Effect of H-1 Virus Infection on RNA Synthesis In NB Cells[1] (35100)

CAROLINE K. Y. FONG, HELENE W. TOOLAN, AND M. SUZANNE HOPKINS
Putnam Memorial Hospital Institute for Medical Research, Bennington, Vermont 05201

H-1 virus, which was originally isolated from human tumors and embryos, is particularly charcterized by the production of a mongoloid-like deformity in newborn hamsters (1). It is one of the parvoviruses, a group of small agents ranging from 18 to 25 mμ (1), containing single-stranded DNA (2, 3).

In a previous study (4) it was demonstrated that H-1, in contrast to most DNA viruses, causes a decrease in thymidine kinase activity and in the incorporation of thymidine into DNA in infected cells. The present study describes the effect of H-1 on the RNA synthesis of such cells.

Materials and Methods. Cells and Virus. A cell line (NB) originally derived from SV40 transformed newborn human kidney cells and obtained through the courtesy of Dr. John Enders, Children's Hospital, Boston, was used in this study. The origin of this cell line and the method of preparation has been described (5). The H-1 virus employed was plaque-purified in Salk "monkey heart" cells (6) and propagated in NB cells.

Infection of Cells. Monolayer cultures of NB cells were infected with H-1 at an input multiplicity of approximately 10 to 15. After 1 hr of virus adsorption, the cells were washed two times with Hanks' balanced salt solution, and fed with Eagle's minimum essential medium supplemented with 5% fetal calf serum. Uninfected cultures were treated in a similar manner but no virus was added.

Rate of RNA Synthesis. For study of rate of RNA synthesis, replicate cultures from uninfected and virus-infected NB cells were pulse-labeled with tritiated uridine (0.5 μCi/ml, 28 Ci/mmole) for 1 hr at 37 at selected intervals. The rate of incorporation of uridine into acid-soluble and acid-insoluble fractions was measured according to the procedures described by Ledinko (7). To determine the number of cells labeled with tritiated uridine during a 1-hr pulse, autoradiographic technique was employed (8).

RNA extraction and acrylamide gel electrophoresis. The effect of H-1 virus infection on different species of RNA synthesis was investigated. Monolayer cell cultures of uninfected and H-1 virus-infected NB cells grown in 60-mm petri dishes were labeled with tritiated uridine (15 μCi/ml, 28 Ci/mmole) for 2 hr at different times after infection. The cells were then washed with Tris-buffered saline containing 0.005 *M* 2-mercapethanol, collected in cold reticulocyte swelling buffer (9) containing the same amount of 2-mercaptoethanol, and stored at −70°. Cytoplasmic RNA was isolated as described by Peacock and Dingman (10). Different species of RNA were separated and analyzed by electrophoresis in 2% acrylamide gels containing 0.5% agarose. The methods for preparation of gels and electrophoresis were used according to Peacock and Dingman (11, 12).

Results. Cytopathic changes in H-1 infected NB cells. At different times after infection, cultures were fixed with Zenker acetic acid fixative, stained with hematoxylin and eosin, and examined under a light microscope. Cytopathic changes in the H-1-infected cells were first detected at 24 hr after infection and were evidenced initially by chromatin condensation. Nuclear vacuolation, chromatin depletion, and disappearance of nucleoli were noted at late stages of infection, approximately 48 hr postinfection.

[1] This investigation was supported by National Cancer Inst., NIH, U.S. Public Health Service Grant CA-07826-05, and by a generous gift from the Given Foundation.

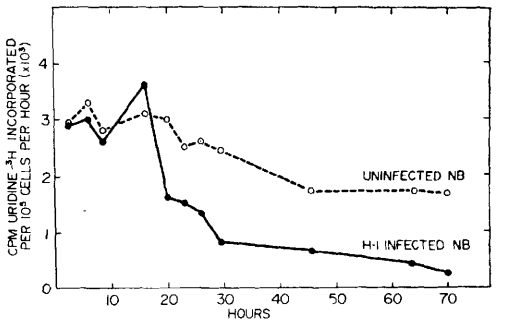

FIG. 1. Rate of incorporation of tritiated uridine into RNA in control and H-1 infected NB cells.

Rate of RNA Synthesis. The rate of overall RNA synthesis of uninfected and H-1-infected NB cells is shown in Fig. 1. In uninfected cells, the rate of incorporation of uridine into RNA decreased gradually after approximately 20 hr. The rate at 45–70 hr was approximately 50% of the rate at 3 hr. In H-1-infected NB cells, there may have been an initial small rise at 16 hr followed by a relatively rapid decrease in the rate of uptake of uridine. The rate at 20 hr was approximately 50% and the rate at 70 hr only 7% of the rate at 3 hr. The rate of uridine uptake into RNA in H-1 infected cells was lower than that in the uninfected controls 20 to 70 hr postinfection (Fig. 1). At 20 hr, the rate of precursor uptake in infected cells was about 50% of that in the control cells; it decreased sharply thereafter. By 70 hr, the rate of uptake of uridine into RNA was only 10% of that in the control.

The incorporation of radioactive precursor into acid-soluble pools was also measured. As shown in Table I, the uptake of uridine into acid-soluble fractions in H-1 infected cells, when compared with the uninfected controls, did not show any appreciable difference until approximately 63 hr after infection. The rate of incorporation of precursor into the acid-soluble pool in H-1 infected cells was only 40% of the control, 63–70 hr postinfection. Thus it appears that the decrease in the rate of RNA synthesis at a late stage of infection is partially due to the decrease in the rate of uptake of uridine into infected cells.

Autoradiographic studies. The rapid decrease in the uptake of uridine into RNA following H-1 infection (Fig. 1) was further investigated using autoradiographic techniques. Cultures were labeled for 1 hr with tritiated uridine as described in Materials and Methods. Most of the grains were located in the nuclei of both uninfected and H-1-infected cells. In addition to the nuclear labeling, a few grains were also seen in some cells in the cytoplasm. A small number of cells were found where the grains were more concentrated in the nucleoli. However, in the majority of the cells, the grains were distributed evenly in the nucleoplasm and nucleoli. The percentage of cells labeled with radioactive precursor in the uninfected and H-1-infected NB cells were determined by counting 1000 cells/each sample tested. The results are shown in Fig. 2. Over 93% of

TABLE I. Uptake of Tritiated Uridine into the Acid-Soluble Fraction of NB Cells Following H-1 Virus Infection.

After infection (hr)	Uptake of tritiated uridine into acid-soluble fractions (cpm/10^5 cells)		Ratio (infected /control)
	NB control	H-1 infected	
2–3	140	128	0.91
15–16	163	192	1.18
19–20	205	208	1.01
21–22	162	177	1.09
25–26	171	126	0.74
39–40	138	114	0.83
45–46	113	99	0.88
63–64	114	44	0.39
69–70	96	38	0.40

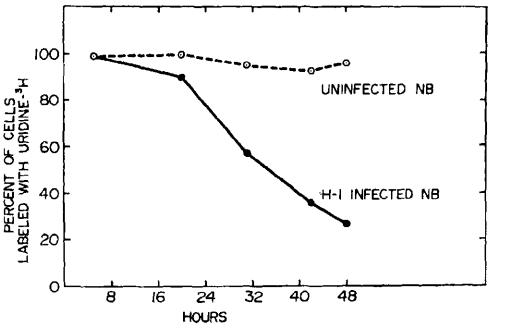

FIG. 2. Percentage of cells synthesizing RNA in uninfected and H-1-infected NB cells as determined by autoradiography. Approximately 1000 cells were counted for each sample.

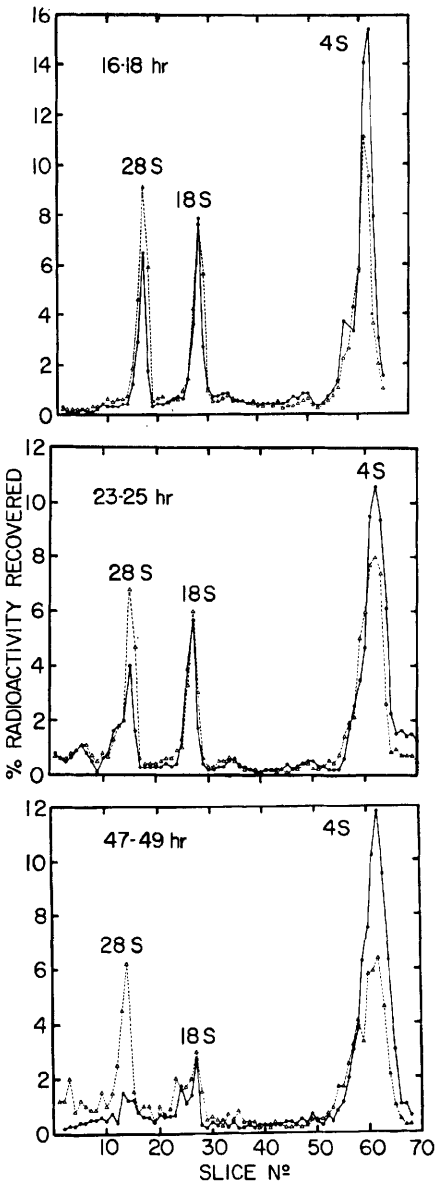

FIG. 3. Labeling patterns obtained by electrophoresis in 2% acrylamide—0.5% agarose for cytoplasmic RNA isolated from uninfected and H-1-infected NB cells labeled for 2 hr with tritiated uridine at indicated times after infection: (●—) H-1-infected; (△- - -) control.

uninfected cells were labeled in 1 hr with tritiated uridine during 4- to 48-hr observation. There was a gradual decrease of cells labeled with uridine in H-1-infected cells after 20 hr postinfection. Only 57% of the cells were labeled at 30–31 hr, and 27% at 47–48 hr postinfection. Therefore, the decrease of RNA synthesis in H-1-infected cells seems to be due to the decrease in number of cells which are capable of synthesizing RNA.

RNA Analysis. In order to study what type of RNA molecules are selectively inhibited or synthesized in H-1-infected cells, RNAs were isolated from the cytoplasm of uninfected and H-1-infected NB cells which had been labeled with tritiated uridine for 2 hr at different times after infection. RNA preparations were analyzed by agarose–acrylamide gel electrophoresis. The results obtained for cytoplasmic RNA from uninfected and H-1 infected cells are shown in Fig. 3. Following H-1 infection, there was a decrease in 28S ribosomal RNA. At 16–18 hr, the 28S RNA of infected cells was about 70% of the uninfected control cells. The difference was more pronounced at 47–49 hr at which time the 28 RNA produced in the infected cells was only 25% of the control. Low molecular weight 4S RNA was selectively stimulated in the infected cells. There was 1.3- to 1.8-fold increase in the percentage of 4S RNA in H-1-infected cells over that of the control 16 to 49 hr postinfection. The percentage of 18S ribosomal RNA showed no significant difference in the infected and the control cells.

Discussion. The selective increase observed in low molecular weight 4S RNA in H-1 virus-infected cells is interesting. Similar observations have also been described for tissues of VEE (Venezuelan equine encephalitis) virus-infected mice (13), and a 5S RNA was augmented in adenovirus-infected KB cells (14).

The mechanism of the H-1 viral inhibitory effect on the RNA synthesis is not understood. Studies of RNAs in poliovirus infections have demonstrated that poliovirus interferes with host messenger RNA; thus protein and RNA synthesis of host cells are suppressed (15). Kehoe and Lust (13) have reported that RNA polymerase activity decreased in murine liver at an early stage of VEE virus infection. These investigators felt that the decrease in RNA synthesis in VEE virus infection was due to the decrease in RNA polymerase activity. Levine and Ginsberg (16) demonstrated that the fiber an-

tigen of adenovirus type 5 inhibited the DNA-dependent RNA polymerase from KB cells starting about 10 hr after infection. Whether the inhibitory effect of H-1 virus on RNA synthesis is also due to the inhibition of enzymes for RNA synthesis remains to be determined.

Summary. The overall rate of incorporation of tritiated uridine into RNA in NB cells rapidly decreased after H-1 virus infection. This decrease in rate of RNA synthesis was apparently due to a decrease in number of cells synthesizing RNA. The 28S ribosomal RNA was selectively inhibited while the low molecular weight 4S RNA was selectively stimulated.

1. Toolan, H. W., Int. Rev. Exp. Pathol. **6**, 135 (1968).

2. Usategui-Gomez, M., Toolan, H. W., Ledinko, N., Al-Lami, F., and Hopkins, M. S., Virology **39**, 617 (1969).

3. McGeoch, D. J., Crawford, L. V., and Follett, E. A. C., J. Gen. Virol. **6**, 33 (1970).

4. Fong, C. K. Y., Ledinko, N., and Toolan, H. W., Proc. Soc. Exp. Biol. Med. **134**, 1199 (1970).

5. Ledinko, N., Hopkins, S., and Toolan, H. W., J. Gen. Virol. **5**, 19 (1969).

6. Ledinko, N., Nature (London) **214**, 1346 (1967).

7. Ledinko, N., Cancer Res. **27**, 1459 (1967).

8. Fong, C. K. Y., Hsiung, G. D., and Bensch, K. G., Virology **35**, 311 (1968).

9. Penman, S., J. Mol. Biol. **17**, 117 (1966).

10. Peacock, A. C., and Dingman, C. W., Biochemistry **6**, 1818 (1967).

11. Peacock, A. C., and Dingman, C. W., Biochemistry **7**, 668 (1967).

12. Dingman, C. W., and Peacock, A. C., Biochemistry **7**, 659 (1968).

13. Kehoe, J. M., and Lust, G. J., J. Infec. Dis. **120**, 411 (1969).

14. Ohe, K., Weissman, S. M., and Cooke, N. R., J. Biol. Chem. **244**, 5320 (1969).

15. Willems, M., and Penman, S., Virology **30**, 355 (1966).

16. Levine, A. J., and Ginsberg, H. S., J. Virol. **2**, 430 (1968).

Received June 8, 1970. P.S.E.B.M., 1970, Vol. 135.