Thymidine Kinase Activity and DNA Synthesis in Cells Infected with the Parvovirus, H-1[1] (34974)

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Enhancement of thymidine kinase activity has been reported to occur in cells infected with many DNA-containing viruses, such as poxvirus (1, 2), herpesvirus (3), pseudorabies virus (4), papovavirus (5, 6) and adenovirus (6, 7). In contrast, Cocuzza (8) noted that thymidine kinase activity decreased in rat embryo cells after H-1 virus infection. H-1 is a member of the conditionally defective parvovirus group (9) which contains single-stranded DNA (10, 11).

Since the enzyme activity described in rat embryo cells after infection with H-1 appeared to be exceptional, we have examined the effect of H-1 infection on the activity of thymidine kinase in two cell lines, SV-40-transformed newborn human kidney (NB), and Salk "monkey heart" (SMH). In addition, the rate of overall DNA synthesis during infection was studied.

Materials and Methods. Cells and virus. The method of cultivation of NB cells (12, 13) and production of H-1 stock virus in NB cells have been described elsewhere (13). SMH cell cultures were prepared as reported previously (14). The H-1 virus employed in these experiments was originally isolated from human tumor tissue (15), and was plaque-purified in SMH cells (14).

Infection of cells. Semiconfluent monolayer NB or SMH cell cultures, containing 1–2 $\times$ 10^6 cells in a 60-mm petri dish were washed twice with Hanks' balanced salt solution, and 0.5 m H-1 virus suspension was added to the cell layer. The multiplicity of infection (PFU adsorbed per cell) was approximately 15–25. After adsorption for 1 hr at 37°, the cells were washed three times, covered with 3 ml of medium consisting of 5% fetal calf serum, and incubated at 37° in a humidified 5% CO_2–air mixture. Control cultures were treated under the same conditions, but no virus was added. At stated times, 2–4 cultures were collected for cell counts (16), or for preparation of the enzyme extract.

In several experiments, in order to eliminate the possible presence of infective SV40 in the NB cell cultures or the virus stock, SV40 neutralizing antiserum, at a final dilution of 1:80, was mixed with H-1 stock virus for 1 hr before infection of cells, and was also added to the culture medium. This procedure did not appear to affect the experimental results.

Determination of the rate of DNA synthesis. NB cells grown on 22 $\times$ 22-mm cover slips placed in 35-mm petri dishes were infected with H-1 as described above. At various times, ^{3}H-thymidine (0.5 μCi/ml, 1.9 Ci/mmole) was added in a total volume of 2 ml to portions of uninfected and H-1-infected cell cultures. The cultures were exposed to ^{3}H-thymidine for 1 hr at 37°, and the rate of DNA synthesis was determined from the incorporation of the label into DNA. The cells were washed twice with cold Tris-buffered saline (16) and then extracted with 0.5 *N* cold perchloric acid for 30 min at 4°. The acid-insoluble material on the coverslips was then washed with absolute ethanol, dried, and counted in 1 ml NCS solubilizer plus 20 toluene-based scintillation fluid. The rate of precursor incorporation into acid-soluble pools was determined as described previously (7).

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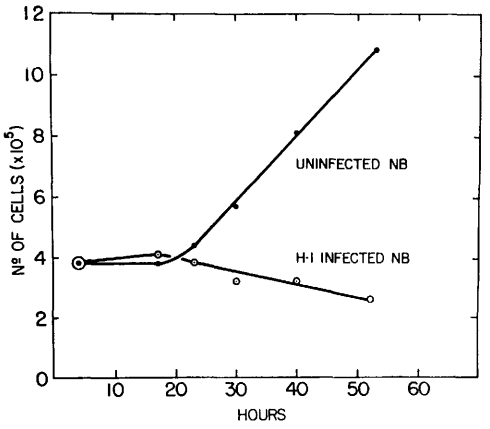

FIG. 1. Effect of H-1 virus infection on the growth of NB cells.

Autoradiography. Coverslip cultures of uninfected and H-1 infected NB cells were exposed for 1 hr at ^{3}H-thymidine (0.5 μCi/ml, 13.4 Ci/mmole) at different times. The cells were then washed, fixed, and prepared for autoradiography as described previously (17). Ilford type 4 nuclear emulsion was used; the exposure time was 17 days at 4°.

Thymidine kinase assay and protein determination. The preparation of cell extracts and the thymidine kinase assay have been described by Ledinko (7). Protein was determined by the method of Lowry (18).

Results and Discussion. The growth characteristics of H-1 in NB cell cultures have been noted (19) and were similar for these experiments. The eclipse period was approximately 10 hr. Synthesis of H-1 was complete at about 50 hr after infection, when approximately 80 PFU were produced per cell. Growth of H-1 in SMH cells was also complete by 2–3 days after infection, but a final yield of only 1–10 PFU per cell was found. Extensive cytopathic changes were observed in both cells at the time when virus maturation was complete or virtually complete.

NB cells infected with H-1 did not divide (Fig. 1). The number of cells in an infected culture declined somewhat after 1 day; at 52 hr postinfection, the cell number was 60–70% of that found at 4 hr. By 52 hr, the uninfected cell number increased by a factor of 2–3.

The rate of total DNA synthesis occurring during H-1 virus growth in NB cells was studied. The rate of incorporation per cell of ^{3}H-thymidine into the DNA of infected cells was markedly inhibited late in infection (Table I). The inhibition observed 40 hr postinfection was approximately 50%, and at 46 hr and later, it was at least 80%. The rate of thymidine incorporation also appeared to be inhibited at 16 hr postinfection. In uninfected control NB cultures, the rate of DNA synthesis generally increased during the experimental period.

The effect of H-1 infection on the rate of ^{3}H-thymidine incorporation into the acid-soluble pool of NB cells was also examined. The incorporation of the DNA precursor into acid-soluble fractions did not appear to be different in infected and uninfected cultures throughout the infectious cycle. These findings suggest that the observed inhibition of ^{3}H-thymidine incorporation into DNA was not related to a decreased uptake of thymidine into the cell.

In order to ascertain whether the decrease in the rate of thymidine incorporation into the DNA of NB cell cultures infected with H-1 was due to a reduction in the number of

TABLE I. Rate of Incorporation of ^{3}H-Thymidine into DNA of NB Cells Infected with H-1 Virus.[a]

Hours after infection	^{3}H-Thymidine incorporated into DNA: Counts per minute per 10^5 cells per hour		
	Uninfected	H-1 infected	H-1 infected /uninfected
2–3	4604	4164	0.90
15–16	9860	4281	0.43
19–20	6021	6556	1.09
21–22	8318	6966	0.84
25–26	10010	8785	0.88
39–40	9946	4910	0.49
45–46	11979	2390	0.20
63–64	9907	285	0.03
69–70	13681	282	0.02

[a] At indicated times, replicate infected and control cultures were exposed to ^{3}H-thymidine (0.5 μCi/ml, 1.9 Ci/mmole) for 1 hr, and the rate of DNA synthesis was determined.

cells synthesizing DNA, or in the rate of DNA synthesis per cell, the number of nuclei labeled with ^{3}H-thymidine was measured by autoradiography (Fig. 2). The nuclei labeled during a 1-hr pulse were arbitrarily divided into heavily or lightly labeled categories; completely black nuclei were designated as heavily labeled. The total percentage of labeled cells in control cultures was 31–37% during the observation period. A small increase in the percentage of labeled cells occurred at 17–32 hr postinfection; at 23–24 hr, approximately 50% of cells synthesized DNA. During the period from 17–32 hr postinfection, the number of lightly labeled nuclei was considerably higher than that found in control cultures. Moreover, at 31–32 hr, the number of heavily labeled nuclei was approximately 60% of that observed in uninfected cultures. These observations indicate that the decrease in the incorporation rate of thymidine was due to a decrease in the rate of DNA synthesis per cell.

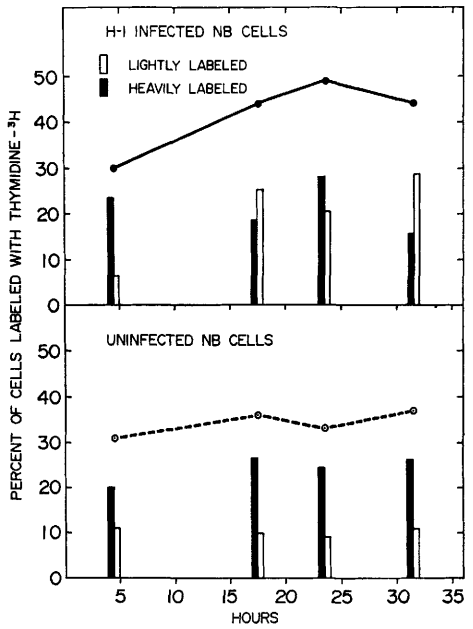

FIG. 2. Percentage of cells synthesizing DNA in uninfected and H-1-infected NB cell cultures as determined by autoradiography. Heavily labeled cells are defined as cells containing such a high concentration of grains that the nucleus appeared totally black. Lightly labeled cells contained fewer grains, so that the nucleus appeared gray. The percentage of each was determined by counting about 2000 cells. The curves represent the total percent of labeled nuclei.

TABLE II. Thymidine Kinase Activity of H-1-Infected NB Cells.[a]

Exp. no.	Hours after infection	Thymidine kinase activity: Uninfected	Thymidine kinase activity: H-1 infected	Ratio thymidine kinase activity infected to uninfected cell extract
1	2	1.35	0.99	0.73
	16	1.17	1.09	0.93
	20	1.14	1.72	1.50
	24	0.99	1.48	1.50
	44	1.25	2.26	1.81
	47	1.30	1.56	1.20
	63	2.89	1.35	0.47
	72	2.52	1.09	0.43
2	4	1.69	2.24	1.33
	16	2.44	3.54	1.45
	24	1.33	3.43	2.59
	48	3.07	4.24	1.38
	72	2.34	0.26	0.11
3	2	0.96	1.17	1.22
	24	1.12	1.85	1.65
	48	1.98	3.25	1.64
	56	2.21	2.60	1.18
	72	2.78	1.04	0.37

[a] The activity of thymidine kinase of the cell extract is expressed as picomoles of thymidine converted to thymidine phosphates per microgram of protein in 30 min.

The thymidine kinase activity of extracts prepared from the control and H-1 infected NB cells was assayed at various times (Table II). At about 20–48 hr postinfection, the activity of thymidine kinase was slightly but consistently higher (1.5- to 2.6-fold) than in the control cells. After this time, the enzyme activity of infected cells decreased considerably, while it exhibited an increase in uninfected cultures undergoing cell multiplication. By 72 hr, the level of thymidine kinase activity of infected cells was only 11–43% of the control cells.

In SMH cells infected with H-1, the increase of thymidine kinase seen in infected NB cells was not observed (Table III). Instead, there was a decline with time in en-

TABLE III. Thymidine Kinase Activity in SMH Cells Infected with H-1 Virus.[a]

Exp. no.	Hours after infection	Thymidine kinase activity: Uninfected	Thymidine kinase activity: H-1 infected	Ratio thymidine kinase activity infected to uninfected cell extract
1	6	1.56	1.95	1.25
	24	1.38	1.20	0.86
	48	2.16	2.37	1.10
	52	1.51	1.77	1.17
	57	1.82	1.27	0.70
	72	0.75	0.34	0.45
2	1	0.83	—	—
	16	0.94	1.07	1.14
	24	0.99	0.96	0.97
	29	1.14	0.55	0.48
	40	0.94	0.47	0.50
	45	1.17	0.47	0.40
	48	1.33	0.36	0.27
	53	1.38	0.21	0.15

[a] Thymidine kinase activity is expressed as picomoles of thymidine converted to thymidine phosphates per microgram of protein in 30 min.

zyme activity relative to the control levels. By 50–70 hr, the enzyme activity of infected cells was 20–50% of the control cells.

The finding of a decrease in thymidine kinase activity in H-1-infected cells at a late stage in infection agrees with the results of Cocuzza *et al.* (8). The small relatively early increase of enzyme activity in infected NB cells, however, did not occur in rat embryo cells, or in SMH cells. Since the total yield of infective H-1 virus produced per cell was considerably lower in SMH cells than in NB cells (19), the increase in thymidine kinase activity in NB cells infected with H-1 may be related to the occurrence of more active viral DNA synthesis in these cells. It has been observed that numerous "empty" particles and relatively very few complete virus particles were present in SMH cells infected with H-1 (19).

To test whether there might be an inhibitor of thymidine kinase in H-1-infected NB cells at a late stage of infection (72 hr), or an enzyme activator at an early stage (24 hr), extracts from infected cells were mixed with extracts prepared from uninfected cells. The enzyme activity of the mixtures was approximately the value expected from the sum of the activities of the individual extracts in the mixture. No enzyme inhibitor or activator was thus detected.

Puromycin, an inhibitor of protein synthesis (7), was employed to learn whether the decrease in thymidine kinase activity in NB cells infected with H-1 depended on new protein synthesis (Table IV). The presence of puromycin ($8 \times 10^{-5} M$) from 14–40 hr postinfection apparently prevented the decrease

TABLE IV. Effect of Puromycin and Actinomycin D on Thymidine Kinase Activity of NB Cells Infected by H-1 Virus.

Hours after infection	Ratio, thymidine kinase of H-1 infected:thymidine kinase of uninfected control: Without inhibitor	Puromycin ($8 \times 10^{-5} M$/ml) added at hr:[a] 4	14	48	Actinomycin (5 μg/ml) added at hr:[b] 4	14
4	1.056	—	—	—	—	—
14	1.126	1.272[c]	—	—	1.058[c]	—
24	1.074	—	1.351[c]	—	—	—
40	0.743	—	1.513[c]	—	—	1.134[c]
48	0.454	—	—	—	1.365[c]	—
62	0.230	—	—	0.557[c]	—	0.844[c]

[a] The amount of puromycin added to replicate infected and control cultures was sufficient to inhibit over 90% of the protein synthesis (7).

[b] Samples were collected at the indicated hour shown in the first column.

[c] The amount of actinomycin D added was sufficient to inhibit 98% of RNA synthesis (7).

in enzyme activity obtained at 40 hr. Puromycin given at 48–62 hr postinfection arrested the additional decrease in activity which occurred during this time.

To determine whether DNA-dependent RNA synthesis was required for the decline of the thymidine kinase activity found in H-1-infected NB cells, actinomycin D (5 μg per ml) was added to cultures at 4 or at 14 hr postinfection (Table IV). The addition of actinomycin D at 4 hr prevented the usual decrease of enzyme activity found at 48 hr. When the drug was added at 14 hr, again the decreases noted at 40 or 62 hr were not observed. These findings suggest that the decrease in thymidine kinase activity found after H-1 infection depended on DNA-dependent RNA as well as *de novo* protein synthesis. The observed decrease appears to be related to virus-induced protein synthesis and may have resulted from an inhibition of enzyme synthesis, enzyme stabilization, or enzyme activation.

Summary. Infection of SV40-transformed newborn human kidney (NB) cell cultures with H-1 virus resulted in a marked drop in the rate of incorporation of ^{3}H-thymidine into DNA relatively late in infection. Autoradiographic measurements revealed that the inhibition of total DNA synthesis was due to a decrease in the capacity of an infected cell to synthesize DNA. Thymidine kinase activity increased 1.5- to 2.6-fold during a period of virus maturation (20–48 hr), but, later in infection, it decreased to 11–43% of the control cell activity. A similar decline in enzyme activity was also observed in "Salk monkey heart" (SMH) cells late after H-1 infection. Extracts prepared from NB cells infected for different times had no effect on the enzyme activity from uninfected cells. The decrease in thymidine kinase activity found after H-1 infection relative to control cell activity was apparently dependent on *de novo* protein and DNA-dependent RNA synthesis.

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