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MACROMOLECULAR SYNTHESIS IN CELLS INFECTED WITH HERPES SIMPLEX VIRUS (*)

by

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A REPORT from this laboratory[1] has shown that in *HEp*-2 cells infected with herpes simplex virus the incorporation of nucleosides into cellular DNA progressively decreases with time after infection. The purpose of this communication is to report on two other aspects of macromolecular synthesis, namely RNA and protein, in infected *HEp*-2 cells.

RNA synthesis. *HEp*-2 cells were infected with strain *MP* of herpes simplex virus and suspended in a maintenance medium according to procedures described elsewhere[2]. Immediately afterwards and at intervals of 30 min-2 h, suspensions consisting of 10^7 infected or of uninfected cells received sufficient tritiated uridine (Nuclear-Chicago, Chicago, Illinois, spec. act. 0.7 c/mmole) to yield 10 micro c./ml. Unlabelled thymidine and deoxycytidine in molar concentrations 10 times greater than that of tritiated uridine were also added. After incubation for 30 min at 34° C, the cells were washed in 0.01 M sodium acetate buffer (*p*H 5.1) containing 10^{-4} M uridine. The RNA was extracted according to a modification[3] of the procedure of Scherrer and Darnell[4], and its absorbancy was read at 260 m The tritiated uridine was measured in a Nuclear-Chicago liquid scintillation spectrometer. For each pulse labelling, the ratio of:

$$\frac{\text{counts/min/unit of absorbancy at 260 m micro (infected cells)}}{\text{counts/min/unit of absorbancy at 260 m micro (uninfected cells)}}$$

was determined and plotted at the mid points of the pulse interval as shown in Fig. 1.

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The data recorded in Fig. 1 show that between 1 and 3 h after infection the incorporation of tritiated uridine into RNA is reduced by a half relative to that of uninfected cells. Between 3 and 6 h after infection, the rate of uptake of tritiated uridine remains relatively level; afterwards, it declines again to approximately 20 per cent of that of uninfected cells. Reduction in RNA synthesis may be a characteristic feature of productive infection of mammalian cells with herpes simplex virus; a similar reduction was observed in canine cells infected with a mutant of the *MP* strain of herpes simplex virus[5].

Protein synthesis. *HEp-2* cells infected at a multiplicity of 10 plaque-forming units/cell were suspended so as to yield 1.2×10^5 cells/ml. of a medium containing a balanced salt solution, 0.3 per cent methyl cellulose (15 c.p.s. U.S.P. grade, Fisher Scientific Co., Fair Lawn, New York), and amino-acids and vitamins at a concentration twice that recommended by Eagle[6]. Immediately afterwards and at intervals of 1-2 h, 10 ml. aliquots of infected and uninfected cell suspensions were centrifuged. The sedimented cells were suspended in a medium identical with that already described here except that the unlabelled arginine was replaced with agrimine labelled with carbon-14 (0.1 micro c./0.73 micro g/ml., New England Nuclear Corp., Boston, Massachusetts). After 1 additional hour of incubation at 34° C, the cells were sedimented and washed with medium containing excess unlabelled arginine. They were then suspended in 3 per cent sodium lauryl sulphate solution containing cold arginine and incubated for 30 min at 37° C. At that time, an aliquot was removed for protein determination by the Lowry method; the proteins in the remainder were precipitated on filter paper disks with trichloroacetic acid. The disks were washed with trichloroacetic acid, dried, and then immersed in toleune base scintillation fluid

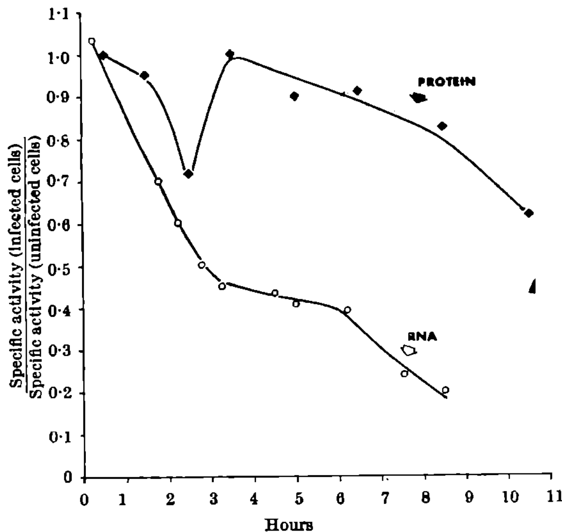

Fig. 1 Pulse labelling of RNA with tritiated uridine, and of protein with arginine labelled with carbon-14, at intervals after infection of *HEp-2* cells with herpes simplex virus. The duration of the pulse was 30 min for tributiated uridine and 60 min for arginine-^{14}C.

for measurement of carbon-14 disintegrations. For each I pulse labelling, the ratio of :

$$\frac{\text{counts/min/mg protein (infected cells)}}{\text{counts/min/mg protein (uninfected cells)}}$$

was determined and plotted at the mid point of the pulse interval as shown in Fig. 1.

Arginine labelled with carbon-14 was chosen because of the report by Tankersley[7], confirmed in this laboratory, that the amino-acid must be furnished in the medium for Herpes virus multiplication. However, experiments with labelled leucine and with media supplemented with labelled yeast hydrolysate yielded results similar to those recorded in Fig. 1. The pattern of protein synthesis in infected cells as revealed by amino-acid incorporation shows three distinct stages: During the first 3 h after infection, protein synthesis decreased to about 70 per cent of control rate. The decrease was observed in all experiments; the rate of decrease, however, varied with the multiplicity of infection. Between 3 and 6 h after infection, incorporation of amino-acid was generally stimulated. Lastly, between 6 and 10 h after infection, incorporation of amino-acid declined to approximately 60 per cent of that of uninfected cells.

The results presented in Fig. 1 reveal two distinct cycles of inhibition of macromolecular synthesis in infected cells. These cycles occur between 0 and 3 h after infection and from 6 h after infection. It is possible that the first cycle is brought about by a product specified by the virus early in infection, whereas the second cycle is caused by a sub-unit of the capsid or by some other product specified after the onset of DNA synthesis[2]. However, the results are insufficient to determine whether the two cycles are the expression of the same or different viral genes·

Inhibition of host macromolecular synthesis has been observed in animal cells infected with a number of RNA [8-10] and DNA [11,12] viruses. In relation to the data presented in this paper, two comments should be made. First, the inhibition produced by herpes simplex virus takes place much sooner after infection than that produced by vaccinia virus [11,12]. The difference may be due to the fact that vaccinia virus replicates in the cytoplasm, whereas herpes simplex replicates in the nucleus. Secondly, inhibition of host RNA and protein synthesis is not a general characteristic of all viral infections[12]. It is uncertain precisely what selective advantages occure to the virus which modifies host metabolism over the one which does not. With respect to herpes simplex, the early inhibition of host RNA synthesis

may be a perequisite for the synthesis of viral constituents. Thus, infection of canine cells, under conditions which preclude early inhibition of host RNA synthesis, results in the production of interferon only. Viral constituents but no interferon, on the other hand, are made in cells the RNA synthesis of which is inhibited very early in infection[5].

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