Effects of Adsorption of u.v.-inactivated Parainfluenza (Sendai) Virus on the Incorporation of Amino Acids in Animal Host Cells

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SUMMARY

Following the adsorption of u.v.-inactivated Sendai virus at high multiplicity on to HeLa-S3 or L-929 cells there was a transient inhibition of uptake of amino acids into the cells, but no inhibition of protein synthesis. The uptake of amino acids into virus-treated cells was maximally inhibited at the end of the adsorption period, but within a few hours returned to control level.

The changes in the uptake of amino acids occurred concomitantly with enhanced potassium uptake and with inhibition of DNA synthesis, but were of longer duration.

The interaction between virus and host cells begins with the adsorption of the virus particles to the surface of the cells. The mode of entry of paramyxoviruses into cells is believed to involve fusion of the virus envelope with the cellular membrane (Meiselman, Kohn & Danon, 1967; Morgan & Howe, 1968; Poste & Allison, 1971), and this event is followed by the release of the virus nucleoprotein into the cytoplasm of the host cell.

The effects of virus infection of animal cells on the macromolecular syntheses in these cells have been reviewed by Martin & Kerr (1968) and Kohn & Fuchs (1973). Inhibition of macromolecular syntheses was observed not only in host cells infected by paramyxoviruses (Ensminger & Tamm, 1970), but also in cells that had adsorbed non-infective u.v.-irradiated viruses (Joklik & Becker, 1964; Huang & Wagner, 1965; Yaoi, Mitsui & Amano 1970; Yamashita, Moritsugu & Shimojo, 1971; Wertz & Youngner, 1972).

The initial effects of adsorption of u.v.-irradiated Sendai virus to HeLa cells on the course of DNA synthesis were reported by Kohn & Fuchs (1973). A transient increase in the incorporation of [^3H]-thymidine into cellular DNA was found to occur 30 to 90 min after virus adsorption, concurrent with an inhibition of incorporation of [^{32}P] into cellular DNA. This seemingly paradoxical result was shown to be due to an actual inhibition of DNA synthesis. The augmented incorporation of [^3H]-thymidine occurred because the endogenous supply of thymidine 5'-phosphate (TMP) was blocked in the methylation step from uridine 5'-phosphate (dUMP); thus during the decreased synthesis of cellular DNA only the exogenous supply of labelled thymidine was utilized by the cells, as was indeed demonstrated by the increase in sp. act. of the DNA (Fuchs & Kohn, 1973). An additional change, detectable in the membrane of cells absorbing u.v.-irradiated Sendai virus, was a transient augmented active transport of [^{42}K] into these cells (Fuchs & Giberan, 1973).

In this communication we describe the effects of adsorption of u.v.-inactivated Sendai virus on the incorporation of radioactive amino acids and protein synthesis in 'infected' host cells.

Cell lines HeLa-S3 and L-929 were grown in Eagle's medium in monolayers. Sendai virus was harvested from the allantoic fluid of 9-day-old chick embryos, and purified and concentrated by differential centrifuging (Fuchs & Kohn, 1971). The final suspension of virus in phosphate-buffered saline (PBS) was inactivated by u.v. irradiation for 4 min (870 ergs/

Table 1. *The effect of adsorption of u.v.-Sendai virus on incorporation of amino acids into proteins in random and synchronized cell cultures*

Expt.		...		TCA precipitates of			
Radioactive precursor				Random cultures L-929		Synchronized cultures HeLa	
	Sp. act. (mCi/ m-mol)	μ Ci/ plate	Cells treated with	ct/min/ 10^6 cells	% inhibition	ct/min/ 10^6 cells	% inhibition
$[^3\text{H}]$ -Leucine	250	3	PBS	5487	51	2155	58
			Virus	2690		915	
$[^3\text{H}]$ -Valine	10000	5	PBS	7170	43	ND	—
			Virus	4064		ND	
$[^{14}\text{C}]$ -Tryptophane	23	5	PBS	ND	—	18050	67
			Virus	ND		6070	
$[^3\text{H}]$ -Thymidine	15.6	10	PBS	—	—	11638	(3.8)*
			Virus	—		44480	

In Expt. 1 the incorporation of $[^3\text{H}]$ -leucine and of $[^3\text{H}]$ -valine was measured in random cultures of L-929 cells treated with u.v.-inactivated Sendai virus. In Expt. 2 synchronized He-La-S3 cells were used during their S-phase (3.5 h post-synchronization). Following adsorption of virus they were labelled with $[^3\text{H}]$ -leucine and $[^{14}\text{C}]$ tryptophane.

* In virus-treated cells thymidine incorporation into the cellular DNA increased 3.8-fold.

s/cm²) and used at a concentration of 640 H.A.U. per 10^6 cells, equivalent to about 500 to 1000 virus particles per cell. The irradiation by the u.v. at the dose used did not change the haemagglutinating activity of the virus suspension, though it completely inactivated the infectivity of the virus.

The effect of adsorption of virus on the incorporation of amino acids into cellular proteins was determined as follows: to monolayers of about 10^6 cells in a dish was added 640 H.A.U. of u.v.-inactivated Sendai virus in 0.2 ml PBS. The cells were incubated for 10 to 15 min. at 37° in a CO₂ incubator; pre-warmed medium containing radioactive amino acids (tryptophane, leucine or valine from Radiochemical Centre, Amersham, Buckinghamshire) was added into the cultures for 30 min. The Eagle's medium used for labelling was made up so that the concentration of the amino acid used as the radioactive label was 1/5 of that prescribed. In control cultures PBS was added instead of virus. At the end of the labelling period, the cells were washed several times with PBS containing an excess of unlabelled amino acids, so that negligible radioactivity remained in the supernatant medium; the cells were then dissolved in 1 % SDS and the acid-precipitable fraction contained in the cells was assayed for radioactivity by liquid scintillation counting.

As a result of adsorption of u.v.-inactivated Sendai virus to HeLa or L-929 cells there was a transient inhibition of incorporation of radioactive amino acids into proteins of the infected cells. This inhibition was evident in randomly growing cells, as well as in synchronized cultures (Table 1). The initial extent of the inhibition was 40 to 70 % as compared to control cells. Maximal inhibition was observed in the first period measured (i.e. during the 30 min from the end of the 10 min adsorption period).

When 30 min pulse labelling was repeated on culture replicas at intervals between $\frac{1}{2}$ and 4 h post-adsorption, the inhibition of incorporation of amino acids in virus-treated cells decreased so that at 2 to 2½ h post-adsorption there was no difference in radioactive counts between the virus-treated and the control cells.

Fig. 1. Uptake of [³H]-leucine into acid soluble pools of HeLa and L-929 cells treated with u.v.-irradiated Sendai virus. Monolayers of HeLa-S₃ or L-929 cells (about 10⁶ cells per plate) were treated with 640 H.A.U. of u.v.-irradiated Sendai virus suspended in Eagle's medium containing only 0.08 mM-leucine (MEM-L) (instead of 0.4 mM). Following adsorption for 10 min, 10 μ Ci of [³H]-leucine (sp.act. 250 mCi/m-mol) were added to the cultures. At the end of time intervals of 1 to 40 min (pulse) the cells were washed with cold Eagle's medium containing an excess of 100-fold of leucine, and extracted with 0.4 M-perchloric acid (PCA). In controls the virus was replaced by MEM-L. ●—●, controls; ●---●, virus treated cells. A, HeLa cells; B, L-929 cells.

The inhibition of incorporation of amino acids into cellular proteins could be due to an inhibition in one of the steps of protein synthesis, or to a block in the entry of the amino acids through the cell's membrane into the cytoplasm. In order to measure the rate of entry, the amino acid pool extractable by 5% perchloric acid from control and virus-treated cells was measured at intervals during the first 30 to 40 min of labelling with amino acids.

Whereas the entry of thymidine into the nucleotide pool was not affected by the presence of adsorbed u.v.-Sendai virus (Fuchs & Kohn, 1971), such virus-treated cells incorporated less amino acids than the corresponding controls (Fig. 1*a, b*), the difference being more marked in HeLa cells than in L-929. Since the net increase in the uptake of amino acids is the balance between entry and loss, we also measured the kinetics of loss of [³H]-leucine from cells pre-labelled for 3 h with this amino acid. The rate of loss determined from accumulation of [³H]-leucine in the culture medium was the same in control as well as in virus-treated cells. Thus the net difference in uptake seems to be due to change in the entry.

Fig. 2. Time course of inhibition of incorporation of [³H]-leucine into acid soluble pool of HeLa-S3 cells treated with u.v.-irradiated Sendai virus. 25 h cultures of HeLa cells (about 10⁶ cells per plate) were prepared. 3 h before the beginning of the experiment the medium in the plates was changed to 2 ml of Eagle's medium depleted of leucine (final conc. 0.08 mM). At 0 time 0.1 ml of a suspension of u.v.-Sendai virus in MEM-L (640 H.A.U.) was added to all test plates, and 0.1 ml MEM-L only to control plates. Following adsorption for 15 min of the virus, the monolayers were labelled for 30 min with 10 μ Ci of [³H]-leucine (0.2 ml.) The labelling was repeated on replicas (in duplicate) of virus-treated and control cells at time intervals of $\frac{1}{2}$, 1, 2, 3 and 4 h. At the end of the pulse, the cells were washed with cold MEM containing 5 mM-leucine, extracted with 0.4 M-PCA and the radioactivity of the extract was measured by scintillation. White bars, control cells; black bars, virus-treated cells.

The inhibition of transport of amino acids into the cellular pool was most pronounced soon after the adsorption of virus (the earliest time interval that it was measured was 30 to 40 min from the addition of the virus). With the progress of time, following the adsorption of the virus, the inhibition of uptake decreased, and after a few hours there was no difference in the uptake between the control and the virus-treated cells (Fig. 2).

The inhibition of incorporation of amino acids into cellular proteins (Table 1) is explicable in the terms of inhibition of uptake into the pool, but could also be a result of a combined effect of both entry into the pool and of synthesis of proteins. It was thus desirable to determine whether protein synthesis *per se* was not involved. This was done in a cell-free system of Allen & Schweet (1962). Control, uninfected HeLa cells or cells treated with u.v.-inactivated Sendai virus (640 H.A.U./10⁶ cells for 20 min) were used. Following adsorption of virus on monolayers, the cells were trypsinized, washed several times in PBS with 10% calf serum, resuspended in 'standard reticulocyte buffer' + 1 mM-DTT and disintegrated in Dounce homogenizer. The homogenate was then fractionated into ribosomes and post-ribosomal cytoplasmic fraction (enzymes, m-RNA, t-RNA) according to Allen & Schweet (1962). The reaction mixture contained buffer, ATP, GTP, phosphoenol pyruvate (PEP), dithiothreitol (DDT), pyruvate kinase, ribosomes and post-ribosomal fraction. The reaction was started by adding 20 μ Ci of [³H]-leucine (sp. act. 250 mCi/m-mol), or 0.1 μ Ci [¹⁴C]-leucine (sp. act. 58 Ci/m-mol), and stopped after 30 min at 37 °C, by the addition of 0.1 M cold leucine. 0.1 ml samples were then deposited on filter paper discs, precipitated by TCA (10%), dried in ethanol and ether and counted in a scintillation spectrometer. We found that the extent of incorporation of [³H]-leucine or [¹⁴C]-leucine into proteins by ribosomes and enzymes isolated from control, or virus-treated, cells was essentially the same (Table 2).

Table 2. Cell-free protein synthesis by extracts from virus-treated HeLa cells

	Ribosomes	Soluble cytoplasmic fraction	Incorporation after 30 min (ct/min)	
			[³ H]-Leucine	[¹⁴ C]-Leucine
Virus-treated cells	+	+	870	7355
			910	9423
Controls	+	+	750	8289
			840	9423

Thus, actual protein synthesis was excluded as a possible factor in the observed changes in incorporation of amino acids. The adsorption of u.v.-inactivated Sendai virus to HeLa or L-929 cells affected the transport of amino acids across the cellular membrane, but not the protein synthesis in these cells.

The multiplicity of infection required for these changes to occur is rather high; it is of the same order of magnitude as that required for fusion of cells by paramyxoviruses (Okada, 1969; Kohn, 1965). In this respect the change in transport of amino acids differs from the thymidine effect, which is elicited even at the low input multiplicity of 10 to 50 virus particles/cell (Fuchs & Kohn, 1971).

We thus observe at least three different transient changes in animal cells absorbing u.v.-inactivated Sendai virus. The first is the increase in the uptake of [⁴²K] into BHK-21 or chicken red blood cells, which lasted from 15 to 45 min after adsorption of the virus. The second is a transient change which resulted in the block in DNA and RNA synthesis in virus-treated cells, lasting from 30 to 90 min after adsorption (Fuchs & Kohn, 1971; Fuchs & Kohn, 1973). The third is the inhibition of the uptake of amino acids in such virus-treated cells which though transient has a longer duration than the two other effects.

One may assume that all these effects are produced by transient changes induced in the cell membrane by the fusion of the envelope of the virus particles with it, though it is not clear yet whether these are independent events, or sequential and linked ones. It is also not clear whether they are related to the observations of Christensen & Riggs (1952) that concentration of glycine by Ehrlich ascites cells was inhibited by potassium.

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REFERENCES

- ALLEN, E. H. & SCHWEET, R. S. (1962). Synthesis of hemoglobin in a cell-free system. I. Properties of the complete system. *Journal of Biological Chemistry* **237**, 760-767.
- CHRISTENSEN, H. N. & RIGGS, T. R. (1952). Concentrative uptake of amino acids by the Ehrlich mouse ascites carcinoma cell. *Journal of Biological Chemistry* **194**, 57-68.
- ENSMINGER, W. D. & TAMM, I. (1970). The step in cellular DNA synthesis blocked by Newcastle disease or mengovirus infection. *Virology* **40**, 152-165.
- FUCHS, P. & KOHN, A. (1971). Nature of transient inhibition of deoxyribonucleic acid synthesis in HeLa cells by Parainfluenza virus I (Sendai). *Journal of Virology* **8**, 695-700.
- FUCHS, P. & KOHN, A. (1973). Virus induced changes in endogenous pathway of thymidine in animal cells. *Israel Journal of Medical Sciences* **9**, 477-480.
- FUCHS, P. & GIBERMAN, E. (1973). Enhancement of potassium influx in baby hamster kidney cells and chicken erythrocytes during adsorption of Parainfluenza I (Sendai) virus. *Federation of European Biochemical Society Letters* **31**, 127-130.
- HUANG, A. S. & WAGNER, R. R. (1965). Inhibition of cellular RNA synthesis by non-replicating vesicular stomatitis virus. *Proceedings of National Academy of Sciences of the United States of America* **54**, 1579-1584.

- JOKLIK, W. K. & BECKER, Y. (1964). The replication and coating of vaccinia DNA. *Journal of Molecular Biology* **10**, 452-474.
- KOHN, A. (1965). Polykaryocytosis induced by Newcastle disease virus in monolayers of animal cells. *Virology* **26**, 228-245.
- KOHN, A. & FUCHS, P. (1973). Initial effects of viral infection in bacterial and animal host cells. *Advances in Virus Research* (in the press).
- MARTIN, E. M. & KERR, I. M. (1968). Virus induced changes in host-cell macromolecular synthesis. In *The Molecular Biology of Viruses*, pp. 15-46. Edited by L. V. Crawford and M. G. P. Stoker. Cambridge: Cambridge University Press.
- MEISELMAN, N., KOHN, A. & DANON, D. (1967). Electron microscopic study of penetration of Newcastle disease virus into cells leading to formation of polykaryocytes. *Journal of Cell Science* **2**, 71-76.
- MORGAN, C. & HOWE, C. (1968). Structure and development of viruses as observed in the electron microscope. IX. Entry of Parainfluenza 1 (Sendai) virus. *Journal of Virology* **2**, 1122-1132.
- OKADA, Y. (1969). Factors in fusion of cells by HVJ. *Current Topics in Microbiology and Immunology* **48**, 102-128.
- POSTE, G. & ALLISON, A. C. (1971). Membrane fusion reaction - a theory. *Journal of Theoretical Biology* **132**, 165-184.
- WERTZ, G. W. & YOUNGER, J. C. (1972). Inhibition of protein synthesis in L-cells infected with vesicular stomatitis virus. *Journal of Virology* **9**, 85-89.
- YAMASHITA, T., MORITSUGU, Y. & SHIMOJO, H. (1971). Suppression of cellular DNA synthesis in growing cells by ultraviolet-irradiated adenoviruses. *Virology* **45**, 687-696.
- YAOI, Y., MITSUI, H. & AMANO, M. (1970). Effect of UV-irradiated vesicular stomatitis virus on nucleic acid synthesis in chick embryo cells. *Journal of General Virology* **8**, 165-172.

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