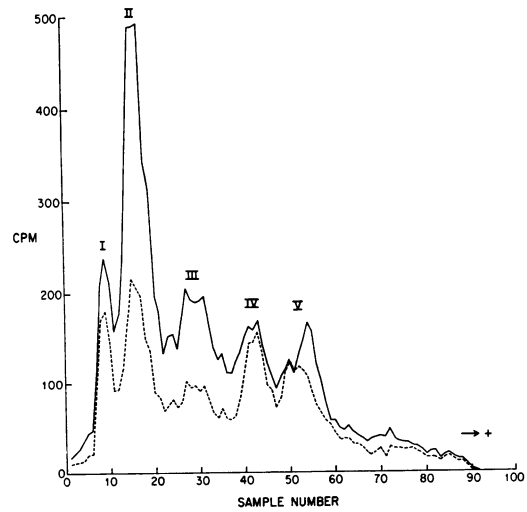

FIG. 8. *Electropherogram of radioactive proteins extracted with 0.25 N HCl from nuclei of pseudorabies virus-infected RK_{13} cells labeled with ^{14}C-tryptophan (solid line) and ^{3}H-arginine (broken line) between 5 and 7 hr after infection. Peaks I through V represent virus-induced proteins.*

It is clear from Fig. 5 that peptides I through IV are virus structural proteins, whereas V most likely is not. We are now attempting to find where these proteins are located in the virion. However, the fact that III and IV migrate with peaks present before infection leads to the interesting possibility that they may be host-derived proteins selectively produced after infection. Although there is no precedence for such a view, this possibility becomes more tenable when it is recalled that the membrane of herpes viruses contains host-cell antigens (12, 16) and is derived from the inner nuclear membrane, which reduplicates during infection (4). Unless there is a large pool of such proteins in these cells, this implies that at least some host-cell membrane proteins continue to be made after infection.

Finally, peptide V is, at present, the molecule of greatest interest to us, since, for several reasons, it appears to be the most likely candidate for a protein which might play a direct role in inhibition of host-cell DNA replication. Experiments concerned with further characterization of all these proteins and an assessment of their possible role in genetic regulation are now underway.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-06246 from the National Institute of Allergy and Infectious Diseases, and by grants from the Life Insurance Medical Research Fund, the American Cancer Society, and the California Institute for Cancer Research.

ADDENDUM IN PROOF

We have recently found (Stevens, *unpublished data*) that peptides I and V are selectively bound to chromatin isolated 6 hr after infection.

LITERATURE CITED

1. Ben-Porat, T., and A. S. Kaplan. 1965. Mechanism of inhibition of cellular DNA synthesis by pseudorabies virus. Virology 25:22–29.
2. Bonner, J., M. E. Dahmus, D. Fambrough, R. C. Huang, K. Marushige, and D. Y. H. Tuan. 1968. The biology of isolated chromatin. Science 159:47–56.
3. Bray, G. A. 1961. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. 1:279–285.
4. Darlington, R. W., and L. H. Moss III. 1968. Herpesvirus envelopment. J. Virol. 2:48–55.
5. Eagle, H. 1959. Amino acid metabolism in mammalian cell culture. Science 130:432–437.
6. Fambrough, D. M., F. Fujimura, and J. Bonner. 1968. Quantitative distribution of histone components in the pea plant. Biochemistry 7:575–585.
7. Hamada, C., and A. S. Kaplan. 1965. Kinetics of synthesis of various types of antigenic proteins in cells infected with pseudorabies virus. J. Bacteriol. 89:1328–1334.
8. Holowczak, J. A., and W. K. Joklik. 1967. Studies on the structural proteins of vaccinia virus. I. Structural proteins of virions and cores. Virology 33:717–725.
9. Kaplan, A. S., and T. Ben-Porat. 1963. The pattern of viral and cellular DNA synthesis in pseudorabies virus-infected cells in the logarithmic phase of growth. Virology 19: 205–214.
10. Levine, A. J., and H. S. Ginsberg. 1968. Role of adenovirus structural proteins in the cessation of host-cell biosynthetic functions. J. Virol. 2:430–439.
11. Luria, S. E., and J. E. Darnell, Jr. General virology, 2nd ed. John Wiley & Sons, Inc., New York.
12. Nii, S., C. Morgan, H. M. Rose, and K. C. Hsu. 1968. Electron microscopy of herpes simplex virus. J. Virol. 2:1172–1184.
13. Penman, S. 1966. RNA metabolism in the HeLa cell nucleus. J. Mol. Biol. 17:117–130.
14. Robbins, E., and T. W. Borun. 1967. The cytoplasmic synthesis of histones in HeLa cells and its temporal relationship to DNA replication. Proc. Nat. Acad. Sci. U.S.A. 57:409–416.
15. Shapiro, A. L., E. Viñuela, and J. V. Maizel. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in S.D.S.-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28:815–820.
16. Shimono, H., and A. S. Kaplan. 1968. Histone synthesis in cells infected with a virulent or an oncogenic virus. Fed. Proc. 27:615.
17. Tankersley, R. W., Jr. 1964. Amino acid requirements of herpes simplex virus in human cells. J. Bacteriol. 87:609–613.
18. Wildy, P., and D. H. Watson. 1962. Electron microscopic studies on the architecture of animal viruses. Cold Spring Harbor Symp. Quant. Biol. 27:25–47.