THE EFFECT OF HYPERTONIC CONDITIONS ON PROTEIN SYNTHESIS IN CELLS INFECTED WITH HERPES VIRUS

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1. Introduction

When HeLa cells are placed in a growth medium containing an elevated concentration of sodium chloride there is a rapid cessation of protein synthesis accompanied by a complete breakdown of polyribosomes [1]. However protein synthesis in HeLa cells infected with poliovirus, vesicular stomatitis virus or vaccinia virus is relatively resistant to hypertonic medium [2,3]. In such infected cells it is well established that there is, in normal medium, an inhibition of host protein synthesis with preferential synthesis of virus-specified proteins. Thus a parallel was drawn between the selective effect on initiation of increased sodium chloride and the suppression of host protein synthesis by these viruses.

A similar examination of simian virus 40-infected cells [4] indicated that in this case virus-infected cell protein synthesis was again more resistant to hypertonic medium than protein synthesis in uninfected cells.

In addition to these experiments in vivo cell-free protein synthesising systems have also been used to examine the mechanism of inhibition of host protein synthesis by virus. These indicated that for some virus messenger RNA species addition of sodium to the cell-free system inhibited the initiation of translation of host protein synthesis whereas it was stimulatory for the translation of the virus messenger RNA [5,6].

On the basis of these and other studies a general mechanism was proposed [6] for the inhibition of cell functions following virus infection. It was suggested that on infection the cell membrane is damaged and that this leads to an alteration in ion transport into the cell. Thus there follows a change in the concentration of monovalent ions inside the cell, this new concentration of these ions being more favourable to the translation of virus-coded messenger RNA than host-coded messenger RNA.

Herpes viruses are also known to inhibit host protein synthesis following infection. After a short period of infection such protein synthesis as occurs is predominantly of virus-specified proteins [7,8]. We have now examined the effect of a hypertonic medium on protein synthesis and polyribosome formation in HeLa cells infected with the herpes virus pseudorabies virus.

2. Methods

HeLa cells and pseudorabies virus were maintained by routine procedures as in [9]. For the analysis of polyribosomes cells were grown in 80 oz. roller bottles. When a monolayer had formed the cells were infected with 20 p.f.u./cell of pseudorabies virus. Control cells were mock-infected. After 1 h at 37°C the medium was replaced with fresh normal medium to remove unabsorbed virus. NaCl was added to make the medium hypertonic 15 min before harvesting. The cells were scraped from the glass, homogenised in 0.01 M NaCl, 0.003 M $MgCl_2$, 0.01 M Tris–HCl, pH 7.4, and the polysomes spread on a 15–30% (w/v) sucrose gradient by centrifugation at 27 000 rev/min for 110 min in a Beckman SW27 rotor. The distribution of polyribosomes was monitored by measuring A_{260} of the gradient.

To analyse protein synthesis cells were grown on coverslips in 50 mm Petri dishes till confluent and

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again infected with virus at 20 p.f.u./cell or mock-infected. After 1h at 37°C the medium was replaced as before. NaCl was added to make the medium hypertonic 30 min before harvesting. [^{3}H]Methionine, 15 μCi, was added to each dish 15 min before harvesting. Coverslips were removed at the appropriate time, washed three times in ice-cold 5% trichloroacetic acid, once in ethanol and their radioactivity measured by liquid scintillation counting. The normal medium in which these HeLa cells are grown is 110 mM in NaCl.

3. Results and discussion

3.1. *Effect of hypertonic medium on polyribosomes*

We find in accordance with previous reports that in HeLa cells grown in hypertonic medium the number of polyribosomes declines. We have compared this reduction in mock-infected cells with that in infected cells after 6 h infection with pseudorabies virus. At this time protein synthesis is predominantly of virus proteins and the number of polyribosomes is already less than that of uninfected cells. One such experiment is illustrated in fig.1. In this we have expressed the number of polyribosomes as the ratio of material sedimenting in a sucrose gradient in the polyribosome region to material sedimenting in the monoribosome region. The amounts are calculated by measuring the area under the curve obtained from a chart recording of the distribution of A_{260} material across the gradient.

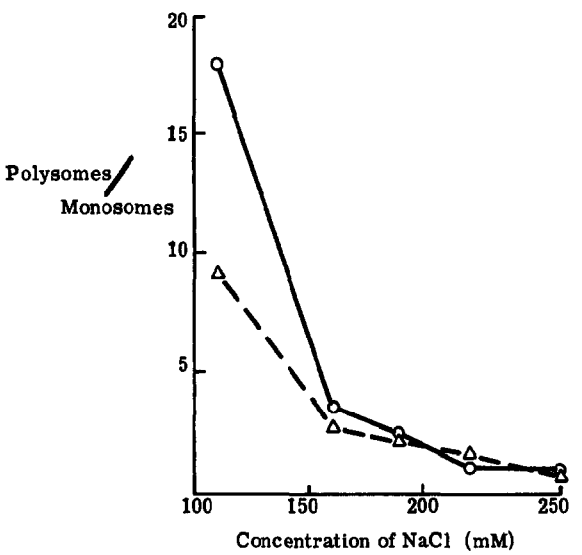

Fig.1. HeLa cells grown in 80 oz. roller bottles were infected with pseudorabies virus (△) or mock-infected (○). The medium was adjusted with NaCl to the appropriate concentration 6 h later and after a further 15 min the cells were scraped from the glass. Polyribosomes were spread on a sucrose gradient and the ratio of polyribosomes to monoribosomes estimated from the distribution of A_{260} material on the gradient. The concentration of NaCl in normal medium was 110 mM.

In fig.1 we show the change in the ratio for cells with differing concentrations of NaCl in the growth medium. It is clear that the polyribosomes in infected cells are at least as sensitive to the hypertonic treatment as those of uninfected cells.

3.2. *Effect of hypertonic medium on protein synthesis*

[^{3}H]Methionine was added to cells at various times after infection or mock infection and its incorporation into acid-insoluble material after 15 min was determined. Table 1 shows the total incorporation per coverslip at 2 h and at 6 h after infection or mock infection in the presence of normal medium.

The incorporation of [^{3}H]methionine in the presence of varying concentrations of NaCl was now examined. Typical results of such experiments at 2 h and 6 h after infection or mock infection are shown in fig.2. For this figure we have set the incorporation in normal medium to 100% for each set of the infected or mock-infected data thus allowing readier comparison of the relative sensitivities to hypertonic medium. It is clear that by this measure also protein synthesis in infected cells is at least as sensitive to the hypertonic treatment as is protein synthesis in normal cells. The increased sensitivity in infected cells at low levels of added NaCl may reflect some virus effect on the cell membrane allowing readier equilibration of extracellular and intracellular monovalent ion concentrations. This effect must be small since at higher levels of salt there is clearly no difference between infected and uninfected cells. For the systems described in the introduction to this paper the difference between infected

Table 1
Incorporation of [^{3}H]methionine into normal and infected HeLa cells

Time after infection or mock infection	[^{3}H]Methionine (cpm/coverslip)	
	Control	Infected
2 h	4423	4594
6 h	4644	2190

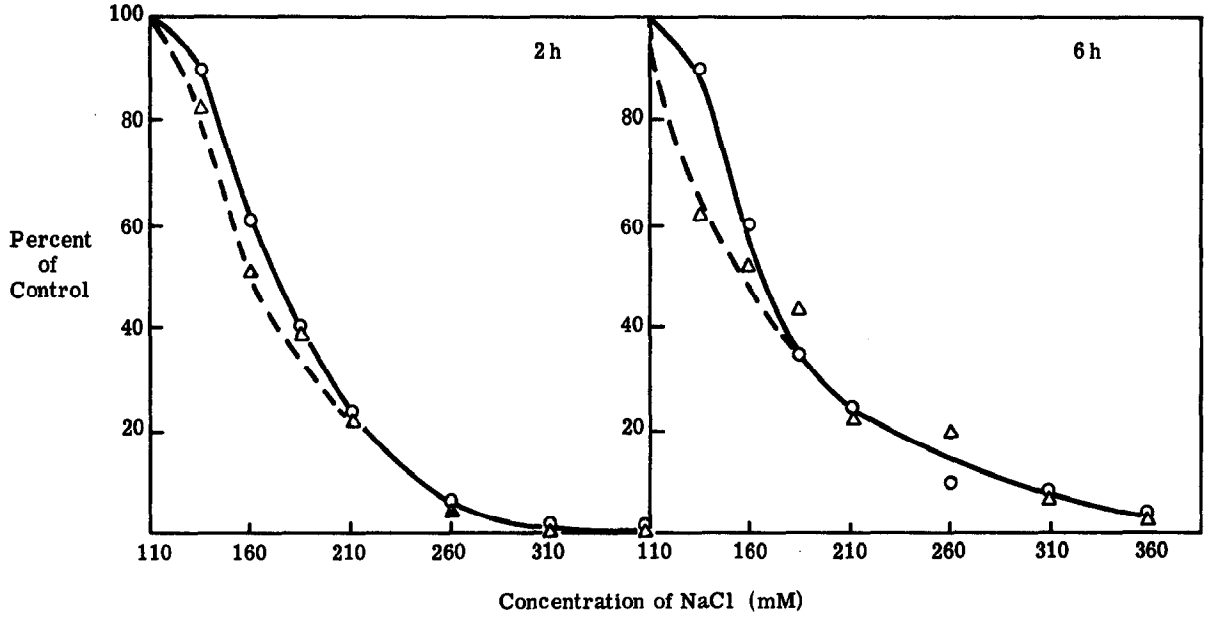

Fig.2. HeLa cells were grown on glass coverslips in 50 mm Petri dishes and infected with pseudorabies virus (△) or mock-infected (○). The medium was adjusted from the normal 110 mM NaCl to the appropriate concentration 30 min before harvesting. [^{3}H]Methionine, 15 μCi, was added to the growth medium 15 min before harvesting. Coverslips were removed from the dishes, washed three times with 5% trichloroacetic acid, once in ethanol and the radioactivity measured by scintillation counting. The incorporation into acid-insoluble material in normal medium is expressed as 100%.

and normal cells was observed in the region of 210–260 mM NaCl.

It is evident from our studies that herpes virus provides an important exception to the hitherto described common effect of virus infections in conferring on protein synthesis increased resistance to hypertonic medium.

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