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# Infectious Process of the Parvovirus H-1: Correlation of Protein Content, Particle Density, and Viral Infectivity

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The infectious particles of the parvovirus H-1 were characterized with respect to protein content, density in CsCl, and specific infectivity. Heavy-full and light-full particles were purified from infected simian virus 40-transformed newborn human kidney (NB) cells and from simian virus 40-transformed hamster kidney (THK) cells. Analysis of the protein content of these particles demonstrated that the ratio of viral protein VP2′ to VP2 was the same in heavy-full and light-full particles derived from the same cell line, but differed significantly between the two hosts. However, the infectivity of the particles from each cell line was the same for all four viral species. Also, in vitro conversion of VP2′ to VP2 did not enhance the particle infectivity of either heavy-full or light-full virus. When the fate of input virus was studied with $^{125}$I-labeled H-1, the conversion of VP2′ to VP2 occurred in a time-dependent manner up to 24 h postinfection. Simultaneous with the proteolytic cleavage, there was a shift in the density of the heavy-full virus to the light-full density. However, protein analysis of the $^{125}$I-labeled light-full virus at various times postinfection indicated that they were not enriched in VP2 when compared with heavy-full virus or the total virus population. Thus, the cleavage of VP2′ to VP2 is not responsible for the shift in density from heavy-full to light-full virus, and although these events might be required for infection, they appear not to be interdependent.

The H-1 virus, a member of the family of autonomous, nondefective parvoviruses, is a small, icosahedral virus which infects eucaryotic cells (11). The single-stranded DNA genome of the H-1 virus is $1.6 \times 10^6$ daltons (12, 16) and is encapsidated by three viral proteins designated VP1, VP2′, and VP2 (alternatively called A, B, and C polypeptides, respectively) (4). The virus particles isolated from parvovirus-infected cells have some very intriguing characteristics with respect to their protein components and their density in CsCl. The full, DNA-containing, virus particles contain all three polypeptides, whereas the empty capsids contain only VP1 and VP2′ (13). VP1 (88,000 daltons) and VP2′ (68,000 daltons) are viral gene products with overlapping sequences such that all of the sequences of the smaller VP2′ are contained within VP1 (15). VP2 (65,000 daltons), on the other hand, is a proteolytic cleavage product of VP2′, the cleavage occurring only in the full virus after the particle is assembled (1, 14). The conversion of VP2′ to VP2 can be achieved in vitro by incubating full virus with a variety of proteases; the empty capsid, however, is resistant to proteolytic cleavage, indicating that structural differences exist between the empty and full capsids (2, 5).

The full virus can be divided into two classes on the basis of their densities in CsCl. Heavy-full (HF) virus has a density of around 1.47 g/cm$^3$, and light-full (LF) virus, which is derived from the HF virus (10), has a density of around 1.43 g/cm$^3$. The relative amounts of these two viral forms appear to be dependent, to a certain extent, on the time postinfection at which the virus is harvested (5): at early times HF virus appears to predominate, whereas at later times LF virus predominates. Similarly, the protein content of the virus purified at various times postinfection appears to be time related such that at early times VP2′ is predominant, and at later times VP2 is the predominant species (14). Several groups have also suggested that the major viral protein is different in HF and LF virus, with VP2′ being the major protein in HF virus and VP2 being the major protein in LF virus (1, 5, 6, 10). These findings have led to the suggestion that the conversion of HF to LF virus is related to or is a direct result of the proteolytic cleavage of VP2′ to VP2. The major evidence against such a correlation is the inability to convert HF virus to LF virus in vitro by cleaving VP2′ to VP2 (2, 5). However, this may be due to the fact that the cleavage site in vivo is different

from that in vitro.

In the present study we have used the H-1 parvovirus in an attempt to correlate the protein content, density, and infectivity of the virus. Our results suggest that neither the proteolytic cleavage of VP2′ to VP2 nor the particle density shift from HF to LF results in a change in the particle/infectivity ratio of the virus. Furthermore, although the proteolytic cleavage and the density shift occur simultaneously inside the cell, these two events appear to be independent of each other.

## MATERIALS AND METHODS

**Production and purification of H-1 virus.** H-1 virus was produced by infecting confluent monolayers of either NB (simian virus 40-transformed newborn human kidney) or THK (simian virus 40-transformed hamster kidney) cells in roller bottles at a multiplicity of infection of 0.1. After cytopathic effect was complete (5 to 7 days postinfection) the cultures were subjected to three freeze-thaw cycles directly in the roller bottles. The lysed cells were collected, adjusted to pH 8.5 with NaOH, and spun at 8,000 × *g* for 30 min to remove the cellular debris. The supernatant was adjusted to 0.5 M in NaCl and 3.4% polyethylene glycol, which caused the virus to precipitate. After standing overnight at 4°C, the virus was collected by centrifugation at 8,000 × *g* for 30 min and taken up in a minimal volume of 20 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA–0.2% Sarkosyl and adjusted to a density of 1.38 g/cm$^3$ with CsCl. The virus was then banded by equilibrium centrifugation at 35,000 rpm for 20 h in an SW60.1 rotor. The bands corresponding to empty capsids and full virus were visualized by vertical light and removed from the tubes by side puncture. To separate HF and LF virus, the full virus was adjusted to a density of 1.43 g/cm$^3$ in CsCl and centrifuged for 48 h at 35,000 rpm in a type 40 rotor. The gradients were eluted from the bottom, and the HF and LF fractions were identified by absorbance at 260 nm and refractive index determinations. The HF virus banded at 1.465 g/cm$^3$, and the LF virus banded at 1.43 g/cm$^3$. The pooled virus was dialyzed into 20 mM Tris-hydrochloride (pH 8.5)–1 mM EDTA. The concentration of virus was determined by the formula $\Sigma^{1\%}_{260} = 94$.

**Plaque assay.** Confluent monolayers of NB cells in 60-mm petri dishes were infected with 0.2 ml of the virus dilution in medium (see below) adjusted to pH 7.0. The virus was allowed to adsorb for 1 h at 37°C with periodic rocking of the dishes. The cells were then overlaid with 8 ml of 0.6% agarose in medium (pH 7.6) and incubated for 5 days at 37°C. The cells were stained with 3 ml of 0.02% neutral red in Hanks balanced salts for 6 h at 37°C, and the plaques were counted (dishes with fewer than five plaques were disregraded).

**Radio-iodination of H-1.** To 100 μl (30 to 50 μg) of H-1 was added 3 μl (200 μCi) of carrier-free Na$^{125}$I followed by 10 μl of chloramine T (5 mg/ml) for 15 min at room temperature. The reaction was terminated by the addition of 10 μl of 1 M β-mercaptoethanol, and the virus was dialyzed extensively against 20 mM Tris-hydrochloride (pH 8.5)–1 mM EDTA.

**Infection with $^{125}$I-labeled H-1.** Plastic 60-mm petri dishes were seeded with 5 × 10$^5$ NB cells in 5 ml of medium (AutoPow minimal essential medium, 30 mM glutamine, 5% fetal bovine serum adjusted to pH 7.6 with 7.5% sodium bicarbonate), and the cells were grown for 24 h at 37°C. The medium was then removed, and the monolayer was infected with 0.2 ml of $^{125}$I-labeled H-1 (5 × 10$^5$ to 10 × 10$^5$ cpm) in the above medium adjusted to pH 7.0. For incubations of greater than 6 h, the cells were refed with 1 ml of medium after 6 h. The cells were harvested differently depending on whether the proteins were to be analyzed by sodium dodecyl sulfate-gel electrophoresis or the virus were to be banded in CsCl. In the first case, the cells were washed twice with 1 ml of phosphate-buffered saline, lysed in 200 μl of 0.1% sodium dodecyl sulfate, scraped out of the dishes, and lyophilized. For density analysis, the monolayers were washed as above, collected in around 1 ml of 20 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA–0.2% Sarkosyl, and frozen. The extracts were then thawed, adjusted to a density of 1.43 g/cm$^3$ with CsCl, and spun to equilibrium in a type 40 rotor as described above for virus purification.

**Cell fraction.** Nuclear and cytoplasmic fractions were separated as described previously (3). For gel analysis, the fractions were lyophilized and taken up in disruption buffer and boiled for 3 to 5 min.

**Polyacrylamide gel electrophoresis.** Polyacrylamide gels were run by the procedure of Laemmli (8). For autoradiography, the gels were dried and exposed to preflashed Cronex-4 film at −70°C with the aid of an intensifying screen. Quantitation of the relative radioactivity in individual viral protein bands was achieved in two ways. Individual bands, located by autoradiography, were cut out of the dried gels and counted directly in a gamma counter, or the autoradiograms were scanned with a Chromoscan MKII, and the relative amounts of each protein were determined by the area under the peaks. Both methods gave the same results.

## RESULTS

**Protein content of the H-1 virus.** H-1 virus was grown in both NB and THK cells and purified as described above. The yield of virus, both empty capsids and full virus, was consistently 8- to 10-fold higher in the NB cells (which yielded around 1 mg of virus protein per roller bottle, of which 90% was empty capsid). We were interested in studying the protein content of the HF and LF virus derived from both cell types, particularly with respect to the relative amounts of VP2′ and VP2. The proteins were studied by polyacrylamide gel electrophoresis as shown in Fig. 1. The NB-derived virus contained almost exclusively VP2′, with only a small amount of the smaller VP2 present (Fig. 1A, lanes 1 and 2). This was true for both HF and LF virus, which had the same ratio of VP2′ to VP2. The virus derived from THK cells, on the other hand,

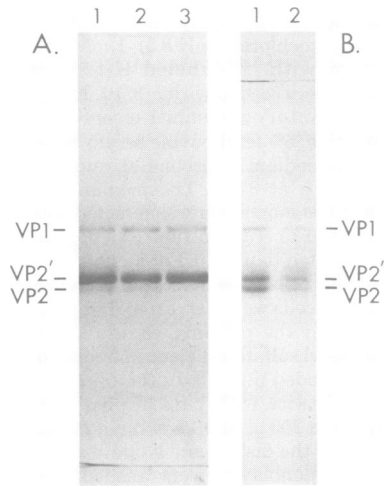


FIG. 1. *Protein analysis of H-1 virus purified from NB and THK cells. H-1 was purified from NB and THK cells as described in the text, and proteins were separated by polyacrylamide gel electrophoresis on 7.5% slab gels followed by staining with Coomassie blue. (A) LF virus (lane 1), HF virus (lane 2), and empty capsid (lane 3) from NB cells. (B) HF virus (lane 1) and LF virus (lane 2) from THK cells.*

contained nearly equal amounts of VP2′ and VP2 (Fig. 1B, lanes 1 and 2). Once again, the ratio of VP2′ to VP2 was the same for the HF and LF virus. The HF and LF virus were clearly separated by the density centrifugation (Fig. 2A), and protein analysis of well-separated gradient fractions (fractions 5 and 14) by polyacrylamide gel electrophoresis (Fig. 2B; lanes 1 and 3) gave the same results as the pooled fractions shown in Fig. 1. Similarly, rebanding of the HF and LF virus separately did not alter these results. Samples of fractions 5 and 14 were also subjected to trypsin digestion to convert the VP2′ to VP2 (Fig. 2B, lanes 2 and 4) which further emphasized the lack of VP2 in the undigested virus. These findings demonstrate that the relative amounts of VP2′ and VP2 are dependent, to a certain degree, upon the cell in which the virus is grown. The fact that HF and LF virus can have the same relative amounts of VP2′ and VP2 and also that two "species" of LF virus can have quite different ratios of VP2′ and VP2 shows that the particle density is independent of the ratio of VP2′ to VP2.

**Infectivity of HF and LF virus.** We next attempted to correlate the infectivity of a particular viral species with its density or protein content or both. To do this we took advantage of the four distinct viral species produced in vivo from NB and THK cells. In the case of the NB-derived virus, the peak fractions 5 and 14 from the CsCl gradient (Fig. 2A) were analyzed. Each of the viral species was plaque assayed, and the particle/infectivity (p/i) ratios were determined by using the absorbance at 260 nm to calculate the number of particles (see above). The p/i

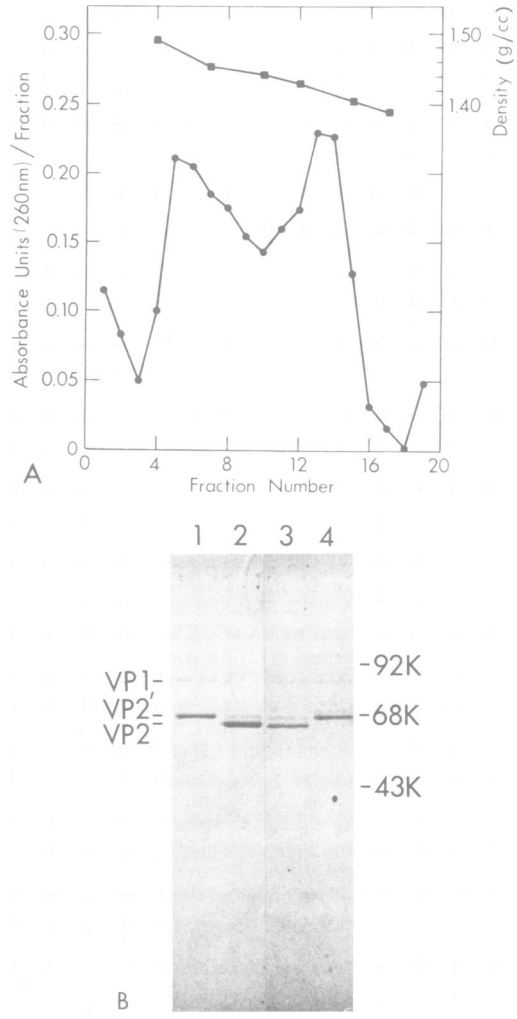


FIG. 2. *CsCl fractionation and protein analysis of H-1 virus. (A) H-1 virus was purified from NB cells as described in the text, banded on a CsCl gradient, and eluted from the bottom. The absorbance at 260 nm was determined for each fraction, and the densities of the fractions were calculated from their refractive index. (B) Fractions 5 and 14 from the gradient in (A) were dialyzed against 50 mM $NH_4HCO_3$ (pH 8.5), electrophoresed on a 7.5% polyacrylamide gel, and stained with Coomassie blue. The proteins of fractions 5 and 14 can be seen in lanes 1 and 4, respectively. Samples of these fractions were made to 5 μg/ml with trypsin and incubated at 37°C for 3 h and electrophoresed in lane 2 (fraction 5) and lane 3 (fraction 14).*

ratios ranged from 500 to 1,000 particles per PFU (Table 1). However, there was no significant difference in the p/i ratios for any of these species. Thus, it appears that the particles are equally infectious regardless of their density or protein content. This was further demonstrated by the data presented in Table 2. In this experiment, HF and LF virus from NB cells were incubated with trypsin at various concentrations under conditions in which VP2′ is converted to VP2. The conversion of VP2′ to VP2 did not increase the infectivity of the particles; in fact, at higher trypsin concentrations the infectivity was significantly reduced, probably due to degradation of the particles.

**In vivo conversion of VP2′ to VP2.** The fact that the conversion of VP2′ to VP2 did not affect the infectivity of the virus suggested the possibility that this conversion, although not required for infection, was a result of the normal infectious process. To study the fate of the input virus protein, NB cells were infected with $^{125}$I-labeled H-1 from NB cells and then harvested at various times postinfection. The labeled protein was then analyzed by polyacrylamide gel electrophoresis and autoradiography. Figure 3 shows the autoradiogram of a dried, Coomassie blue-stained gel of total cell protein harvested between 1 and 24 h after infection with $^{125}$I-labeled H-1 (lanes A through E). The success of the infection was evidenced by the appearance of a strong Coomassie blue-stained band in the region of VP2′ as the infection proceeded (data not shown). It is clear from this autoradiogram that VP2′ is converted to VP2 in a time-dependent manner. The conversion of VP2′ to VP2 was quantitated as described above, and the amount of VP2 relative to VP2′ at various times after infection is shown graphically in Fig. 4. The appearance of VP2 was nearly linear up to 6 h postinfection and reached a maximum of around 65% of the sum of VP2′ plus VP2 after 24 h. This conversion may require entry of the virus into the cell since no conversion was seen at 4°C, at which temperature the virus attaches to the cell surface but is not internalized (9). However, the inhibition of conversion at 4°C may simply be due to a direct temperature effect on the kinetics of the cleavage reaction.

When cells infected with $^{125}$I-labeled H-1 were subfractionated, a majority of the virus was as-

TABLE 1. *p/i ratios of HF and LF virus from NB and THK cells*[a]

| Cell line | Virus | p/i ratio |
|---|---|---|
| NB | HF | 655 |
| NB | LF | 500 |
| THK | HF | 495 |
| THK | LF | 980 |

[a] The HF and LF H-1 virus from NB and THK cells were plaque assayed to determine their infectivity. The number of virus particles in each preparation was determined from the absorbance at 260 nm as described in the text. The p/i ratio is the number of virus particles per PFU.

TABLE 2. *Effect of trypsin digestion on p/i ratio of HF and LF virus*[a]

| Trypsin concn (μg/ml) | p/i ratio | |
|---|---|---|
| | HF | LF |
| 0 | 500 | 350 |
| 0.04 | 570 | 220 |
| 0.40 | 420 | 250 |
| 4.0 | 24,000 | 2,900 |
| 40.0 | 160,000 | 170,000 |

[a] HF and LF virus derived from NB cells were incubated with various concentrations of trypsin at 37°C for 3 h. The virus from each of the reactions was then plaque assayed, and the p/i ratios were determined as described in footnote *a* of Table 1.

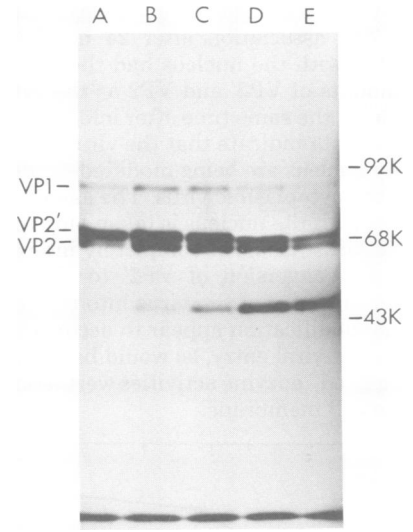


FIG. 3. *In vivo conversion of VP2′ to VP2. NB cells were infected with $^{125}$I-labeled H-1 (5 × 10$^5$ cpm per 60-mm petri dish), and the cells from one dish were harvested in 0.1% sodium dodecyl sulfates as described in the text, at various times after infection as indicated below. The total cell extracts were run on a 7.5% polyacrylamide gel which was stained with Coomassie blue, dried, and then autoradiographed. The $^{125}$I-labeled protein extracts harvested 1, 3, 6, 12, and 24 h postinfection are seen in lanes A through E, respectively. In the left margin are indicated the positions of the viral proteins, and in the right margin are indicated the positions of marker proteins phosphorylase A (92K), bovine serum albumin (68K), and ovalbumin (43K) which were run in an adjacent lane.*

sociated with the nuclear fraction after 6 h, with nearly 70% association after 24 h. The virus associated with the nucleus had the same relative amounts of VP2′ and VP2 as the cytoplasmic virus at the same time after infection (Table 3). These data indicate that the virus associated with the nucleus are being modified at the same rate as the cytoplasmic virus. The association of the virus with the nucleus is taken only to mean that the virus has been completely internalized (9). Thus, conversion of VP2′ to VP2 is not required for entry of the virus into the cell, nor does the modification appear to occur preferentially during viral entry, as would be expected if the "required" enzyme activities were associated with the cell membrane.

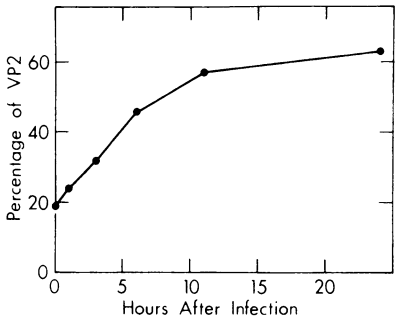


FIG. 4. *Time course of the in vivo conversion of VP2′ to VP2. The conversion of $^{125}$I-labeled VP2′ to VP2 at various times after infection was quantitated either by scanning autoradiograms or by cutting out the labeled bands from the dried gels and counting them directly as described in the text. The conversion is presented as the increase in the percentage of VP2, which was calculated as (VP2/VP2′ + VP2) × 100. Each point represents the average of three to six separate experiments.*

TABLE 3. *Subfractionation and analysis of $^{125}$I-labeled H-1 input virus*[a]

| Time after infection (h) | Fraction | % Label | % VP2′ |
|---|---|---|---|
| 6 | Cytoplasmic | 41 | 50.7 |
| 6 | Nuclear | 69 | 51.5 |
| 6 | Unfractionated | 100 | 53.9 ± 4.2 |
| 24 | Cytoplasmic | 33 | 43.8 |
| 24 | Nuclear | 67 | 35.9 |
| 24 | Unfractionated | 100 | 36.5 ± 10.2 |

[a] NB cells were infected with $^{125}$I-labeled H-1 and harvested at either 6 or 24 h after infection as described in the text for separation of nuclear and cytoplasmic components. The percentage of VP2′, calculated as (VP2′/VP2′ + VP2) × 100, was determined as described in the legend to Fig. 3. The percentage of VP2′ from unfractionated cell lysates is an average of six separate experiments, and the standard deviations are included.

Along with the time-dependent conversion of VP2′ to VP2, there is also the appearance of a breakdown protein with an apparent molecular weight of 48,000 to 50,000 daltons. This protein was first seen at 3 h after infection, and by 24 h after infection it was the major protein species, comprising 40 to 50% of the total virus protein (Fig. 3). This protein is particularly intriguing for several reasons. First, the molecular weight matches that previously reported for a putative third viral capsid protein, "VP3" (4, 7). Second, this protein remained associated with the capsid structure on banding in CsCl (see Fig. 6) and was associated with both HF and LF virus. The exact origin of this protein cannot be determined from these experiments; however, it should be pointed out that the ratio of VP1 to VP2′ plus VP2 does not change in proportion to the appearance of "VP3" as would be expected if "VP3" were derived only from VP2′ and VP2. Furthermore, there is not enough VP1 to account for all of the "VP3" which appears. Therefore, "VP3" is either derived from all of the capsid proteins, or just VP2′ and VP2, with a concomitant degradation of VP1 by some other mechanism such that the ratios of VP1, VP2′, and VP2 remain constant. If the appearance of "VP3" were the result of a single cut in the capsid protein(s), we would expect the appearance of another protein of 15,000 to 20,000 daltons (from VP2′ or VP2) or a protein of 35,000 to 40,000 daltons (from VP1) or both, provided that these portions of the proteins were labeled. The fact that none of these species has been detected suggests that "VP3" is the result of more than one cleavage with a core structure being specifically protected.

**Conversion of HF to LF virus during infection.** $^{125}$I-labeled H-1 virus was again used to infect NB cells as described above, except that the virus was then analyzed on CsCl gradients to determine any change in the density distribution of the virus with time after infection. Figure 5 shows the CsCl gradient analysis of the $^{125}$I-labeled virus at 6 and 24 h after infection along with the original input virus. As can be seen, there is a clear shift of the $^{125}$I label to the LF virus density with 80% of the total virus at the lower density after 24 h. Since this conversion of HF to LF virus occurs at the same time that the VP2′ is being converted to VP2, we wanted to see whether these two events were independent as suggested above for the unlabeled virus preparations. To do this, HF and LF virus were purified from $^{125}$I-labeled H-1-infected cells at 3, 12, and 24 h after infection (as in Fig. 5), and the proteins were analyzed by polyacrylamide gel electrophoresis and autora-

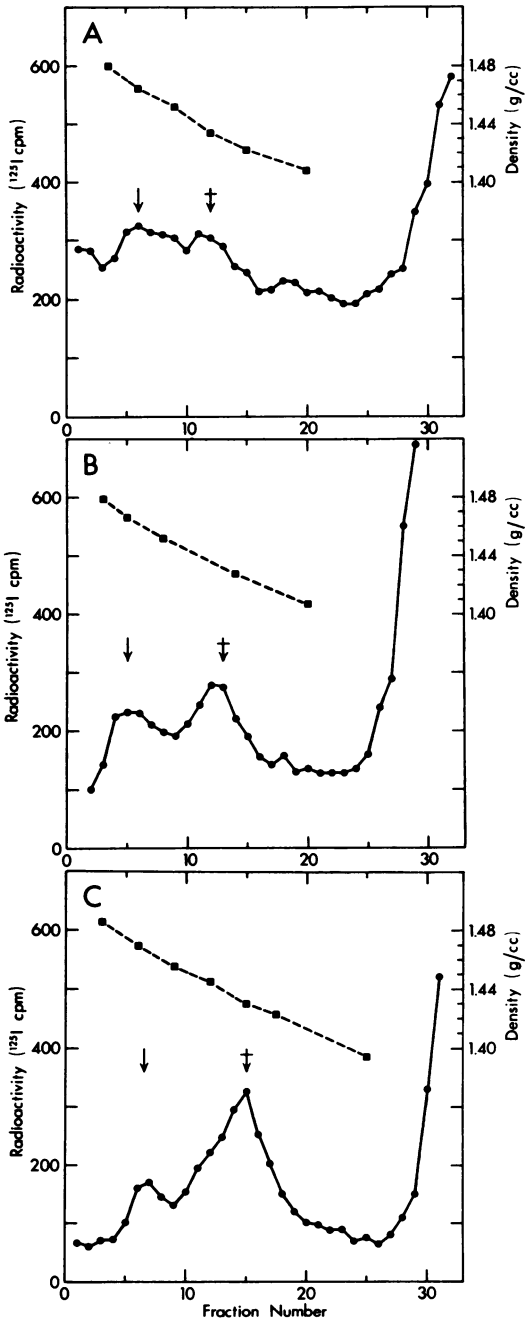


FIG. 5. *Conversion of HF to LF virus in vivo. NB cells were infected with $^{125}$I-labeled H-1 ($5 \times 10^5$ cpm per dish), and the cells were harvested in 20 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA–0.2% Sarkosyl at either 0 (A), 6 (B), or 24 h (C) after infection. After freeze-thawing, the extracts were diluted to 7 ml in the same buffer, adjusted to 1.43 g/cm$^3$ in CsCl, and centrifuged at 35,000 rpm for 48 h in a type 40 rotor. Gradient fractions were collected from the bottom and counted. The densities of individual fractions were determined by refractometry. The arrows indicating HF (↓) and LF (‡) virus are marked at 1.465 g/cm$^3$ and 1.43 g/cm$^3$, respectively.*

diography. The protein content of the LF virus was markedly different at the different harvest times (Fig. 6). The percentage of VP2 in these three samples as well as the HF virus from the same harvests was calculated (Table 4). The LF particles had essentially the same VP2 content as the HF virus and the total virus population at each time period shown. Furthermore, the data in Fig. 6 once again point out that three different LF populations can have very different contents of VP2′ and VP2. Therefore, although the conversion of VP2′ to VP2 and the conversion of HF virus to LF virus occur simultaneously, they are independent processes.

## DISCUSSION

The primary purpose of this study was to analyze the infectious process of the H-1 parvovirus and to correlate this process with the structure of the virus. In particular we were interested in probing the relationship between viral den-

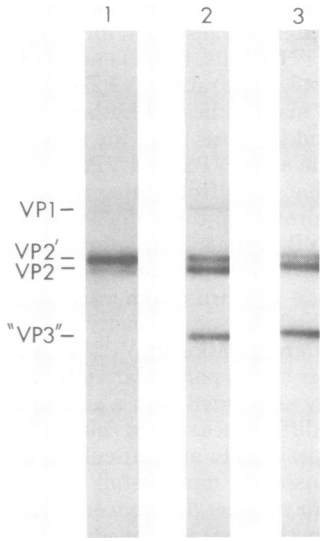


FIG. 6. *Protein content of $^{125}$I-labeled LF virus at various times after infection. NB cells were infected with $^{125}$I-labeled H-1 and harvested at 3, 12, and 24 h after infection, and the virus was fractionated as described in the legend to Fig. 5. The LF virus from each time period was then run on a 7.5% polyacrylamide gel, and the proteins were visualized by autoradiography. Lanes 1, 2, and 3 correspond to samples harvested at 3, 12, and 24 h, respectively.*

TABLE 4. *Comparison of the VP2 content of HF and LF virus at various times after infection*[a]

| Time after infection (hours) | % VP2[b] | | |
|---|---|---|---|
| | HF | LF | Total[c] |
| 3 | 32.2 | 23.5 | 32.5 ± 6.9 |
| 12 | 51.6 | 62.9 | 57.4 ± 6.4 |
| 24 | 66.4 | 71.3 | 63.5 ± 10.2 |

[a] NB cells were infected with $^{125}$I-labeled H-1 virus and harvested as described in the legend to Fig. 6.

[b] The percentage of VP2 was calculated as (VP2/VP2′ + VP2) × 100 as described in the legend to Fig. 3.

[c] The percentage of VP2 in the total, unfractionated virus is that seen in Fig. 3, and the standard deviations are included.

sity, protein content, and infectivity. Previous reports by Clinton and Hayashi (1) and Richards et al. (10) for minute virus of mice and by Kongsvik et al. (5, 6) for H-1 have shown that the HF virus contains VP2′ as the predominant structural protein, whereas LF virus contains predominantly VP2. The results that we have presented here suggest that, for H-1 virus grown in NB or THK cells, there is not a direct correlation between the ratio of VP2′ to VP2 and viral density. This is indicated both by the fact that the HF and LF virus from the same cell appear to have the same VP2′ and VP2 content and by the fact that virus from different cell lines have different protein patterns for virus of the same density (NB- versus THK-derived virus). Furthermore, when the fate of $^{125}$I-labeled virus was studied, the LF virus from various times after infection had the same density in CsCl but distinctly different ratios of VP2′ to VP2. It thus seems clear that a particular viral density is not necessarily associated with a given protein content. The differences seen in this study and those reported previously are difficult to understand. As is discussed in more detail below, these differences may reflect differences in the host cells from which the virus is derived. In different cells the virus is subjected to different modification reactions, some of which may have significant effects on the viral protein, viral structure, or both. It should be pointed out here that the data presented do not eliminate the possibility that the density shift from HF to LF virus is associated with or caused by the specific cleavage of a small number of selected VP2′ molecules in the capsid. Although cleavage of nearly all of the VP2′ molecules in vitro did not result in a concomitant shift in density (2, 5), the cleavages may not have been in the exact position required.

Attempts to correlate virus structure to the infectious process are very difficult since so many seemingly independent events are occurring simultaneously. Previously, Clinton and Hayashi (2) showed that HF and LF particles from minute virus of mice were equally infectious. Similarly, all of the viral species measured here had the same p/i ratio, indicating that none of the combinations of protein content and density conferred a selective advantage to the virus. However, shortly after entering the cell, the H-1 virus is modified by proteolytic cleavage and a density shift. It is very possible that these modifications are required for virus infection, but considering the number and heterogeneity of the virus population it is impossible at this point to determine which events are required for the infectious cycle.

The appearance of a 48,000- to 50,000-dalton cleavage product is very interesting for a number of reasons. First, the molecular mass matches that previously reported for "VP3" (4, 7). Second, the capsid structure remains intact to a certain degree even when nearly 50% of the viral protein has been cleaved to "VP3." The fact that the cleavage occurs at all suggests that the virus structure is changing and that the normally resistant proteins are being "exposed" to cleavage enzymes. Third, the resistant "VP3" must represent the parts of the virus proteins that are most intimately involved in the capsid structure, being able to hold the structure together after extensive cleavage of the original proteins. Knowing the nature of this cleavage to "VP3" would enhance our understanding of the protein structure of the capsid.

The data presented here indicate that within an H-1-infected cell, several events are occurring concomitantly and, in some cases, independently. I have looked at the virus formed during a multiple-round infection, and I also have studied the fate of the virus particles upon entry into the cell. These two populations are not the same and have not necessarily been subjected to all of the same conditions within the environment of the cell. However, it seems clear that the H-1 virus within the cell is undergoing modifications which are resulting in the conversion of VP2′ to VP2 and of HF virus to LF virus. If one looks at the total virus population within the cell at any time, the relative amounts of the various viral forms will be dependent upon the rate of virus assembly (by whatever mechanism this occurs) and the rates of viral modification. If the viral synthesis reactions are occurring more rapidly than the modification reactions, one would expect a population of virus enriched in VP2′ and HF particles (with a VP2′-containing HF particle being the earliest viral particle). The reverse would also be true. With the NB cells studied here, the rate of virus production is very high

compared with that seen in THK cells. As a result, the ratio of VP2′ to VP2 is much higher in NB cells than in THK cells. This would also indicate that the rates of the modification reactions in the THK cells are not reduced (compared with those of NB cells) to the same degree that the rate of virus production is decreased. Similarly, it has previously been observed by others (1, 5) that the proportion of LF particles is higher at later times after viral infection. This would result from the fact that late in the viral infection the production of virus slows down and eventually ceases when the cell dies. If the viral modification continues, a greater proportion of LF particles will appear as the rate of the modification reaction increases with respect to the rate of viral synthesis. Similarly, the differences in the protein content of HF and LF virus found by others (1, 5, 6, 10) may reflect differences in the relative rates of the synthesis and modification reactions in the particular system used. Since the average age of the LF population will, by definition, be older than in the HF population, the VP2 content may be higher as a result of the age difference. The age differences would also be dependent on the relative rates of the reactions mentioned above. Thus, it would be possible to have differences in the protein content of these two viral densities without a cause-and-effect relationship.

At present it is not possible to determine whether the postinfection modifications of H-1 are nonessential side reactions of the infection or are required for the infectious process. I am presently probing H-1 protein-protein and protein-DNA interactions with the hope of better correlating the structural and functional properties of this virus.

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