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Virus-specific Ribonucleic Acid Synthesis in KB Cells Infected with Herpes Simplex Virus

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The production of virus-specific ribonucleic acid (RNA) was investigated in KB cells infected with herpes simplex virus. A fraction of RNA annealable to virus deoxyribonucleic acid (DNA) was found in these cells as early as 3 hr after virus inoculation. Production of this species of RNA increased up to 6 or 7 hr after infection, at which time elaboration of virus messenger RNA (mRNA) declined. At 24 hr after infection, the rate of incorporation of uridine into this RNA was approximately one-half of the rate present at 6 hr after inoculation. Nucleotide analysis of the RNA annealable to virus DNA was compatible with that expected for virus mRNA. Centrifugation showed considerable spread in the size of the virus-induced nucleic acid, the bulk of this RNA sedimenting between 12 and 32*S*. Incorporation of uridine into cell mRNA, ribosomal precursor RNA, and soluble RNA was suppressed rapidly after infection. As is the case with most other cytocidal viruses investigated to date, virus-induced suppression of cell RNA synthesis appears to be a primary mechanism of cell injury.

Herpes simplex virus, and the related pseudorabies virus, have been employed as model agents in a number of in vitro studies on synthesis of cytocidal mammalian deoxyribonucleic acid (DNA) viruses and the effect of these parasites on host cells (2, 16, 23). These viruses accumulate in the nuclei of infected cells, suggesting that the virus nucleic acid is synthesized in this location, and mature particles are extruded through the cell membrane (3). For herpes simplex, the kinetics of DNA and antigen synthesis in relationship to appearance of infectious particles have been reported by other investigators (16, 19). This paper describes studies of the relationship of virus messenger ribonucleic acid (mRNA) synthesis to virus DNA, antigenic protein, and infectious-particle production in KB cells infected with herpes simplex. The effect of virus infection on synthesis of cellular ribonucleic acid (RNA) is also described.

MATERIALS AND METHODS

Tissue culture. All experiments were performed with suspension cultures of KB epithelial cells grown in Eagle's minimum essential medium (modified for suspension culture) supplemented with 5% fetal calf serum. This strain of KB cells originally came for the laboratory of Harry Eagle in 1960 and was obtained from Harold S. Ginsberg in 1962.)

The kinetic studies of virus synthesis were performed on suspension cultures maintained at 1.5 $\times$ 10^5 to 4 $\times$ 10^5 cells per ml by daily feeding. The doubling time for these cells varied between 22 and 28 hr. No pleuropneumonia-like organisms were found in this cell line by culturing at numerous times during the course of these studies.

Virus and virus infection. The herpes simplex virus was the same strain used in previous studies (4). Virus stocks were prepared by inoculation of monolayer cultures of KB cells in 900-ml bottles with 2 $\times$ 10^6 plaque-forming units (PFU) of virus. The infected cells were harvested after 48 hr and concentrated by centrifugation (10 min at 800 $\times$ *g*). Virus stocks with a titer of 5 $\times$ 10^7 to 10^8 PFU/ml were prepared as described in a previous publication (4). Plaque titrations were performed in plastic petri dishes (60 by 15 mm; Falcon), with BHK-21 (baby hamster kidney) cells. These monolayer cultures were inoculated with 1 ml of virus suspension. After 2 hr of adsorption, the inoculum was decanted from these plates, and 5 ml of BHK-21 medium (11) suspended in 0.5% Ionagar (Colab) was applied. An overlay of neutral red (1:10,000) was applied at 96 hr, and the number of plaques was determined 120 hr after infection. Purified virus was used to infect suspension cultures and for isolation of virus DNA. Virus was purified either by calcium phosphate column chromatography (24) or by potassium tartrate gradient centrifugation (10). Complement fixation tests for soluble virus antigen(s) were performed with human convalescent antiserum as described previously (5).

Nucleic acid inhibitors. Actinomycin D (Merck Sharpe and Dohme) was used as an inhibitor of DNA-dependent RNA synthesis (15). The concentration used in these studies (0.05 μg/ml) was five times greater than that necessary to reduce virus

production to 0.1% of that present in untreated control cultures. 5-Iododeoxyuridine (5-IUDR) (Nutritional Biochemicals Corp., Cleveland, Ohio) was used in a concentration of 100 μg/ml as an inhibitor of the formation of normal virus DNA (16). This concentration of 5-IUDR was 10-fold greater than the concentration required to reduce the final titer of virus in treated cultures to 0.1% of the titer present in untreated control cultures at the end of 24 hr of incubation.

Radioisotope labeling, isolation, and centrifugation of RNA from infected cells. Pulse-labeling for kinetic studies of RNA in infected cells was performed in Eagle's minimum essential medium with tritiated or ^{14}C-labeled uridine for periods of 15 min. Two-liter suspension cultures of cells were infected with 12 to 20 PFU of virus per cell. At this multiplicity, 84 to 93% of the cells were infected at the end of 2 hr as shown by infectious-center assays. At selected times, 200-ml samples were withdrawn from the culture for radioactive labeling with 10 μc of ^{14}C-uridine or 500 μc of tritiated uridine. At the end of the labeling period, the cells were poured onto frozen medium, centrifuged for 10 min at 800 × *g*, and washed once in cold medium. The cells were suspended in 10 ml of 0.02 M sodium acetate buffer (*p*H 5.1). Total cell RNA was extracted by addition of 0.5% dodecyl sulfate and three cycles of extraction with an equal volume of 85% redistilled phenol at 60 C (22). After release of radioactively labeled RNA by phenol treatment, the nucleic acid was precipitated with 2 volumes of cold ethyl alcohol and pelleted at 12,000 × *g* for 15 min; the precipitate was dissolved in 1.0 ml of phosphate-buffered saline (PBS). One-half of this specimen was layered onto a linear gradient of 10 to 30% sucrose (w/v) buffered with 0.005 M tris(hydroxymethyl)aminomethane (Tris) chloride (*p*H 7.3) and centrifuged at 45,000 × *g* (21,000 rev/min) for 16 hr in the SW 25.1 rotor of a Spinco L-2 centrifuge. (The remaining one-half of the sample was retained for duplicate centrifugation runs.) After centrifugation, the tube was punctured and samples were collected from the bottom. The distribution of optical density in the gradient was determined in a Beckman DU spectrophotometer at 260 mμ. Carrier RNA (50 μg) was then added to each sample, and the nucleic acid was precipitated with 2 volumes of absolute ethyl alcohol. The precipitates were collected and washed on Millipore RA filter pads (pore size, 1.2 μ). Carbon-14 counts were determined in an end-window Geiger counter (Nuclear-Chicago Corp., Des Plains, Ill.). Tritium counting was performed in a liquid scintillation counter (Nuclear-Chicago). Quenching was corrected by using an external standard-channels ratio computation for each separate sample. The counting effiiciency averaged 39% for tritium-labeled RNA.

Isolation of virus DNA. Purified virus DNA for annealing with RNA from infected cells was obtained by concentrating the cells from 90 to 120 900-ml bottles, releasing the virus from the cells by homogenization at 4 C in the 50-ml container of a Sorvall Omnimixer operated at top speed, and submitting the released virus to two cycles of centrifugation at 100,000 × *g* for 90 min in a Spinco L-2 centrifuge. The pellets from the second centrifugation were suspended in 0.15 M sodium chloride buffered with 0.02 M sodium phosphate (*p*H 7.15) and treated with deoxyribonuclease (Worthington Biochemical Corp., Freehold, N.J.) at a concentration of 100 μg/ml for 1 hr at 37 C. The virus suspension was centrifuged again at 100,000 × *g* for 90 min and the pellet was resuspended in 0.05 M sodium phosphate buffer (*p*H 6.2). Virus was purified by chromatography on calcium phosphate (Brushite) columns measuring 2.5 by 30 cm (24). Virus was again centrifuged for 90 min at 100,000 × *g* and suspended in 20 ml of 0.15 M sodium citrate buffered at *p*H 7.2. The pellet was dispersed in this medium with a Ten Broeck homogenizer. Absolute ethyl alcohol (22 ml) was added to this suspension, and the material was centrifuged for 10 min at 14,000 × *g*. The supernatant fluid was decanted, and 10 ml of absolute ethyl alcohol and 10 ml of freshly redistilled ether were added to the pellet. This suspension was again centrifuged at 14,000 × *g* for 10 min, and the precipitate was washed once with absolute ethyl alcohol. The pellet was then suspended in 10 ml of saline-sodium citrate (1:10 SSC), and 0.5 ml of 10% sodium dodecyl sulfate (w/v) and 0.25 ml of mercaptoethanol were added. The tube was shaken and allowed to stand for 30 min at room temperature. Pronase (5 mg) was then added, and the preparation was incubated for 1 hr at 37 C. Freshly redistilled phenol (10 ml) was then added, and the tube was shaken for 3 min at room temperature (14). The suspension was cooled in a bath of melting ice and centrifuged at 12,000 × *g* for 10 min. Sodium acetate (3 M) containing 0.001 M ethylenediamine-tetraacetate (EDTA) was added to produce a final concentration of 0.3 M sodium acetate. Two volumes of absolute ethyl alcohol at −20 C was then added, and the precipitating DNA was spooled onto a glass rod. The DNA was resuspended in 7 ml of SSC and held overnight at 4 C. Ribonuclease was added to a final concentration of 20 μg/ml, and the solution was incubated for 1 hr at 37 C. This preparation was treated with 3.5 ml of phenol four times at room temperature as above. The DNA was precipitated with 2 volumes of absolute ethyl alcohol and redissolved in SSC; 3 M sodium acetate in 0.001 M EDTA was added to produce a final concentration of 0.3 M sodium acetate. The DNA was then precipitated by the addition of 0.54 volume of isopropanol (12). This precipitate was resuspended in 2.5 ml of SSC, a drop of chloroform was added as preservative, and the preparation was stored at 4 C. The mean amount of virus DNA obtained from 150 bottles was 2.34 mg. To test the purity of DNA prepared by this method, a tracer quantity of RNA from infected cells which had been pulse-labeled for 15 min with ^{14}C-uridine, and an equal number of radioactive counts in ^{32}P-labeled purified virus DNA, were added to the homogenate of crude virus prior to extraction of virus DNA; 100 μg of DNA isolated from homogenates containing these labeled compounds was subjected to alkaline hydrolysis in 0.5 M potassium hydroxide. The counts soluble or pre-

cipitable by 66% ethyl alcohol were then determined in a liquid scintillation counter; 0.45% of the total corrected counts were found in carbon-14 solubilized by alkali treatment. This was regarded as evidence that the DNA obtained by this method contained negligible quantities of contaminating cell RNA.

Isolation of cell DNA. Cell DNA was purified from 5×10^8 suspension-culture cells. At harvest, the cells were washed once in PBS, suspended in 15 ml of PBS, and disrupted by the addition of 1.5 ml of 5% sodium deoxycholate. The lysate was treated once with an equal volume of freshly distilled phenol, and pancreatic ribonuclease was added to produce a final concentration of 50 μg/ml. After 1 hr of incubation at 37 C, the phenol treatment was repeated three times. DNA was then precipitated from solution with two volumes of ethyl alcohol redissolved in SSC, and precipitated once with 0.3 M sodium acetate and 0.54 volume of isopropanol as described previously for virus DNA. The average recovery of DNA from 5×10^8 cells was 6.22 mg.

Nucleotide analysis of viral and cellular DNA. Nucleotide composition of purified ^{32}P-labeled DNA from KB cells or herpesvirus was determined by incubating 250 μg of the DNA with 100 μg of pancreatic deoxyribonuclease in 1.0 ml for 16 hr at 37 C; 500 units of purified Russel's viper phosphodiesterase (Calbiochem) was then added, and the mixture was incubated for 7 hr at 37 C. A portion of this mixture was subjected to paper electrophoresis at 50 v/cm in 0.05 M ammonium formate (*p*H 3.6) for 1.5 hr. The ultraviolet-absorbing spots were identified spectrophotometrically, and the radioactivity retained in these spots was counted in a liquid scintillation counter.

Annealing of DNA and RNA. Virus or cell DNA in SSC was adjusted to a concentration of 0.5 to 1.0 mg/ml, and was heated in a boiling-water bath for 15 min to produce strand separation. DNA (150 μg) was mixed with 30 to 40 μg of RNA from infected or control cells (in a total volume of 1.4 ml) and 0.5 ml of potassium chloride in 0.005 M Tris (*p*H 7.3); 0.7 ml of this solution was diluted immediately to 15 ml with 0.5 M potassium chloride buffered with Tris (*p*H 7.3). All of the diluted specimens were retained overnight at 4 C to serve as unannealed controls for nonspecific background absorption controls. The remaining 0.7 ml in each tube was placed in a water bath at 61 C for 16 hr to permit hybridization between DNA and the homologous RNA. The incubated specimens were then cooled to room temperature and diluted to 15 ml with 0.5 M potassium chloride. Pancreatic ribonuclease (previously heated to 70 C for 10 min to destroy deoxyribonuclease activity) was added to all tubes (including the refrigerated control specimens) in a final concentration of 2 μg/ml. The tubes were incubated at 37 C for 1 hr and cooled in ice. Each specimen was then filtered slowly through a Bac-T-Flex filter (10 mm in diameter; Carl Schleicher & Schuell Co., Keene, N.H.). The filters were washed five times with 5 ml of Tris-buffered potassium chloride. Counts trapped on these pads were determined in a liquid scintillation counter. The net amount of RNA annealed was determined by subtracting the counts retained on the pad of the unincubated control from the total number of counts retained on the pad of the annealed specimen. The counts in replicate samples of the same mixture agreed within 10% in both the control and incubated specimens. Nonspecific absorption to the pads was computed as the percentage of total counts in the unannealed specimen which adhered to the filter pad. This amounted to 0.2 to 0.26% of the total added counts. The quantities of DNA used for these hybridization studies were at least twofold greater than that necessary to anneal the maximal percentage of RNA from solution, indicating that abundant DNA sites were available for hybridization with homologous RNA. Nucleotide analysis of the annealed RNA was conducted by labeling infected or control cells with ^{32}P in phosphate-free medium, eluting the annealed RNA from the pads after hybridization, and subjecting it to alkaline hydrolysis in 0.5 M KOH. The nucleotides were separated by high-voltage electrophoresis at 50 v/cm, and the retained counts in each nucleotide were determined in a liquid scintillation counter.

RESULTS

Experiments with nucleic acid inhibitors. These studies were performed to investigate the time relationship between critical RNA production, virus-specific DNA synthesis, and the virus multiplication cycle.

A single 1,000-ml suspension culture of KB cells was infected with herpes simplex. After 2 hr of adsorption, the cells were washed three times in medium and reconstituted in 1,000 ml of new suspension medium. At selected intervals thereafter, 75-ml portions were withdrawn. This volume was divided into 25-ml fractions. Actinomycin D in a final concentration of 0.05 μg/ml was added to one fraction, 5-IUDR in a final concentration of 100 μg/ml was added to a second fraction, and the third fraction was harvested for freezing and subsequent titration. All fractions were subjected to six cycles of freezing and thawing to disrupt the cells. Virus and complement-fixation titers were then determined.

Resistance to actinomycin D appeared about 4 hr after infection, and resistance to 5-IUDR appeared about 1 hr later (Fig. 1). Addition of either of these inhibitors later than 12 hr after infection failed to reduce the titer of infective virus at 24 hr in comparison with the level present in control cultures. These experiments suggested that mRNA appeared in these cells approximately 4 hr after infection, preceding DNA synthesis by about 1 hr. Infectious particles showed an increase in these cells at 7 hr after infection (Fig. 1). The rise in complement-fixing

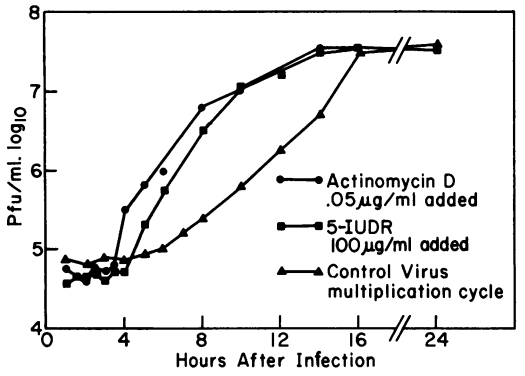

FIG. 1. *Inhibition of herpes simplex virus formation by actinomycin D and 5-IUDR added at selected intervals after infection. Suspension cultures were infected with 12 to 20 PFU of virus per cell. The virus was allowed to adsorb for 1 hr; unadsorbed virus was removed by washing the cells three times. Actinomycin D or 5-IUDR was added at the indicated time, and the cultures containing the inhibitors were incubated for a total of 24 hr from the time of infection. The control multiplication cycle was determined by harvesting samples of the suspension culture at the times shown. Results are the geometric means of four experiments.*

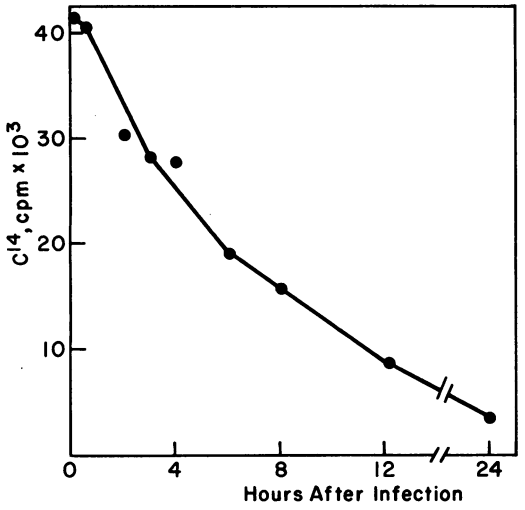

FIG. 2. *Uridine incorporation into RNA of 2,000-ml suspension cultures of KB cells infected with 12 to 20 PFU of virus per cell. The virus was permitted to adsorb for 1 hr, the cells were then centrifuged and restored to their original volume and 200-ml samples were removed and labeled by treatment for 15 min with 10 μc of ^{14}C-uridine at the times shown. The number of counts incorporated into total cell RNA is presented. (Each point represents the mean of three experiments.)*

antigen(s) occurred simultaneously with the increase in virus titer. We were unable to detect a rise in soluble complement-fixing antigen prior to the rise in herpesvirus titer, in contrast to the work of Gold et al. (6, 19).

Radioisotope incorporation into total cell RNA of infected cells. ^{14}C-labeled uridine was used for pulse-labeling of infected cultures (15-min periods) at chosen times during a single replication cycle of herpes simplex. Labeling of 200-ml samples with 10 μc of ^{14}C-uridine was followed by isolation of the total cell RNA. Suppression of total RNA synthesis in KB cells began rapidly after inoculation with herpes simplex (Fig. 2). At the end of 12 hr, total RNA synthesis had fallen to a level one-fourth of that present in uninfected cells.

Analysis of RNA synthesized, during herpes infection, by density gradient centrifugation. These studies were performed to investigate: (i) the possibility that a new species of virus-directed RNA might be measurable because it possessed different sedimentation properties from cell RNA, and (ii) the possibility that differential suppression of certain fractions of the cellular RNA might occur after infection of cells with herpes simplex. The conditions for labeling and isolation of the RNA were the same as those described in the preceding paragraph. Figure 3

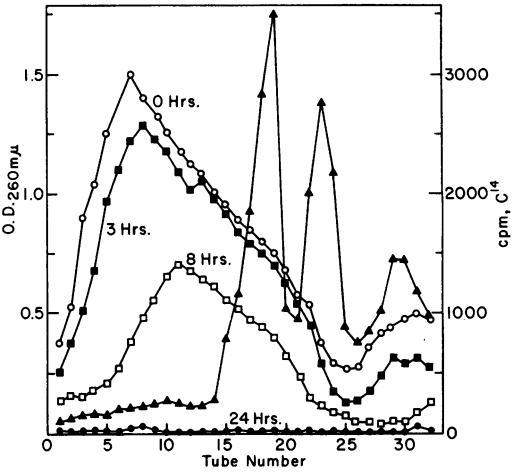

FIG. 3. *Sedimentation properties of RNA from herpes-infected KB cells. Suspension cultures were infected as in Fig. 2; 200-ml samples were withdrawn at the indicated times and labeled by treatment for 15 min with 20 μc of ^{14}C-uridine. Total RNA was isolated by the hot-phenol method, and a portion of the RNA from each labeling period was subjected to sucrose density gradient centrifugation in 10 to 30% sucrose with 0.002 M magnesium chloride buffered at pH 7.3 with 0.005 M Tris chloride for 16 hr at 45,000 × g. Fractions were collected by puncturing the bottom of the tube, the optical density at 260 mμ was read for each fraction, and the counts incorporated into RNA were determined in liquid scintillation. This is a single representative experiment.*

shows the labeling patterns obtained at selected times in the virus replication cycle. For brevity, only the centrifugation patterns obtained with RNA labeled at the time of infection, and at 3, 8, and 24 hr after inoculation, are shown. The large peak of radioactive labeling at 45*S* in the gradient pattern is typical of the distribution of radioactivity in RNA labeled for pulse periods of 15 min (22). This label has previously been shown to move into the 28 and 16*S* ribosomal peaks in pulse-chase experiments and is thought to represent ribosomal precursor RNA (22). These studies showed diminution in labeling of all species of cellular RNA. In addition, it is apparent from these studies that no new virus-induced RNA fraction was measurable in infected cells by this technique. Labeling and gradient centrifugation studies were also performed at 5, 10, 12, and 16 hr after inoculation of virus. These studies confirmed the findings depicted in Fig. 3.

Annealing of RNA from infected cells to virus and cellular DNA. The preceding studies suggested that virus-induced RNA might be produced in relatively small quantities, and that its presence might be masked, therefore, by persistent incorporation of uridine into cell RNA. Studies were then undertaken to separate virus and cell mRNA from the bulk of cell RNA by hybridization with homologous DNA at elevated temperature followed by entrapment of the hybridized nucleic acids on nitrocellulose filters (13). To carry out these experiments, it was necessary to purify cell and virus DNA. The nucleotide analysis of these nucleic acid preparations (Table 1) agreed closely with those described previously (2, 18). In the annealing studies, unlabeled DNA from one of these sources was permitted to combine with tritium-labeled RNA from infected cells. The complexes were trapped on filters and washed repetitively; the dried filter discs were counted in a liquid scintillation counter. Significant labeling of RNA annealable to virus DNA was present at the time of first measurement, 3 hr after infection (Fig. 4). Labeling in this fraction progressed rapidly to a maximum at 6 to 7 hr after inoculation, falling at 24 hr to approximately one-half of the labeling present at the time of maximal incorporation. The rate of labeling in RNA annealable to cell DNA fell rapidly after infection, reaching a level 12 hr

TABLE 1. *Nucleotide analysis of cell DNA, herpes simplex DNA, cell RNA, and RNA annealable to virus DNA*

Nucleic acid[a]	Guanylic acid	Cytidylic acid	Adenylic acid	Uridylic (thymidylic) acid
	%	%	%	%
Cell DNA	21	22.3	27.8	28.9
Total cell RNA	26.3	30.7	21.1	22.9
Virus DNA	33.4	34.6	15.7	16.3
RNA annealable to virus DNA (labeling period)				
6–8 hr	31.0	30.5	18.2	10.3
8–10 hr	32.1	30.3	18.1	19.5
10–12 hr	32.5	30.4	18.0	19.1
12–14 hr	32.6	30.5	18.3	18.6

[a] DNA preparation: 40-hr labeling period in monolayer cultures, with 0.5 mc of ^{32}P per bottle (twenty 900-ml bottles per experiment); DNA was hydrolyzed by venom phosphodiesterase, and the nucleotides were separated by paper electrophoresis (1.5 hr in 0.05 M ammonium formate at *p*H 3.6). The nucleotide ratios were computed by identifying the ultraviolet spots spectrophotometrically and counting the radioactivity in a liquid scintillation counter. RNA preparation: 2-hr labeling period with 1,000-ml suspension culture in phosphate-free medium labeled with 25 mc of ^{32}P; RNA was hydrolyzed by treatment with 0.5 M potassium hydroxide for 16 hr. Nucleotides were separated by paper electrophoresis as with DNA; the ultraviolet-absorbing spots were identified, and the radioactivity in the nucleotides was counted in a liquid scintillation counter.

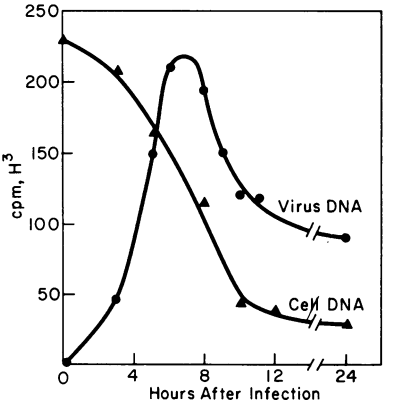

FIG. 4. *Pulse-labeled RNA from infected cells annealable to virus or cell DNA. A 2,000-ml suspension culture of KB cells was infected with 12 to 20 PFU of virus per cell. After 1 hr of adsorption, the cells were centrifuged and resuspended; 200-ml samples were labeled for 15 min with 0.5 mc of tritiated uridine at the times shown. The total cell RNA was isolated and annealing was performed by use of 150 μg of cell or virus DNA and 40 μg of RNA from infected cells. After annealing for 16 hr at 61 C, the solutions were treated with ribonuclease and filtered through nitrocellulose pads. The radioactivity retained on the pads was counted in a liquid scintillation counter. Results are the mean values obtained from two separate experiments.*

after inoculation which was approximately 20% of the rate present in uninfected cells.

Nucleotide analysis with ^{32}P-labeled RNA from herpes-infected cells in the annealing experiments showed reasonably good agreement between the nucleotide composition of the virus DNA and the complexed RNA. As seen in Table 1, this agreement was not complete; RNA annealed to virus DNA showed a guanylic-cytidylic acid content of 63%, whereas the virus DNA possesses a 68% guanylic-cytidylic acid content. The reason for this disparity is not immediately apparent; it may be due to contamination with RNA retained on the pad by simple absorption or to incomplete equilibration of the labeled nucleotide pools.

Sedimentation characteristics of RNA annealable to virus DNA. Studies were performed to estimate the relative size of the RNA annealable to virus DNA. It was the aim of these experiments to investigate the degree of heterogeneity existing in herpes-specific RNA.

The RNA in infected cells was labeled by treatment for 2 hr with ^{32}P in phosphate-free medium. Total RNA was then isolated and centrifuged in sucrose; 1-ml samples were collected by puncturing the bottom of the centrifuge tube. Optical densities at 260 mμ were read, and the tubes were pooled into four fractions. Each of these fractions contained the same number of optical density units of RNA. The fractions were precipitated with 2 volumes of absolute ethyl alcohol and centrifuged at 12,000 × *g* for 15 min. The pellets were dissolved in 0.2 ml of PBS, and the RNA from each fraction was tested for its capacity to anneal to virus DNA. The maximal percentage of annealing occurred with RNA from the fraction sedimenting between 12 and 24*S*, with a lesser percentage of annealing occurring between the 24 and 32*S* RNA and virus DNA (Table 2). A small amount of RNA sedimenting faster than 32*S* was also annealable to virus DNA.

TABLE 2. *Sedimentation properties of RNA annealable to virus DNA*[a]

Labeling period	*S* value of RNA	Annealable to virus DNA[b]
hr		%
6–8	32	2.2
	24–32	4.3
	12–24	6.1
	4–12	1.2
8–10	32	1.9
	24–32	7.2
	12–24	11.7
	4–12	0
10–12	32	2.7
	24–32	6.3
	12–24	9.2
	4–12	0.4

[a] The RNA of one 1,000-ml infected suspension culture was labeled with 10 mc of ^{32}P in phosphate-free medium for 2 hr. Total cell RNA was extracted and centrifuged (16 hr at 45,000 × *g*. Samples were collected from the bottom of the tube. Each of the four pooled RNA fractions from a particular labeling period contained an identical number of optical density units of RNA.

[b] Net percentage of total counts annealed to DNA and trapped on the filters.

DISCUSSION

These data indicate that a new species of RNA appears in herpes-infected KB cells, with a nucleotide composition similar to that in the virus DNA. These findings are in keeping with studies on cells infected with vaccinia virus (1, 21) and adenovirus (17), suggesting that genetic information is transcribed from the DNA genome of these mammalian viruses to an intermediate RNA prior to transltiaon into polypeptide synthesis. Experiments with actinomycin D, an inhibitor of DNA-dependent RNA synthesis, and 5-IUDR, an inhibitor of the normal formation of DNA, suggest that this production of RNA is the first virus-directed synthetic event after infection of KB cells with herpes simplex. This interpretation is dependent on the following assumptions: that these two inhibitors act rapidly after addition to the cells, that the inhibited step in synthesis is arrested rather than simply retarded, and that virus-specific RNA and DNA are relatively stable once they are synthesized. The resu ts of direct measurement of virus-specific RNA synthesis in this study appear to be sound, approximating closely the results of experiments with actinomycin D. The thesis is offered, therefore, that the inhibitor studies are indicative of the true sequence of nucleic acid synthesis in herpes infection.

Within the period of time extending from 6 to 14 hr after virus inoculation, the sedimentation patterns, and presumably the size of the RNA molecules annealable to virus DNA, remained remarkably constant. This implies that, in the time spans examined, no alteration from synthesis of "early" mRNA to "late" mRNA was demonstrated. Previous work with vaccinia demonstrated that significant alterations in the sedimentation properties of pulse-labeled cytoplasmic RNA occur during the course of infection

of cells with this virus (1). The nature of the RNA molecules made during T2 infection of *Escherichia coli* has been evaluated by testing the capacity of RNA made early during the course of bacteriophage replication to compete with RNA synthesized late in the cycle for hybridization to T2 DNA (7); according to this test, RNA made 15 to 19 min after infection is different from the virus-specific RNA synthesized during the first 6.5 min in the cycle of bacteriophage replication. Experiments recorded in this paper need to be extended to examination of virus-directed RNA produced in the first few hours after infection, to define precisely whether the type of mRNA synthesized during infection with herpesvirus is altered as the course of the virus multiplication cycle progresses. This work demonstrates that rapid inhibition of all cellular RNA synthesis occurs after herpes infection of KB cells. Studies with polio and vaccinia viruses under conditions that prevent virus nucleic acid production have shown that inhibition of cellular protein synthesis by these viruses is not dependent on replication of the genome of the virus (8, 20). In the case of poliovirus infection, there is good evidence that cell RNA synthesis is inhibited by a component of the input virus (8). The results of this study are compatible with the possibility that a constituent of the infecting virus is responsible for the deterioration of cell RNA production after inoculation with herpes simplex. On the other hand, the processes of virus synthesis and cell RNA inhibition overlap. Definition of the specific cause of decay in cell RNA synthesis in herpes-infected cells will, therefore, require studies which separate virus adsorption and uncoating from virus synthesis. Separation of these events may be possible by infecting the cells with virus inactivated by nitrogen mustard or ultraviolet light.

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