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Sequential Formation of Vaccinia Virus Proteins and Viral Deoxyribonucleic Acid Replication

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In vaccinia virus-infected cell cultures, cellular protein synthesis was inhibited 50% at 2 hr postinfection (PI) and 80 to 90% by 4 hr PI. Input virus was responsible for this inhibition. Five early proteins, coded for by the viral genome, could be detected at 2 to 3 hr PI. Normally, their synthesis did not continue beyond 6 hr PI, at which time synthesis of a different set of proteins began. When DNA replication was blocked, synthesis of these early proteins continued until 9 to 12 hr PI. The bulk of the proteins which were incorporated into mature virus were synthesized at 8 hr PI and thereafter. The time of their formation was close to the time at which virus maturation occurred. However, 15% of the protein found in mature virus was synthesized early in the infectious cycle. The quantity of "early viral protein" which was not incorporated into mature virus was almost as large as the quantity of viral protein which did appear in mature virus. The "early" and "late" proteins could be shown to have separate and distinct immunological properties. The role of this large quantity of "early" protein is discussed.

The complex morphological structure of vaccinia virus can be demonstrated by electron microscopic examination (2, 6, 17). A centrally located, electron-dense nucleoid body, two dense lateral bodies, a double membrane layer, a threadlike protein structure, and an outer membrane envelope are observed. Antigenic analysis has confirmed the complex structure which is revealed by the electron microscope. Eight precipitin lines have been observed after partial dissociation of purified vaccina virus (19). In addition, when infected tissue is examined, an additional 10 precipitin lines are found that are distinct from those observed in the purified virus particle (18). These findings are consistent with a viral genome of 1.6×10^8 daltons (16).

The present study was undertaken to characterize more fully the various viral proteins and to establish the sequential pattern of formation of these proteins in a single cycle of virus replication. In addition, the relationship of deoxyribonucleic acid (DNA) replication to the synthesis of specific viral proteins was examined.

Previously, it was shown that at 4 to 5 hr postinfection (PI), and thereafter, there is an almost complete suppression of cellular protein synthesis (13); thus, at these times labeled amino acids are only incorporated into viral proteins. However, for the first 5 hr of the infectious cycle, there is continued synthesis of cellular proteins, and for this reason it is not possible to study the formation of early viral proteins directly. Several indirect methods which do not require the isolation of viral proteins have been used to study the synthesis of viral proteins at these early times. The levels of enzymes involved in the synthesis of deoxynucleotide triphosphates and their subsequent polymerization to DNA have been determined. The formation of thymidine kinase, which is induced in response to virus infection, commences 1 hr PI and is complete by 4 hr PI (5). Similar kinetics of induction of enhanced levels of deoxynuclease and DNA polymerase have also been observed (4). By use of the analogue *p*-fluorophenylalanine, a vaccinia virus protein whose synthesis does not start until 4 hr PI and whose formation coincides with, or precedes by no more than 0.5 hr, the formation of infectious virus has been demonstrated (10). In contrast, when total viral proteins are examined by immunological procedures, a constant rate of viral protein synthesis is observed from 2 to 13 hr PI (13). These indirect observations suggest sequential formation of vaccina viral proteins.

By combining chemical techniques with the quantitative precipitin technique (12, 13), agar diffusion (1), and radioautography, it has been possible in the present work to examine the synthesis of a large number of immunologically distinct viral proteins throughout the infectious cycle. The results clearly demonstrate that viral proteins are made sequentially during a normal cycle of virus replication. In contrast, when replication of the viral genome is blocked, a fixed reading of the viral genome results in continued synthesis of the "early proteins." The proper functioning of a "switch-off" mechanism is dependent on DNA synthesis.

MATERIALS AND METHODS

Conditions for cell growth. A subclone of the HeLa S3 cell line was grown in suspension culture in Eagle's medium (3) supplemented with 5% whole or dialyzed horse serum. The cell has a generation time of 18 hr.

Conditions for virus infection. The conditions for simultaneous infection of HeLa cells with vaccinia virus strain WR have been described, as have procedures for the plaque assay on chick embryo monolayers (11). With this strain of vaccinia virus, replication starts at 5 hr, and the cycle is complete at 13 to 15 hr PI. A final yield of infectious particles of 100 to 200 plaque-forming units (PFU) per cell is obtained.

Immunological procedures. The antiserum was prepared with purified vaccinia virus (8) which had been grown on the chorioallantoic membrane. Virus (5×10^8 PFU) was injected intradermally at 10 sites on the rabbit's back, and 1 month later the rabbit was given four intravenous injections (4×10^8 PFU per injection) at weekly intervals.

One sample of vaccinia antiserum, which was used in the agar diffusion studies, was furnished to us by G. Appleyard. His kindness and generosity are gratefully acknowledged. Procedures used for agar diffusion were those described by Crowle (1). The quantitative precipitin procedure was described previously (12, 13).

RESULTS

Time course of formation of total viral proteins. The antiserum used in these studies was prepared by inoculation of infectious virus into rabbits. After inoculation, there was active replication of virus in the rabbit, and so a full spectrum of antibodies to both early and later proteins was obtained. If virus replication induces the synthesis of new proteins coded for by the cell's genome, it is likely that these proteins would not be antigenic. Thus, the antiserum used would be expected to detect only those proteins coded for by the viral genome. However, it will not detect all of these proteins with equal efficiency. This will result from differences in the inherent antigenicity of the various viral proteins and from the fact that widely different quantities of viral protein are made and will stimulate production of variable amounts of specific antibodies.

The kinetics of viral protein synthesis were determined with cells infected in the presence of 5-fluorodeoxyuridine (FUDR) and then washed free from unadsorbed virus in the presence of the analogue. The culture was then divided, and thymidine (10^{-6} M, final concentration) was added to one part of the culture. This concentration of thymidine reverses the effect of the inhibitor and permits the synthesis of a full yield of virus with normal kinetics of formation (15). Uniformly labeled L-phenylalanine-^{14}C (2 $\mu c/\mu$mole; 0.01 mM, final concentration) was added to both cultures at 2 hr PI. At various times thereafter, equal samples were removed, and the incorporation of phenylalanine-^{14}C into total protein was determined by precipitation with trichloroacetic acid. Incorporation of the isotope specifically into viral protein was measured by a quantitative precipitin test. The results presented in Table 1 and Fig. 1 demonstrate that, in the normal cycle of infection, the absolute quantity of viral protein made from 2 to 3 hr PI was as great as the quantity synthesized during any subsequent 1-hr interval. This finding was confirmed by the experiment with pulse labeling described below. By 13 hr, a time which is close to the completion of the infectious cycle, the rate of viral protein synthesis was decreased.

When viral protein synthesis was studied under conditions where DNA replication was completely inhibited, there was no significant inhibitory effect on the quantity of viral protein synthesized until 4 hr PI (Fig. 1). Thereafter, the rate of viral protein synthesis was suppressed. The yield of viral protein synthesized by 13 hr PI in the absence of DNA synthesis was 54% of the quantity that was formed at the same time (completion of the virus cycle) in a normal infectious cycle. The above findings are in excellent agreement with results previously reported (13).

The results of the experiment just described were confirmed by a similar experiment in which cultures similar to those used above were pulsed with uniformly labeled L-phenylalanine-^{14}C for 1-hr periods throughout the infectious cycle. At the end of the labeling period, the cells were collected, washed, and analyzed to determine the total quantity of protein and the quantity of virus-specific proteins made during the 1-hr exposure period. The results are presented in Table 2. The surprising observation that viral protein synthesis proceeded as rapidly early in the cycle as it did

TABLE 1. *Immunological precipitation of viral proteins*

Condition	Period of isotope incorporation (hr PI)	Counts/min precipitated by[a]			Counts/min incorporated into viral protein[b]	Counts/min incorporated into cell protein	Counts/min[c] incorporated into cell protein in uninfected culture	Per cent inhibition[d] of cell protein synthesis
		Immune serum	Pre-immune serum	5% trichloroacetic acid				
DNA synthesis possible	2–3	738	204	1,380	627	753	1,490	49.5
	2–4	1,257	268	3,090	1,083	2,007	4,140	52.7
	2–6	3,569	588	5,560	3,334	2,226	9,500	95.9
	2–9	4,915	1,080	7,030	4,531	2,499	15,860	95.7
	2–13	6,344	1,475	8,540	5,885	2,655	24,250	98.1
DNA synthesis inhibited	2–3	729	166	1,400	639	761	1,490	48.9
	2–4	1,102	246	2,180	965	1,215	4,140	82.9
	2–6	1,646	432	3,450	1,388	2,062	9,500	84.2
	2–9	3,528	1,085	7,660	2,846	4,814	15,860	72.5
	2–13	4,048	1,406	8,950	3,144	5,806	24,250	88.2

[a] Adsorption of virus (input multiplicity 30 to 50 PFU/cell) and washing to remove unadsorbed virus were carried out in Eagle's medium containing 0.01 mM L-phenylalanine, 10^{-6} M FUDR, and 5% dialyzed horse serum. Cells were resuspended in the same medium at 4×10^5 cells per milliliter at 1.25 hr PI. The culture was divided, and thymidine (10^{-6} M, final concentration) was added to one part. The cells were collected at 37 C by centrifugation and resuspended at 2 hr PI in the same medium containing 0.01 mM L-phenylalanine-*U*-^{14}C (2 $\mu c/\mu$mole). Samples were removed at the indicated times. Cells were collected by centrifugation, resuspended in phosphate-buffered saline, and sonically treated. Trichloroacetic acid was added to one portion. A second portion was centrifuged at 900 rev/min for 15 min, and the quantity of viral protein in the supernatant fluid was determined. An indirect precipitin reaction has been used in which vaccinia virus immune rabbit serum (or preimmune rabbit serum) was first added to infected cell sonic extracts, and then a sheep-antirabbit γ-globulin antiserum was added. The quantity of preimmune serum was adjusted so that the total quantity of protein in the final antigen-antibody precipitate was the same as that obtained with the immune serum.

[b] The incorporation into viral protein was calculated by subtracting the preimmune from the immune, and correcting this for the fraction of viral protein nonspecifically precipitated by the preimmune serum. Assuming viral and cellular proteins to be nonspecifically precipitated to the same extent, the equation is:

$$\text{viral protein} = \frac{\text{immune} - \text{preimmune}}{1 - \dfrac{\text{preimmune}}{\text{total (trichloroacetic acid)}}}$$

The validity of the above assumption is shown by the fact that the same fraction of total counts is precipitated by the preimmune serum in both the infected and uninfected cultures.

[c] The uptake of L-phenylalanine-*U*-^{14}C was determined with uninfected cells that had been manipulated in the same manner as the infected cells.

[d] The per cent inhibition of cellular protein synthesis is based on the increment between consecutive time periods rather than on the total amount of newly synthesized protein.

at the later times (4 to 10 hr PI) was confirmed in this experiment. In the presence of DNA synthesis, at 4, 6, and 9 hr PI, 64 to 72% of all protein that was made was shown to be viral protein. At the end of a 1-hr period of isotope incorporation, some of the labeled precursor is found in incomplete chains, and in viral protein which has not assumed a proper immunological configuration. The quantitative precipitin procedure would not be expected to detect isotope incorporated into these proteins. As much as 74% of the isotope could be directly demonstrated as present in immunologically detectable viral protein. It seems likely that the isotope which is not precipitated is also present in viral proteins, but fails to react for the above reasons. In both this and the preceding experiment (Table 1), the fraction of the newly synthesized protein which was specifically precipitable with vaccinia antiserum was less when DNA synthesis was blocked than in a normal cycle of infection. Two possible explanations may account for such results. (i) In the absence of DNA replication, cell protein synthesis may not be as effectively inhibited as in its presence. (ii) The inhibition of cell protein synthesis may be the same under either condition for virus replication, but there may be continued synthesis of only the early proteins when DNA synthesis is inhibited, and these early proteins are not as effectively precipi-

tated by the antiserum as are the late proteins. Of the two possibilities, the latter seems the more probable. This is inferred from the fact that the kinetics of inhibition of cell protein synthesis is similar under both conditions of virus replication (see below).

Inhibition of cellular protein synthesis resulting from virus infection. In infected cultures, the difference between the total incorporation of isotope into protein and the quantity of isotope in viral protein which is specifically precipitated by antiserum (see Table 1) gives a measure of the quantity of cell protein that continues to be synthesized. In Fig. 2, the quantities of cell protein made in infected cultures have been plotted at different times in the infectious cycle relative to the quantity of cell protein made in an uninfected culture.

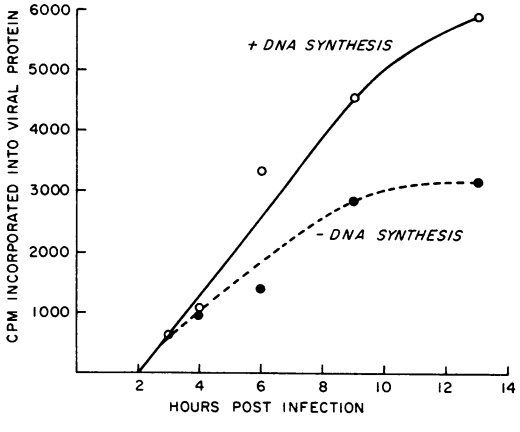


FIG. 1. *Viral protein synthesis as determined by immunological precipitation.*

The results are expressed as the per cent inhibition of cell protein synthesis. In infected cultures, both in the presence and absence of DNA replication, the rates of cell protein synthesis are markedly inhibited by 2 hr. By 4 to 5 hr, they are inhibited at least 80 to 95%, and these same levels of inhibition are observed throughout the remainder of the infectious cycle. Since the precipitin reaction does not measure all of the viral protein present in a sample (see above), estimates of the quantity of cellular proteins which are made in infected cultures are high, and the extent of inhibition of cellular protein synthesis (80 to 95%) is a minimal figure.

Kinetics of formation of proteins that are incorporated into mature virus particles. One method of showing that the designation of proteins as either "early" or "late" proteins is a meaningful biological classification is by examination of mature virus particles isolated after pulsing cultures for different times in the infectious cycle. L-Phenylalanine-^{14}C was added at 2 hr PI to a series of replicate infected cultures. At different times thereafter, a 100-fold excess of the unlabeled amino acid was added to stop further incorporation of isotope. At 24 hr PI, all cultures were harvested, and purified vaccinia virus was isolated (8) from each sample. The results are shown in Table 3. Of those proteins incorporated into mature virus particles, 74% were made at 8 hr PI and thereafter. The time of synthesis of these proteins was similar to the time at which maturation of virus occurred (90% of the infectious virus was synthesized between 8 and 14 hr PI). However, 65% of the total quantity of viral protein was synthesized prior to 8 hr PI (see Fig. 1). It

TABLE 2. *Immunological precipitation of viral proteins*[a]

Conditions	Period of isotope incorporation (hr PI)	Counts/min precipitated by			Counts/min incorporated into viral protein	Per cent of counts precipitable with antiserum
		Immune serum	Preimmmune serum	5% trichloro-acetic acid		
DNA synthesis possible	2–3	738	204	1,380	627	45.4
	4–5	828	199	1,160	759	65.4
	6–7	628	139	800	592	74.0
	9–10	496	100	670	465	69.4
	12.5–13.15	210	66	320	181	56.6
DNA synthesis inhibited	2–3	729	166	1,400	639	45.6
	4–5	465	102	1,030	463	39.1
	6–7	382	122	840	304	36.2
	9–10	369	109	730	306	41.9
	12.5–13.5	293	102	740	222	30.0

[a] The procedures are the same as described in Table 1, except that L-phenylalanine-*U*-^{14}C was added to replicate cultures at different times in the infectious cycle. After a 1-hr period of exposure to the isotope, the cells were collected and analyzed. One series of cultures contained 10^{-6} M FUDR, and the second series contained 10^{-6} M FUDR and 10^{-6} M thymidine.

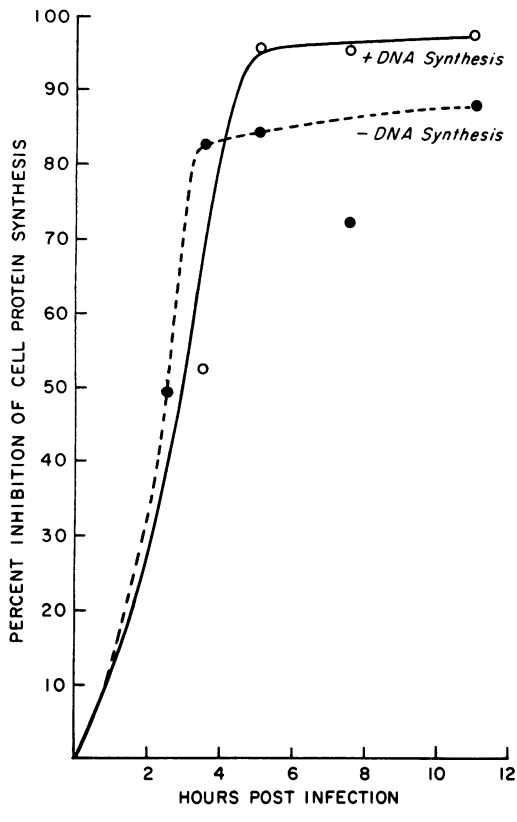


FIG. 2. *Inhibition of cell protein synthesis in vaccinia-infected cultures.*

TABLE 3. *Incorporation of* L-*phenylalanine-U*^{14}*C into purified vaccinia virus*[a]

Period of isotope incorporation (hr PI)	Properties of purified virus I			
	Counts/min per ml	OD (260 mμ)	Counts/min per OD unit	Relative specific activity
2–4	44	0.878	50	14
2–6	38	0.718	53	15
2–8	62	0.672	92	26
2–10	112	1.055	106	30
2–12	110	0.661	166	48
2–24	280	0.808	347	100

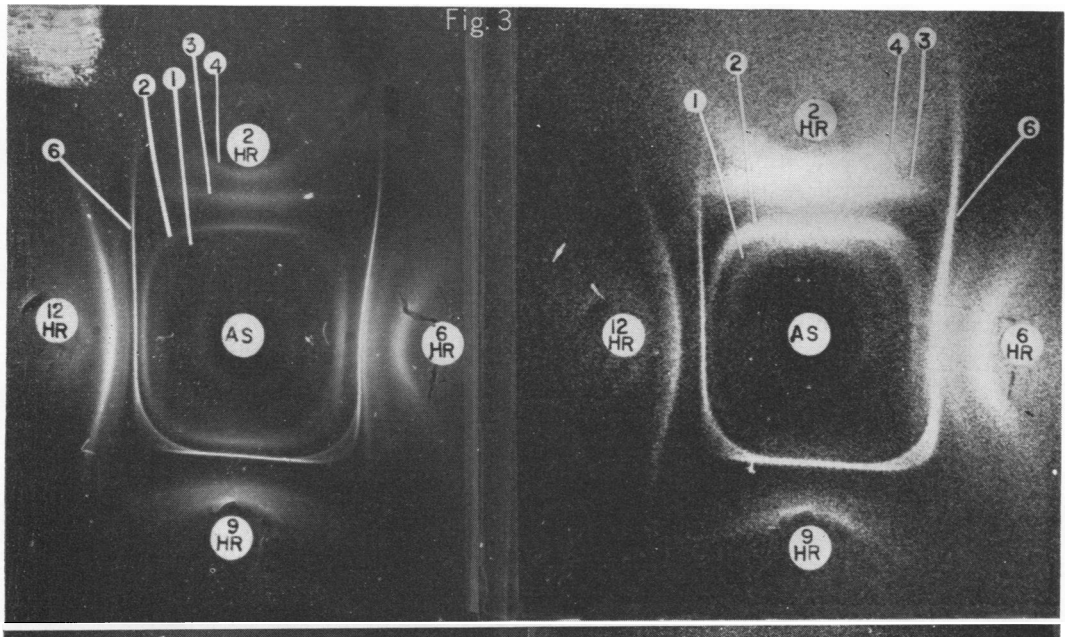
[a] Virus was purified as described previously (8). The optical density at 260 mμ and the incorporation of isotope into the purified virus suspension were measured. Optical density unit, 1 ml of material with an optical density of 1 (at 260 mμ, 1-cm light path).

was considered possible that proteins identical to those found in mature virus were made at the early time periods but were not incorporated into virus. However, the discrete immunological nature of the early and late proteins could be demonstrated (see below) and established that this was not the case.

Examination of viral proteins synthesized at different times in a normal cycle of virus replication. By agar diffusion, it was possible to study viral protein synthesis at early and late times in the infectious cycle. Cellular protein which failed to form an insoluble antigen-antibody complex could be eliminated by washing the agar at the completion of the reaction.

Infected cells were labeled with L-phenylalanine-^{14}C for 1-hr periods at various times PI. The cells were then harvested and disrupted by sonic vibration, and the low-speed supernatant fluids were analyzed by agar diffusion. After 2 days at 25 C, the agar was washed with 0.85% saline to remove protein which had not given rise to precipitin lines. It was then stained, and the stained pattern on the microscope slide was used as a negative to prepare an enlargement. The microscope slide was then placed in contact with X-ray film which was developed after periods of exposure for 1 to 6 days. The X-ray film was also used to prepare an enlargement of identical size to that prepared with the microscope slide. The results are shown in Fig. 3. By using longer periods for diffusion, a more complex pattern of viral antigens was obtained, and several components which appeared as single precipitin lines could be shown to be made up of two or more species of protein. (For example, it is shown that VP-6 is made up of at least two components, and, under appropriate conditions, they are resolved.) We have, however, limited ourselves to these data for clarity in presentation, and, as will be shown, these problems of heterogeneity do not bear on the conclusions that are drawn.

Visual inspection of the protein pattern in Fig. 3A reveals a relatively simple pattern at 2 to 3 hr PI. Under optimal conditions, five bands could be observed at that time. VP-1 was not readily seen in the protein pattern (Fig. 3A), but its presence was clearly revealed in the radioautograph (Fig. 3B). By 6 to 7 hr PI, the protein pattern was more complex, and changed only slightly thereafter. The fusion of two precipitin lines from adjacent samples made it possible to recognize common antigens which were present at different times. One common antigen, which is designated VP-6, was present at 6, 9, and 13 hr, but was not present at 2 hr. Two other viral antigens, VP-1 and VP-2, were seen at all time periods; this was also true of a third protein, VP-4, which was located near the antigen well and so that its common identity at the different time periods was not revealed as clearly. The radioautograph



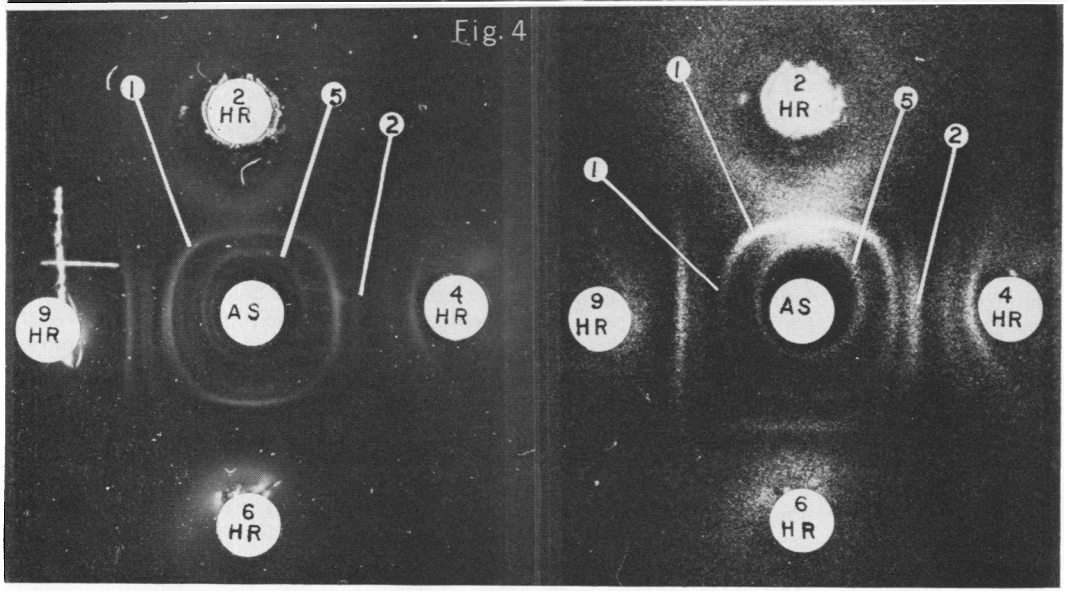


FIG. 3 and 4. *Viral protein synthesis as determined by agar diffusion. Agar diffusion was carried out according to Crowle's procedure (1). Ponceau S was used for staining of the proteins. Exposure time for autoradiography was 6 days with Ansco High Speed X-ray Film. Those proteins which are designated by the same number in Fig. 3 and 4 have been shown to be identical when tested by agar diffusion in adjacent wells and run against vaccinia antiserum.*

demonstrates that protein VP-1 was synthesized by 2 to 3 hr PI. Although protein VP-1 was present in the cultures at later times, there was no evidence of its synthesis at these later times. VP-2 was synthesized at 2 and 6 hr, but not at 9 and 12 hr. By tracings with a Joyce Chromascan (*unpublished data*), it was observed that VP-3, which was present at 2 and 6 hr, was not present at the later times. VP-6 which was seen at 6, 9, and 12 hr was synthesized at each of these times.

The results of a similar experiment with proteins obtained at different times after infection in the absence of DNA replication are shown in Fig. 4 with the corresponding radioautograph. It is

clear that VP-1 and VP-2, which are made at 2 to 6 hr in a normal cycle, under these conditions continued to be made for 9 hr (and also for 12 hr). Characterization of VP-1 and VP-2 in Fig. 3 and 4 was carried out by agar diffusion, in which those samples shown in Fig. 4 were placed in wells and the samples from Fig. 3 were placed in the alternate wells. Fusion of precipitin lines established their common identity. An additional protein, VP-5, which is not observed easily in Fig. 3, can be seen in Fig. 4. In the absence of DNA synthesis, it continues to be made for the 9-hr period.

DISCUSSION

Previously, we reported that a block in the normal transport of cellular RNA from the nucleus to the cytoplasm results from vaccinia virus infection (11). Under these conditions, cell protein synthesis would only persist until that mRNA which was present in the cytoplasm at the start of the infectious cycle had been degraded. In the present study, it is shown (Fig. 2) that in infected cultures, by 4 to 5 hr PI, cell protein synthesis is inhibited 90%. A rapid inhibition of cell protein synthesis is also observed when viral DNA replication is blocked. The half-life of mRNA in HeLa cells has been reported as 3 to 4 hr (7). The speed with which inhibition of cell protein synthesis is achieved in vaccinia-infected cultures indicates that mechanisms besides an inhibition in the transport of cellular mRNA from the nucleus to the cytoplasm operate to inhibit cellular protein synthesis. Cell protein synthesis is also inhibited when cells are infected with vaccinia virus in the presence of actinomycin D (14). It follows, therefore, that the inhibition of cell protein synthesis is controlled by the input virus.

The study described in the present paper depends upon the preparation of an antiserum which can detect all classes of viral protein. This is possible with vaccinia virus, since there is virus proliferation after the injection of virus into rabbits. Further, by preparing antiserum in rabbits with purified vaccinia virus which had been grown in eggs, antibodies against HeLa cell proteins were not formed. This was shown by the absence of precipitin lines when proteins from uninfected cells and viral antiserum were examined by agar diffusion. Proteins which are elicited by virus infection but are coded for by the cell's genome would not be expected to be antigenic. This latter point is important, since it enables us to examine only those early proteins which are coded for by the viral genome.

By use of the quantitative precipitin reaction, it was shown that viral protein synthesis proceeded at a linear rate in a normal cycle of infection starting at 2 hr PI. Of those proteins incorporated into mature virus, 74% were made after 8 hr PI. With a yield of 2,000 to 5,000 virus particles per cell, it was calculated that late synthesis of proteins is largely concerned with the formation of viral protein components that are incorporated into mature virus. It is clear that viral protein synthesis is a sequential event. However, it was of interest to determine whether those proteins whose synthesis started early in the infectious cycle continued to be made throughout the entire cycle. The agar diffusion and radioautographic studies of viral proteins synthesized at different times in the infectious cycle resolved this problem. With viral proteins pulse-labeled at different times, it was possible to examine the properties of identical proteins at various times in the infectious cycle. Thus far, several different patterns of synthesis of proteins have been observed. There is no evidence that any species of protein is made continuously throughout the infectious cycle. The early proteins are synthesized until 6 hr PI. Three of these early proteins were observed to persist throughout the entire cycle, but with no evidence of their continued synthesis at the later times. A fourth early protein, VP-3, was no longer present at the later times. Failure to detect this early protein at 9 and 12 hr PI may result from its active degradation or its precursor role and the distinct immunological nature of the product that is formed.

Although it is clear that the proteins which are synthesized early are distinct from those formed at the later times, the biological role of these early proteins is not clear. The levels of several enzymes involved in the replication of DNA are enhanced after virus infection (4, 5). It was during the early period (from 1 to 4 hr PI) that such increases were observed. Unequivocal evidence that these proteins are coded for by the viral genome has not been presented. Further, there are no data to indicate whether these enzymes represent more than an insignificant fraction of the viral protein that is synthesized during the early period. It would seem obvious to equate the early proteins that are observed by immunological procedures in the present study with these enzymes. However, the quantity of early protein formed makes such an interpretation questionable. By 8 hr PI, 65% of the final viral protein yield has been formed. Only a small fraction of this protein is incorporated into the mature virus. It is between 8 and 13 hr that the protein constituents of the mature virus are synthesized. That quantity of protein which is made early is in large excess of the quantity which would be required to function enzymatically. Additional studies are required to characterize "early" proteins. Because of the immunological procedures employed, it can

be stated that "early" proteins observed in the present study are coded for by the viral genome. Although these data are the most direct evidence that the early proteins induced by an animal virus are coded for by the viral genome, they do not exclude the possibility that additional proteins are coded for by the cell's genome in response to virus infection.

At the early times, the pattern of protein synthesis in a normal cycle of infection is almost identical to that observed in the absence of DNA synthesis. At the later times in the normal cycle, however, a new spectrum of proteins is made. In contrast, in the absence of DNA synthesis, early proteins continue to be made throughout the 12-hr period we have examined. Thus, inhibition of viral DNA replication inhibits the proper functioning of a switch-off mechanism which normally operates to block synthesis of early proteins beyond 6 hr PI. This inhibition also restricts gene expression to a more limited part of the viral genome than would occur in a normal cycle. In order for a normal sequence of events with proper functioning of the switch-off mechanism to occur, we believe that there is a requirement for viral DNA replication rather than a need to achieve some critical level of DNA. The fact that failure of the switch-off mechanism is observed at different input multiplicities in the absence of DNA synthesis supports this idea. However, during a cycle of virus replication, there is a 100- to 500-fold increase in viral DNA, so the possible role of critical levels of DNA cannot be completely ruled out by adjusting the multiplicity of infection. This especially is true because the late proteins are made at 6 hr PI and thereafter, and viral DNA replication is complete by 6 hr PI (9).

DNA synthesis is complete by 6 hr, and those proteins which are incorporated into mature virus are made after this time. Thus, the pattern of proteins synthesized when viral DNA replication is inhibited by FUDR at the start of cycle, and the pattern obtained when it stops in a normal cycle of infection, are clearly distinct.

A possible unique role of the input virus, as distinct from progeny virus, is suggested by these studies. The input virus is capable of coding for early proteins, and can synthesize large quantities of these proteins. Whether, in a normal cycle of infection, progeny virus also performs this same function or whether this role is restricted to the input virus is not presently known.

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