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Analysis of Uridine Incorporation in Chicken Embryo Cells Infected by Vesicular Stomatitis Virus and Its Temperature-Sensitive Mutants: Uridine Transport

NICOLE GENTY

Institut de Microbiologie, Université de Paris-Sud, Centre d'Orsay, 91405 Orsay, France

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The shut-off of RNA synthesis in chicken embryo cells, after infection with vesicular stomatitis virus, is partially due to a reduced capacity of the infected cells to transport uridine. Permeability to uridine decreases exponentially after infection. This loss of ability to transport uridine may be caused either by structural components of the input virions or may result from the expression of the viral gene products. In the latter case, only minor levels of viral transcription is sufficient to modify cellular permeability, since, even at low multiplicities, RNA$^-$ temperature-sensitive (ts) mutants of vesicular stomatitis virus bring about a significant diminution of uridine incorporation in cells infected under nonpermissive conditions. Experiments with mutants of group III suggest that the M protein of the viral envelope may play a role in the sequence of events that modifies uridine transport. In addition to this cause of the diminution of incorporation of uridine by infected cells, another mechanism is noted which requires protein synthesis.

Many reports indicate a rapid and significant inhibition of macromolecular syntheses in cells infected with a wide variety of viruses (12, 17). In the case of vesicular stomatitis virus (VSV), Huang and Wagner (8) have shown that UV-irradiated virions, as well as normal virions, inhibited RNA synthesis in Krebs II ascites cells just after infection. This shut-off has also been demonstrated in other cell types infected with live or UV-irradiated VSV, albeit with variable efficiency (25, 28, 29).

As nonreplicating particles are able to induce a rapid decline in the rate of cellular RNA synthesis, it has been postulated that structural components of the infecting particles are involved in the inhibition of cellular metabolism. This fact does not rule out the possibility of an inhibitory protein newly synthesized on an intact cistron of the UV-irradiated viral genome (8).

To distinguish between the effects caused by components of the input virions and effects linked to the intracellular expression of the viral genome, uridine incorporation has been examined in cells infected with temperature-sensitive (ts) mutants of VSV blocked at different steps of their growth cycle under nonpermissive conditions.

These experiments call for a system in which (i) uridine incorporation decreases rapidly after infection to enable one to detect the effect of the input virions, and (ii) uridine incorporation is affected at low multiplicities of infection (MOIs), whereby the role of structural components of the input particles is more likely to be minimized by comparison to the effect of newly synthesized viral material. Chicken embryo cells (CEC) meet these requirements (5).

Since uridine is frequently used as a precursor for measuring de novo RNA synthesis, possible effects of virus infection upon uridine transport is an important problem to be investigated.

MATERIALS AND METHODS

Cells and media. Primary CECs were prepared from 10-day-old embryos and cultured in plastic petri dishes (60 mm in diameter) at 37 C for 20 h, by which time approximately 7×10^6 cells formed a complete monolayer. The medium used both for growth and in the reported experiments consisted of Eagle minimal essential medium (Eurobio) supplemented with 8% calf serum and 8% tryptose phosphate and buffered with Tris (pH 7.6).

Viruses. Wild-type (wt) VSV (Indiana strain) and ts mutants were propagated at low MOIs in CEC; no T particle production could be detected by the usual techniques (16). Virus preparations were harvested 17 to 20 h postinfection, freed from cell debris by low-speed centrifugation, and pelleted by centrifugation (45,000 $\times$ *g* for 90 min). Virus titration was performed on CEC by the plaque assay method (3).

The ts mutants have been isolated and described by Flamand (3). They have been classified into five complementation groups. The reversion rate of the mutants used was lower than 0.01%, and their leakiness was less than 0.02%.

Chemicals. [5-^{3}H]uridine (23 Ci/mmol) was purchased from CEA (Saclay, France). Cycloheximide was obtained from Serva (Heidelberg, Germany). Soluene is a sample solubilizer from Packard (France).

Infection of cells. Virus inocula (0.2 ml) were plated on CEC and allowed to adsorb for 45 min at room temperature, the unadsorbed inoculum was removed, and the cells were washed with saline and overlayed with 2 ml of prewarmed culture medium. The monolayers were then incubated in a constant-temperature water bath.

The MOI was calculated from separate estimates of the PFU per 0.2 ml of inoculum relative to the number of cells per plate.

Uridine incorporation. At different times after infection, culture medium was replaced by prewarmed radioactive medium (containing 1 μCi of [^{3}H]uridine per ml). At the end of a 30-min pulse, incorporation was stopped by placing the petri dishes on ice, and monolayers were washed rapidly three times with chilled saline buffer. If the acid-soluble pools were to be determined, the cell monolayers were extracted twice with 2 ml of chilled 5% trichloracetic acid. The extracts were combined, and a 0.5-ml portion was mixed with 10 ml of Bray scintillation fluid.

To obtain the corresponding acid-insoluble fractions, cells were scraped off the petri dishes, and the precipitates were washed with an equivolume mixture of ethanol-ether, dissolved in 0.2 ml of Soluène, and added to 10 ml of Bray fluid. Radioactivity was measured in an Intertechnique K4 liquid scintillation spectrophotometer and corrected for quenching by means of an external standard.

Uridine uptake. To analyze the rate of penetration of [^{3}H]uridine in CEC, cultures were incubated with gentle rotation in a constant-temperature water bath. The cells were pulse-labeled for 60 s by addition to the culture media (through a small hole in the cover of the petri dish) of 0.5 ml of Eagle minimal essential medium containing [^{3}H]uridine.

At the end of the 1-min pulse, the medium was decanted rapidly, and the petri dish was immersed immediately and sucessively in two fresh solutions of chilled saline buffer. The monolayer was then overlayed with 2 ml of chilled saline buffer to determine the remaining extracellular radioactivity; these values were less than 5% that found in the intracellular space. Washing required only 15 s. No more label was eluted from the cells when washings were performed for longer periods (regardless of the presence or absence of cold uridine in washing buffer).

Intracellular nucleoside and nucleotide pools were extracted with 2 ml of ice cold 5% trichloroacetic acid. The extract was collected after shaking the plate briefly. About 80% of the total acid-soluble radioactivity was recovered in this first extract. A second extraction was performed, extracts were combined, and a 0.5-ml portion was added to 10 ml of Bray scintillation fluid. This technique gave very reproducible results (±5%).

All cell incubations were performed at 39. 5 C, a restrictive temperature for the ts mutants.

The incorporation of [^{3}H]uridine in acid-insoluble material, or the uptake of uridine into soluble pools, is expressed, for each corresponding time point, as the ratio of disintegrations per minute per monolayer of infected cells to disintegrations per minute per monolayer of mock-infected cells. Each experimental value represents the average of three monolayers.

RESULTS

Uridine incorporation in CEC infected by wt VSV at different MOIs. Uridine incorporation into acid-insoluble material during 30-min pulses declined exponentially in infected cells by comparison to uninfected cells, and this decrease was a function of the MOI (Fig. 1). This incorporation loss can be characterized by its half-life (viz., the time at which the incorporation of exogenous uridine into acid-precipitable material was 50% that observed in mock-infected cells). The half-life of incorporation decreased from about 2 h (at a MOI of 1) to less than 1 h for a MOI of 100 (Fig. 1A). Biphasic

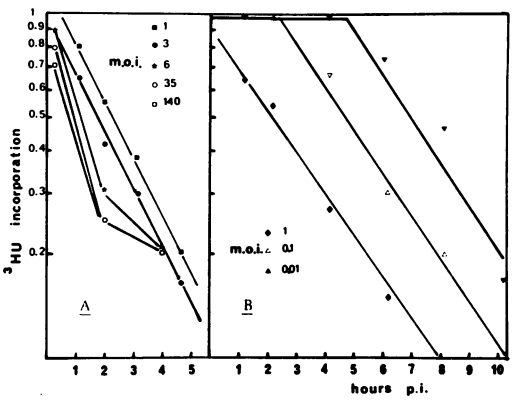

FIG. 1. *Effect of varying MOIs of wt VSV on [^{3}H]uridine incorporation into the acid-precipitable material of infected CEC. Monolayer cultures of CEC were infected with VSV and then overlayed with culture medium. At different times postinfection (p.i.) (in this and subsequent experiments time 0 refers to the end of the adsorption period), culture medium was replaced by prewarmed radioactive medium (containing 1 μCi of [^{3}H]uridine per ml; specific activity, 23 Ci/mmol). At the end of a 30-min pulse, the acid-precipitable fraction was determined. The data are recorded as the ratio of [^{3}H]uridine incorporation by infected cells divided by the incorporation by mock-infected cells at each time point and plotted against the midpoints of the 30-min pulse intervals. The results of two different experiments are plotted. (A), MOI ≥ 1; (B), MOI ≤ 1.*

curves were always obtained for multiplicities higher than five. For MOI lower than 1 (Fig. 1B), the initial incorporation continued unchanged and then (depending on the initial virus MOI) exponentially declined with time. The slopes of those later curves are identical.

Uridine incorporation in infected cells treated with cycloheximide. To determine whether the depression of uridine incorporation in acid-insoluble material after VSV infection was due to components of the initial infecting particles or to newly synthesized VSV gene products, cells were treated with cycloheximide immediately after the adsorption period.

For a MOI of 2 the half-life of the incorporating system was 6 h in the cycloheximide-treated cells, compared to a half-life of 2 h in the absence of the drug (Fig. 2A). The difference between the two sets of conditions disappeared as the MOI was increased (Fig. 2B; MOI of 35).

It was noted that even in absence of protein synthesis the decline in the rate of uridine incorporation, as measured by the 30-min pulse incorporations into acid-insoluble material, was exponential. At the higher MOI in the presence of cycloheximide, no biphasic curves were obtained (compare Fig. 1A and 2B).

Uridine incorporation in CEC infected by ts mutants of VSV. The inhibition of uridine incorporation into acid-insoluble material obtained on CEC infection by selected (RNA$^-$) ts mutants belonging to group I (ts I-5), group II (ts II-52), and group IV (ts IV-100) at nonpermissive temperatures (39.5 C), compared to wt infection each at a MOI of 2, is shown in Fig. 3. The inhibition slopes obtained were essentially similar for each mutant and for the wt virus (Fig. 3).

A different pattern of inhibition was obtained for the (RNA$^+$) mutant belonging to group III (ts III-23; Fig. 4). At a MOI of 5, the half-life of incorporation was 5 h, compared to slightly more than 1 h for the wt at the same MOI. At a MOI of 0.5, the efficiency of the uridine incorporation of ts III-23-infected cells decreased very slowly, and after 3 h the effect of infection on uridine incorporation virtually disappeared (compare wt values in Fig. 1).

Other group III ts mutants (ts III-76 and ts III-89) gave the same results, indicating that the effect was group specific. It has been shown that the group III gene products correspond to the viral M protein (14, 19).

A revertant of ts III-23 has been selected (R$^+$/ts III-23). The effect of this revertant on the incorporation of uridine in CEC is strikingly similar to that of the wt for the same MOI of 5 (Fig. 4).

Uridine uptake by infected cells. A cause for the decrease in uridine incorporation could be a failure in uridine uptake by the infected cells. This hypothesis was investigated by comparing uridine uptake into acid-soluble cell material during 1-min pulses in cells infected with wt, ts

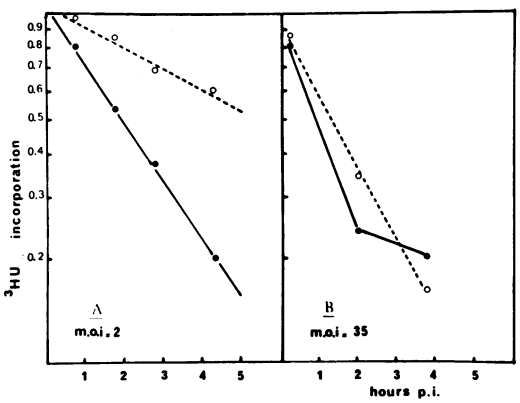

FIG. 2. *Inhibition of uridine incorporation in acid-precipitable material in CEC infected with wt VSV: requirement for protein synthesis and the effect of different MOIs. CECs were infected with VSV at a MOI of 2 (A) or 35 (B). The cultures were then incubated in medium with (dotted lines) or without (solids lines) 10 μg of cycloheximide per ml. At different times postinfection (p.i.), cells were pulse labeled with [^{3}H] uridine (1 μCi/ml; see legend to Fig. 1). Acid-precipitable radioactivity per infected monolayer is expressed as a fraction of the acid-precipitable material from mock-infected cells (likewise, with or without cycloheximide treatment).*

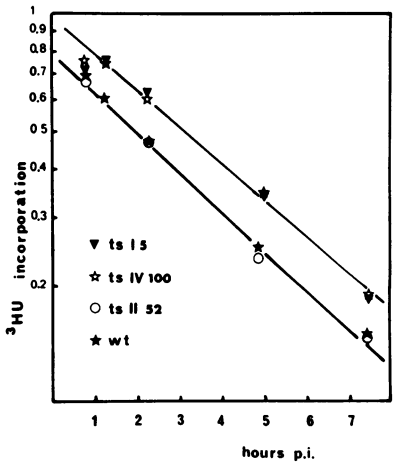

FIG. 3. *[^{3}H]uridine incorporation in acid-precipitable material of CEC infected at a MOI of 2 with wt or (RNA$^-$) ts mutants of VSV. The experimental details are the same as those given in Materials and Methods and in the legend to Fig. 1. p.i., Postinfection.*

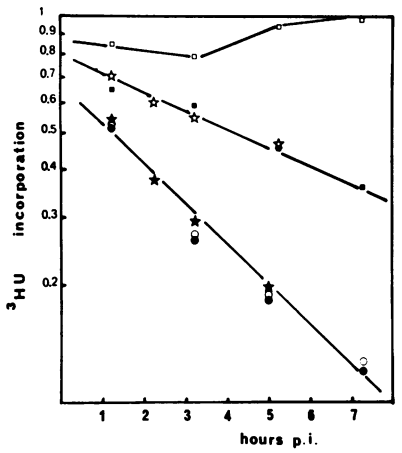

FIG. 4. *[^{3}H]uridine incorporation in acid-precipitable material of CEC infected with ts III-23, a revertant (R^+/ts III-23), and wt VSV. The experimental details are the same as those described in the legend to Fig. 1 and Materials and Methods. Results of two different experiments are plotted. (Experiment I) Symbols: □, ts III-23, MOI of 0.5; ■, ts III-23, MOI of 5; ○, VSV wt, MOI of 0.5; ●, VSV wt, MOI of 5. (Experiment II) Symbols: ☆, ts III-23, MOI of 5; ★, R^+/ts III-23, MOI of 5. p.i., Postinfection.*

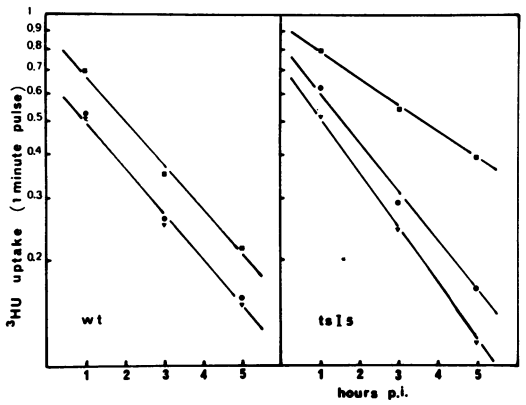

FIG. 5. *Effects of varying MOIs on [^{3}H]uridine uptake by CEC infected with VSV wt and ts I-5, respectively. At different times postinfection (p.i.), 0.5 ml of Eagle medium containing [^{3}H]uridine (5 μCi/ml) was added to the culture media; after 60-s pulses the cells were rapidly washed with saline and extracted twice with 2 ml of cold 5% trichloroacetic acid. The rate of uptake of [^{3}H]uridine into the trichloracetic acid-soluble fractions of CEC infected with VSV wt (left panel) or ts I-5 (right panel) is expressed as a fraction of the uptake by mock-infected cells. Symbols: ■, MOI of 5; ●, MOI of 50; ▼ MOI of 500.*

I-5, ts III-23, and in mock-infected cells. To determine whether a modification of permeability was related to the MOI, the uptake of precursor was examined in cells infected at MOIs ranging from 5 to 500.

Uridine uptake in acid-soluble cell material was depressed at an exponential rate throughout the viral cycle for both wt as well as ts I-5 infected cells (Fig. 5). However, the phenomenon was less sensitive to MOI changes in the case of the wt (particularly for MOI of 50 and 500). In the case of the RNA$^-$ ts I-5 mutant, the inhibition of uptake was less pronounced at low MOI (compare similar wt MOIs) with a distinct difference in the slopes for ts I-5 MOIs of 50 and 5.

The half-life of uridine uptake into acid-soluble fractions (about 3 to 4 h for wt and ts I-5 infected cells at MOI of 1) was considerably greater in cells infected with ts III-23 (Table 1). For instance, at a MOI of 3, a half-life of 10 h was obtained for the group III mutant.

Estimate of the rate of RNA synthesis in infected cells. From these results, it appeared that the restricted rate of uridine incorporation into acid-insoluble material by VSV-infected cells (Fig. 1 to 4) could be at least partly related to a reduction in the uptake of exogenous uridine. To examine this possibility, the uptake of labeled uridine during 30-min pulses as well as its subsequent incorporation into acid-precipitable material was determined in cells infected with wt and the mutants ts I-5 and ts III-23. Figure 6 shows the results of such experiments in terms of the reduction of uridine uptake in the acid-soluble pools (dotted lines) or uridine incorporation into acid-precipitable material (solid lines).

On the supposition that the intracellular pool of uridine-labeled acid-soluble material does not change volume after viral infection, and

TABLE 1. *Half-life of uridine uptake in CEC infected at different MOIs with VSV wt, ts I-5, or ts III-23*[a]

wt		ts I-5		ts III-23	
MOI	Half-life (h)	MOI	Half-life (h)	MOI	Half-life (h)
500	1	500	1	100	4
50	1	50	1.5	30	4
5	2	5	3.5	10	8
1	3	1	4.5	3	10

[a] The values were obtained by plotting semilogarithmically the fraction of label in virus-infected cell acid-soluble pools by comparison to mock-infected cells during 1-min pulses of [^{3}H]uridine at different times postinfection. (The ability to take up [^{3}H]uridine remained constant in the mock-infected controls.) This decrease (see Fig. 5) was used for mutant or wt infections (at different MOIs) to calculate the half-life of uridine uptake as defined by the time at which the infected cells took up 50% of the uridine adsorbed by mock-infected cells.

that the de novo pyrimidine synthesis is the same in infected cells as in mock-infected cells, the specific radioactivity of the acid-soluble pool can be used to calculate the rate of synthesis of pulse-labeled RNA by dividing the uridine incorporation into acid-precipitable material by the label in the corresponding acid-soluble fraction. The ratios of these calculated values for infected and mock-infected cells, at each time point, are given by the dashed lines in Fig. 6. The results indicate a smaller reduction in the overall rate of RNA synthesis after viral infection than was otherwise suggested by comparison of the incorporation of radioactive precursor into acid-precipitable material.

Similar experiments performed on wild-type infected or mock-infected cells treated with cycloheximide showed that in this case the modification of cellular permeability to uridine uptake is sufficient to explain the observed exponential decrease of uridine incorporation in acid-insoluble material by the infected cells (Table 2).

Inhibition of synthesis of cellular RNA by UV-irradiated wt VSV. An analysis of the decrease in uridine incorporation in CEC infected with UV-irradiated VSV is presented in Fig. 7.

The 90-min half-life of incorporation of label into acid-insoluble material observed when CEC were infected with VSV at a MOI of 3 (Fig. 7, A) increased to 4 h when the virus was previously irradiated with UV light (Fig. 7, B).

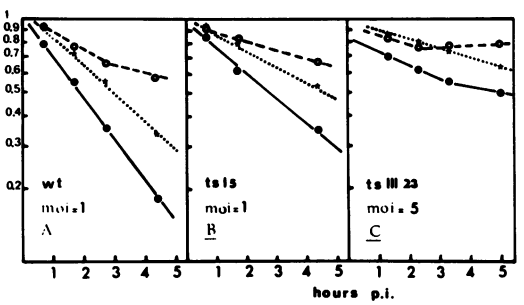

FIG. 6. *Uridine incorporation into acid-soluble and acid-precipitable fractions of CEC infected with VSV (wt, ts III-23, or ts I-5). Pulses (30 min) were carried out at different times postinfection (p.i.) using CEC infected with VSV wt (A), ts III-23 (B), and ts I-5 (C), respectively. The rate of incorporation of [³H]uridine into trichloracetic acid-soluble (dotted lines) or -insoluble fractions (solid lines) are plotted as fractions of the radioactivities found in the corresponding fractions of mock-infected controls. The dashed lines represent the ratios obtained when each experimental value of uridine acid-precipitable radioactivity was divided by the acid-soluble radioactivity of the corresponding sample.*

TABLE 2. *Incorporation of [³H]uridine in CEC infected with VSV wt virus in the presence of cycloheximide*[a]

Virus (MOI)	Post-infection time (min)	Acid precipitable (counts/min per monolayer)	Acid soluble (counts/min per monolayer)	Acid precipitable/acid soluble
0	30	74,600	475,000	0.157
0	120	93,000	496,000	0.188
6	30	67,400	405,000	0.166
6	120	48,000	254,000	0.189
35	30	65,600	425,000	0.154
35	120	31,900	170,000	0.187
140	30	51,100	355,000	0.143
140	120	30,700	180,000	0.170

[a] Thirty-minute pulses of [³H]uridine (1 μCi/ml) were used in wt virus infections to determine the incorporation of label at different times post-infection in acid-soluble or -insoluble material (see text). The infections were performed in the presence of cycloheximide (10 μg/ml).

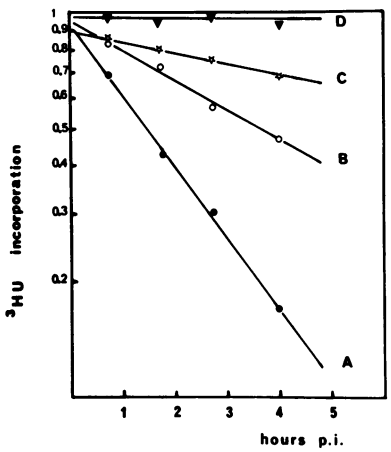

FIG. 7. *Analysis of uridine incorporation in CEC infected with UV-irradiated VSV. CEC monolayers were infected (MOI of 3) with either wt VSV (A) or virus which had been exposed to UV irradiation (10,000 ergs) (B, C, and D). Cells were pulse labeled for 30 min with [³H]uridine at different times postinfection (p.i.). Both acid-precipitable and -soluble fractions were determined. The acid-insoluble radioactivity incorporated in infected cells is expressed as the fraction of the radioactivity incorporated in equivalent mock-infected controls. (A) CEC infected with wt VSV; (B) CEC infected with UV-irradiated VSV; (C) CEC infected with UV-irradiated VSV and treated with cycloheximide (5 μg/ml) just after infection; (D) the values used to derive (C) were divided by the corresponding acid-soluble fractions to give the relative RNA syntheses in the UV-irradiated VSV, cycloheximide-treated, CEC-infected cells by comparison to the mock-infected controls.*

When the cells were treated with cycloheximide just after infection with the UV-irradiated virions, the inhibition effect was smaller (Fig. 7, C). Moreover, when the relative rates of pulse-labeled RNA synthesis were calculated for these cycloheximide-treated, UV-irradiated VSV or mock-infected cells, it was found that there was no difference between the infected or mock-infected cells (see footnote to Table 2).

DISCUSSION

An immediate, exponential, and MOI-dependent inhibition of uridine incorporation in acid-insoluble material is observed in CEC infected with VSV. The half-life of this incorporation, which is about 2 h (for a MOI of 1), decreases to less than 1 h at higher MOIs (Fig. 1). The biphasic curves observed at multiplicities higher than 5 may be due to uridine incorporation into viral-directed RNA synthesis, since it is not observed when cells are treated with cycloheximide (Fig. 2B). When the MOI is 1, or less than 1, the curves show the same slope after an initial lag, depending on the MOI (Fig. 1). The similarity of those slopes suggests that the same phenomena may be involved. The initial lag in low multiplicity experiments is probably related to secondary infections by progeny virus, since cells infected with ts mutants at nonpermissive temperatures do not show this delayed inhibition (5).

As demonstrated earlier by Flamand and Bishop (4), I have observed that usually less than 10% of the PFU of the inoculum adsorbs to the cell monolayer, irrespective of the viral concentration of the inoculum (data not shown). Since the particle to PFU ratios of VSV preparations are usually much greater than 1 (often around 5 or 10 to 1, see Flamand and Bishop [4]), and an immediate inhibition of uridine incorporation into acid-insoluble material is observed at MOIs of 1 (Fig. 1) or even 0.5 (Fig. 4), it is possible that some of the noninfectious but adsorbed viral particles are able to induce modification of cellular metabolism without inducing plaques. This observation is in agreement with the results of Marcus and Sekellick (16), which showed that VSV stocks contain noninfectious particles capable of killing cells, but defective in their capacity to form plaques.

When CECs were infected with ts mutants of groups I, II, and IV, I obtained a similar inhibition of uridine incorporation into acid-insoluble material compared to the one observed with wt (Fig. 3). Mutants of group III, however, apparently induce a weaker inhibition (Fig. 4).

Holloway et al. (7) have shown that for a MOI of 25 all the ts mutants of VSV caused a marked inhibition of RNA synthesis in mouse L cells comparable to that produced with wt virus.

One or several components of the infective particles may be responsible for the modification of uridine incorporation in infected cells. The half-life of incorporation into acid-insoluble material increases from 2 to 6 h if protein synthesis is blocked (Fig. 2A). However, this reduction can be overcome at higher MOIs (Fig. 2B).

It appears that at least at low MOIs the inhibition phenomena are amplified by the expression of the introduced genomes, since in the case of UV-irradiated virions this amplification is reduced (Fig. 7).

I payed special attention to uridine penetration into the cellular acid-soluble pool, since the adsorption of virions may cause cell surface alterations leading to changes in cellular permeability (15, 22, 26, 27). From my data the concept emerges that infection of CEC by VSV induces an alteration of the cell membrane. Uridine uptake declines exponentially after infection (Fig. 5). There is a saturation effect, since for MOIs of 50 and 500 the curves showing the inhibition of uptake are identical.

Results obtained with mutants of group III (Table 1) suggest the possibility that the M protein (14, 19) of the viral envelope may play a role in the sequence of events that modify uridine transport. The preferential in vitro association of this M protein with the HeLa cell membrane has been demonstrated by Cohen and Summers (2).

Similarities and differences between ts I-5 and the wt are probably related to the fact that in complementation group I transcription is severely depressed (9, 20, 24) although it still occurs (4). A few transcriptional events may therefore suffice to modify cellular permeability. By the same token, a low MOI induces a detectable inhibition.

The decrease of uridine uptake after infection leads first to a diminution of the specific radioactivity of the acid-soluble pool and then to a diminution of ^{3}H-labeled UTP incorporation into the newly synthetised RNA (Fig. 6).

I have suggestive evidence indicating that, if protein synthesis is blocked just after infection (Table 2; Fig. 7), the apparent cause of shut-off phenomenon is the diminution of the penetration of precursor into the acid-soluble pool and therefore of the specific radioactivity of the RNA newly synthetised in the infected cells.

This demonstration stresses the importance

of the fact that in some systems the rate of incorporation of extracellular precursor into RNA cannot be taken by itself as a necessarily valid indication of the rate of RNA synthesis. Similar conclusions were drawn by Kramer et al. (13) on the basis of studies on analysis of RNA turnover and by Kay and Handmaker (10), who studied RNA synthesis in stimulated lymphocytes.

In other systems, e.g., HeLa cells or L cells infected with VSV (data not shown), I have not observed a rapid decline in uridine penetration even at high MOIs. The reason is not known, but this could explain why more than 2 h are necessary to show a shut-off of RNA synthesis in those systems.

It is obvious that in CEC infected with VSV the modification of cell permeability is partially responsible for the decrease in the efficiency of the uridine incorporation, but does not entirely explain it (except when infected cells are treated with cycloheximide). In addition to the inhibition of uridine uptake an actual inhibition of incorporation of this precursor in RNA molecules seems to occur (Fig. 6). I do not know if this reflects a defect in the different phosphorylation steps as observed in vaccinia-infected L cells (11) or an actual inhibition of rate of RNA synthesis.

At least two mechanisms may be involved in the modification of uridine incorporation into CEC infected by VSV (Fig. 6). Wertz and Youngner (26) have shown that two phenomena are involved in the inhibition of protein synthesis in L cells infected by VSV. I have demonstrated that in our system the initial, multiplicity-dependent, UV-insensitive inhibition is the modification of cellular permeability to uridine.

Cell surface alterations have often been invoked as a result of viral infection (1, 18) as well as in transformed cells (30), but these modifications appear rather late in the course of the viral cycle. Here I observe an immediate and progressive alteration of cell membrane permeability that may be induced by the mere adsorption of the virion, since the uridine uptake decreases exponentially even if the viral cycle is blocked by cycloheximide.

The modification of uridine uptake after infection could be interpreted in two ways. As proposed by J. P. Changeux, an impulse from localized interaction between a membrane receptor site and a ligand (here the virion) could be progressively propagated over the cell membrane, changing the activity of the transport entities which are presumably widely distributed over the periphery of the cell membrane. According to the fluid mosaic model of membrane structure, surface proteins are free to diffuse in a lipid matrix and thus to assume a random and homogenous distribution over the cell surface (23). If in my system VSV penetrates the cell by fusion of the viral envelope with the plasma membrane (6), I can imagine the spreading of viral molecules from the point of insertion, and then the progressive inhibition of the transport units which are responsible for uridine uptake or uptake and phosphorylation, as kinases may play an important role in the uptake of nucleosides by the cells grown in tissue culture (21).

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