DNA and Histone Synthesis in Reovirus-infected Cells

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SUMMARY

Reovirus infection inhibits the incorporation of $^3$H-thymidine into cellular DNA. We have now investigated several aspects of this inhibition in L-929 cells early (8 h) after infection at high multiplicity (200 to 250 p.f.u./cell). Using equilibrium sedimentation analysis of DNA sequentially labelled with density and radioactive analogues of thymidine, we find a 52% reduction in the amount of DNA synthesized with no change in rate of replication fork movement in infected cells. Gel electrophoresis of histones labelled with $^3$H-lysine shows that infection inhibits their synthesis by 76% several hours before overall cellular protein synthesis is inhibited. There is also a reduction of nearly 50% in the size of the thymidine triphosphate pool, as measured by enzymic assay. The proportion of exogenous nucleotide in the pool is the same as in uninfected cells since there is no change in the buoyant density of DNA labelled during a short pulse with $^3$H-bromodeoxyuridine. The uptake of thymidine is reduced, but its phosphorylation to thymidine triphosphate is normal. The findings provide direct evidence that DNA synthesis is inhibited early in infection. This inhibition is accompanied by other derangements of thymidine and chromatin metabolism suggesting that there is an early and specific attack by reovirus on nuclear function in infected cells.

INTRODUCTION

Infection of L cells with reovirus leads to the inhibition of thymidine incorporation into DNA (Gomatos & Tamm, 1963). This takes place early in infection before the inhibition of protein synthesis and the onset of cell rounding (Ensminger & Tamm, 1969a). The reduction in thymidine incorporation, which starts 6 h after infection, probably represents an inhibition of DNA synthesis, although the amount of DNA synthesized during a short pulse in unsynchronized infected cells has not been measured. Cells infected 2 h before the release of a fluorodeoxyuridine block synthesize almost 60% less DNA in the 6 h following release compared to uninfected cells (Ensminger & Tamm, 1970). Beyond 9 h post infection, the cells synthesize no new DNA (Ensminger & Tamm, 1970) and this correlates with the reduction of thymidine incorporation (Cox & Shaw, 1974). The late effects (9 h post infection and after) of reovirus on DNA synthesis may be attributed to non-specific inhibition in dying cells. The early effects may result from a specific action of reovirus on cellular synthesis.

A number of studies have attempted to localize the early block in DNA synthesis in infected cells. There is a moderate increase in the distance between active initiation sites (Hand & Tamm, 1974); this suggests that the initiation of DNA synthesis on individual
replication units is inhibited. However, the interval between initiation sites is not a stringent measure of DNA synthesis and the increased interval cannot account for the overall reduction of thymidine incorporation into DNA. Surprisingly, other studies have been unable to demonstrate abnormalities in various aspects of thymidine metabolism and DNA synthesis in infected cells. The proportion of cells in S phase is normal (Gomatos & Tamm, 1963), as is the activity of several enzymes involved in the synthesis of DNA precursors (Ensminger & Tamm, 1969b). The amount of thymidine incorporated into the acid-soluble pool is unchanged (Shaw & Cox, 1973) and there is no breakdown of preformed DNA (Ensminger & Tamm, 1969b; Shaw & Cox, 1973). The maturation of nascent DNA chains to the full size of bulk DNA takes place normally (Ensminger & Tamm, 1969b), and there is no decrease in the rate at which the new DNA chains elongate (Hand & Tamm, 1972).

Although a reduction in thymidine incorporation is often a reflection of decreased DNA synthesis, there are many experimental situations in which this is not the case (Hauschka et al. 1972; Brownstein et al. 1975; Grunicke et al. 1975). If reovirus does inhibit cellular DNA synthesis early in infection, then it should be possible to demonstrate this inhibition in experiments that measure synthesis in a more rigorous fashion than the incorporation of thymidine and the interval between active initiation sites. In addition, alterations in other aspects of thymidine and chromatin metabolism should be evident, since there is a tight correlation between these and DNA synthesis (reviewed by Hauschka, 1973; Elgin & Weintraub, 1975). In this report, we show there is an actual decrease in DNA synthesis in infected cells at early times after infection, as well as an inhibition in histone synthesis and a reduction in the size of the thymidine triphosphate pool. Uptake of thymidine into the cell is also inhibited.

**METHODS**

**Cells and virus.** We have described elsewhere the methods used for the growth and maintenance of the L-929 cell line in monolayers (Hand, 1975) and for growth and assay of reovirus type 3, Dearing strains (Hand & Tamm, 1971).

**Infection of cells with reovirus.** Logarithmically growing cells were infected with reovirus at a multiplicity of 200 to 250 p.f.u./cell. Absorption was allowed to take place for 2 h in one-tenth the usual amount of medium. Control cell cultures were sham-infected.

**Extraction of DNA.** Cellular DNA was extracted as described elsewhere (Hand, 1976a) using a modification of the Marmur phenol–chloroform extraction (Britten et al. 1974). The extracted DNA was sheared to fragments of 8 to 9 × 10⁶ daltons by passage four times through a 25-gauge needle.

**Equilibrium sedimentation of denatured DNA.** This was performed as described elsewhere (Hand, 1976a). DNA in an alkaline solution of CsCl of density 1.75/ml was centrifuged for 36 to 60 h at 33,000 rev/min in a fixed angle rotor. A marker for normal density was provided in all tubes by DNA uniformly labelled with ¹⁴C-thymidine. The gradients were analysed for density and radioactivity.

**Extraction of histones.** The procedure of Winocour & Robbins (1970) was followed. Cells labelled with radioactive lysine were washed, scraped from the Petri dishes, and resuspended in hypotonic buffer (0.02 M-tris-HCl, 0.01 M-NaCl, 0.002 M-mercaptoethanol, 0.0015 M-MgCl₂, pH 8.0) for 15 min at 4 °C. They were disrupted with a Dounce homogenizer and the nuclei collected by centrifugation. After thorough washing of the nuclei, acid-soluble proteins were extracted from them with 0.25 N-H₂SO₄ for 60 min at 4 °C. The extract was clarified by centrifuging at 1000 g for 10 min. Sodium dodecyl sulphate (SDS) was added
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Polyacrylamide gel electrophoresis. This was performed using the method of Laemmli (1970). Dialysed nuclear extracts containing labelled proteins were subjected to electrophoresis on 10% polyacrylamide-SDS gels for 3.5 h at 3 mA per gel. The gels were sliced into 1 mm sections, and the radioactivity in individual slices determined after addition of NCS solubilizer and toluene-based scintillation fluid.

Measurement of the total cellular thymidine triphosphate pool. An in vitro DNA polymerase assay was used to measure the concentration of thymidine triphosphate in acid-extracts of cells (Solter & Hanschumacher, 1969; Lindberg & Skoog, 1970). The procedure has been described in detail elsewhere (Hand, 1976a). Perchloric acid extracts of cells, after neutralization and precipitation, were used to supply limiting quantities of thymidine triphosphate for the Escherichia coli DNA polymerase (EC 2.7.7.7) reaction. The reaction was followed by the incorporation of 3H-deoxyadenosine triphosphate, present in non-limiting quantities, into acid-precipitable material. The number of cells for each extract was estimated by measuring the protein content (Lowry et al. 1951) of a sample before extraction.

Paper chromatography. This is described elsewhere (Hand, 1976a). Cells, labelled for 5 min with 3H-thymidine (20 μCi/ml), were extracted with trichloroacetic acid. The extracts, along with markers of purified thymidine and its nucleotides, were prepared for chromatography and the chromatograms developed with 1 M-ammonium acetate (pH 5.0)–95% ethanol (3:7) (Plagemann, 1971), at 37 °C for 6 h. Migration was determined by measuring the radioactivity in 1 cm strips of the chromatograms and comparing this with the position of the markers visualized by u.v. light.

Transport of thymidine into the cell. Thymidine transport was measured as described elsewhere (Hand, 1976a). Groups of cell monolayers were exposed to 3H-thymidine (2.5 μCi/ml) at a concentration from 5 × 10⁻⁸ to 5 × 10⁻⁷ M for 5 min. Acid-soluble and acid-precipitable 3H incorporation was measured in separate monolayers. Total incorporation was determined by summing the two and correcting for the specific activity of the 3H-thymidine.

RESULTS

DNA synthesis and replication fork movement

To measure DNA replication fork movement in infected cells, we used a modification of the method developed by Painter & Schaefer (1969). This analyses the density of DNA that has been sequentially labelled with density and tritium analogues of thymidine. After sequential labelling, a proportion of the radioactive DNA will be in end-to-end association with density-labelled DNA. If the DNA is extracted and sheared, radioactive fragments of two types will be formed, those with both density and radioactive label, and those with radioactive label alone. The proportion of each can be determined by equilibrium sedimentation under denaturing conditions. With faster rates of replication fork movement, there will be a higher proportion of fragments with radioactive label only. With slower rates of fork movement, more fragments will be substituted with both density and radioactive analogue.

This experiment can also be used to measure DNA synthesis in addition to the rate of fork movement. The DNA was uniformly labelled with 14C-thymidine for one generation before the cells were infected. At the appropriate time after infection, the density and tritium labels were administered in the presence of fluorodeoxyuridine. Since this compound blocks the synthesis of endogenous thymidine nucleotides, all thymidine incorporated during the tritium pulse will have been derived from exogenous sources. Therefore the ratio of
Fig. 1. Buoyant density of DNA sequentially labelled with bromodeoxyuridine and 3H-thymidine to measure replication fork movement in cells 8 h after reovirus infection. Cells were labelled with 14C-thymidine (0.005 μCi/ml) for 16 h. The radioactive medium was removed and the cells infected. At 4.75 h after infection, the growth medium was supplemented with bromodeoxyuridine (2 × 10⁻⁶ M) and fluorodeoxyuridine (2 × 10⁻⁶ M). At 7.75 h after infection this medium was replaced with one containing 3H-thymidine (5 × 10⁻⁶ M, 20 μCi/ml) and fluorodeoxyuridine (2 × 10⁻⁶ M) for an additional 30 min. Thus, the mid-point of the radioactive pulse was 8 h after infection. At the end of the pulse, the DNA was extracted and sedimented to equilibrium in alkaline CsCl. • • •, d/min; ○○○ 14C, d/min; ▲, density. (a) Uninfected cells; (b) reovirus-infected cells; (c) gradient from (a) with 3H disintegrations normalized by equating the peak 14C fraction with the equivalent 3H fraction and multiplying all other 3H fractions by the equating factor (Painter & Schaefer, 1969); (d) gradient from (b) with 3H disintegrations normalized.

3H:14C in the DNA is proportional to the amount of DNA synthesized during the tritium pulse.

Cells were labelled overnight with 14C-thymidine and then infected. At 4.75 h after infection, the cells were treated with bromodeoxyuridine in the presence of fluorodeoxyuridine. Three hours later, the medium containing the density analogue was replaced with medium containing 3H-thymidine and fluorodeoxyuridine and the cells pulsed for an additional 30 min. The mid-point of the 3H-thymidine pulse was 8 h after infection.

After labelling, the DNA was extracted and centrifuged to equilibrium in alkaline CsCl. When the gradients were analysed, we found that less 3H-thymidine had been incorporated into DNA from infected cells than into control DNA (Fig. 1a, b). The ratio of 3H:14C was 6.47 in the controls, and 3.14 in DNA from infected cells. This indicates that the amount of DNA synthesized during the 3H pulse was reduced 51.5% in infected cells.

To determine the rate of fork movement, we measured the proportion of DNA with greater than normal density. To do this, the 3H counts in the gradient were normalized by equating the peak 14C fraction with the equivalent 3H fraction, and then multiplying all other 3H fractions in the gradient by the equating factor (Painter & Schaefer, 1969). From
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these normalized gradients, the proportion of DNA of heavy density can be estimated from the net number of $^{3}$H counts sedimenting to the denser side of the $^{14}$C peak. In control cells, 0.24 of the DNA is heavy; in infected cells, the proportion is 0.27 (Fig. 1c, d). In three replicate experiments, the proportion of heavy DNA from infected cells was within 12% of the controls.

This type of experiment detects inhibition of DNA fork movement caused by cycloheximide (Hand & Tamm, 1977) and mengovirus infection (Hand & Oblin, 1977) and the degree of inhibition correlates very well with the results obtained using fibre autoradiography (Hand, 1976b). The lack of significant change in the proportion of heavy DNA observed here in reovirus-infected cells shows there is little change in the rate of fork movement in these cells.

Histone synthesis

The synthesis of histones is tightly co-ordinated with that of DNA (reviewed by Elgin & Weintraub, 1975). If reovirus causes a decrease in DNA synthesis in infected cells, then the synthesis of histones should also be decreased at times before overall cellular protein synthesis is inhibited.

An earlier study (Ensminger & Tamm, 1969a) showed that incorporation of $^{3}$H-leucine into acid-soluble nuclear proteins is not inhibited up to 12-5 h after infection. In the present study, we measured histone synthesis using polyacrylamide gel electrophoresis to determine the amount of $^{3}$H-lysine incorporated into acid-soluble proteins.

Monolayers of cells were pre-labelled with $^{14}$C-lysine for 48 h and then infected. From 6 to 8.5 h after infection, they were labelled with $^{3}$H-lysine. The $^{14}$C incorporation was used as a standard to permit comparison of the amount of histone synthesis during the $^{3}$H pulse in control and infected cells. The cell nuclei were isolated and extracted with acid to obtain the labelled histones and the extracts were then subjected to electrophoresis on 10% polyacrylamide-SDS gels.

Analysis of the gels from control cells showed two peaks that incorporated large amounts of both $^{14}$C- and $^{3}$H-lysine (Fig. 2a). These contain the five classes of histones (Winocour & Robbins, 1970). In separate experiments, we found that radioactive tryptophan was not incorporated into the proteins of either peak (data not shown). The gels from reovirus infected cells showed a decrease of 76% in the amount of $^{3}$H-lysine incorporation into histones as determined by the ratios of $^{3}$H:$^{14}$C in those peaks (Fig. 2b).

Overall protein synthesis was estimated from the ratio of $^{3}$H:$^{14}$C in acid-precipitable material from cells labelled for 48 h prior to infection with $^{14}$C-leucine and then from 6 to 8.5 h after infection with $^{3}$H-leucine. To estimate cell-specific protein synthesis, cell extracts labelled in the same fashion were subjected to electrophoresis on 10% polyacrylamide-SDS gels and the $^{3}$H:$^{14}$C ratios in those regions of the gel not containing reovirus-specific proteins were calculated. The results of these experiments and the histone synthesis experiment are compared in Table 1. The inhibition of total and cell-specific protein synthesis is minimal or moderate and is very similar to the results obtained by other investigators (Ensminger & Tamm, 1969a; Zweerink & Joklik, 1970). The inhibition of histone synthesis is more marked showing that the histone class of proteins is specifically inhibited earlier than cellular proteins.

Size of the thymidine triphosphate pool

An increase in the size of the thymidine triphosphate pool could cause a dilution of exogenous thymidine in the pool with a resulting decrease in the specific activity of thymidine incorporated into DNA. To rule out this explanation for decreased thymidine
Fig. 2. Histone synthesis in cells 6 to 8·5 h after reovirus infection. Cells were labelled with 14C-lysine (3·2 × 10⁻⁴ M, 0·5 μCi/ml) for 48 h. The radioactive medium was removed and the cells infected. At 6 h after infection, the medium was replaced with one containing 3H-lysine (3·7 × 10⁻⁴ M, 25 μCi/ml). After a 2·5 h labelling period, acid-soluble nuclear proteins were extracted and subjected to electrophoresis on 10% polyacrylamide-SDS gels. •, 3H, ct/min; ○—○, 14C, ct/min. (a) Uninfected cells; (b) reovirus-infected cells.

Table 1. Histone and protein synthesis 6 to 8·5 h after reovirus infection

<table>
<thead>
<tr>
<th>Cells</th>
<th>Histones</th>
<th>Total protein</th>
<th>Cell protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13·6</td>
<td>4·2</td>
<td>4·7</td>
</tr>
<tr>
<td>Reo-infected</td>
<td>3·3 (76)</td>
<td>4·0 (5)</td>
<td>3·5 (26)</td>
</tr>
</tbody>
</table>

* Ratio of 3H counts incorporated 6 to 8·5 h after infection to 14C counts incorporated during the 48 h prior to infection. The ratio was calculated for histones from incorporation of radioactive lysine into the histone peaks of a polyacrylamide gel, for total protein from incorporation of radioactive leucine into acid precipitable material, and for cell protein from incorporation of radioactive leucine into regions of a polyacrylamide gel not containing reovirus proteins. Values in parentheses are the percent inhibition in infected cells.

incorporation in infected cells, we measured the size of the pool. *Escherichia coli* DNA polymerase was used to assay limiting quantities of thymidine triphosphate in acid-extracts of cells 8 h after infection. The assay measured the total cellular pool of thymidine triphosphate in cells that had not been exposed to exogenous thymidine. We found that in
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Fig. 3. Buoyant density of DNA pulse-labelled with $^3$H-bromodeoxyuridine from cells at 8 h after infection with reovirus. Cells were labelled with $^{14}$C-thymidine (0.005 μCi/ml) for 16 h. The radioactive medium was removed and the cells infected. At 7.75 h after infection, the cells were labelled with $^3$H-bromodeoxyuridine ($1 \times 10^{-4}$ M, 10 μCi/ml) for 30 min. The DNA was extracted and sedimented to equilibrium in alkaline CsCl. • — •, $^3$H, d/min; ○ — ○, $^{14}$C, d/min; ▲ — ▲, density. (a) Uninfected cells; (b) reovirus-infected cells.

Fig. 4. Distribution of $^3$H-thymidine among thymidine and its nucleotides in acid-soluble extracts of infected cells. Cells were labelled with $^3$H-thymidine (20 μCi/ml) for 5 min at 8 h after infection. The extracts were analysed by descending paper chromatography and liquid scintillation counting. The positions of thymidine and its nucleotides, co-chromatographed as markers, are indicated at the top: (a) Uninfected cells; (b) reovirus-infected cells.

Table 2. Thymidine triphosphate pool 8 h after reovirus infection

<table>
<thead>
<tr>
<th>Cells</th>
<th>Cells/plate* ($\times 10^6$)</th>
<th>dTTP pool† (mol./cell $\times 10^{-18}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.0</td>
<td>22.5</td>
</tr>
<tr>
<td>Reo-infected</td>
<td>6.0</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* The cell count was estimated from the protein content of the cells on the plate. Under these conditions of culture, 1 mg of protein equals $3 \times 10^6$ cells.
† Assayed using *E. coli* DNA polymerase to measure limiting concentrations of thymidine triphosphate in acid extracts of cells. The polymerase reaction was followed by the incorporation of $^3$H-deoxyadenosine triphosphate into calf thymus DNA.
reovirus-infected cells, the thymidine triphosphate pool was reduced nearly 50\% (Table 2). Essentially identical results were obtained in three replicate experiments.

The contribution of exogenous nucleotide to the thymidine triphosphate pool

To eliminate completely the possibility that pool dilution contributes to the inhibition of thymidine incorporation, it is necessary to demonstrate that the proportion of exogenous nucleotide in the pool is the same in infected and control cells. We therefore analysed the density of DNA labelled with \(^{3}H\)-bromodeoxyuridine during a short pulse in the absence of fluorodeoxyuridine, reasoning that since this density analogue was incorporated by the same pathway as exogenous thymidine, a decrease in the proportion of nucleotide in the pool would be reflected as a decrease in the density of the substituted DNA. The administration of exogenous nucleosides, either thymidine or bromodeoxyuridine, can inhibit endogenous thymidine nucleotide synthesis by inhibiting ribonucleotide reductase. At a concentration of \(1 \times 10^{-5} \text{ M-dTTP or BrdUTP}\), the degree of enzyme inhibition is slight, being...
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Table 3. Thymidine transport 8 h after reovirus infection*

<table>
<thead>
<tr>
<th>Cells</th>
<th>$V_{\text{max}}$ (mol x $10^{-12}$/10^6 cells/min)</th>
<th>$K_m$ (M x $10^{-6}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.13</td>
<td>0.156</td>
</tr>
<tr>
<td>Reo-infected</td>
<td>4.55</td>
<td>0.686</td>
</tr>
</tbody>
</table>

* The data in Fig. 5 was used to calculate formulae for the straight lines in the double-reciprocal plot by regression analysis; the $V_{\text{max}}$ values were then determined from the reciprocals of the $y$-intercepts and the $K_m$ values from the negative reciprocals of the $x$-intercepts.

11 % and 22 % respectively (Meuth & Green, 1974). A concentration of $1 \times 10^{-5}$ M-bromo-deoxyuridine was used in our experiment, as one that would give reasonably high substitution in labelled DNA and yet have only a minimal effect on endogenous synthesis.

Cells were pulse-labelled with 3H-bromodeoxyuridine at 8 h after infection. The DNA was extracted and sedimented to equilibrium in alkaline CsCl. 14C-pre-labelled DNA from the same cells was used as normal density marker. There is a decrease in the amount of 3H incorporated into DNA of infected cells, but no change in the density of the DNA (Fig. 3). This shows that the proportion of exogenous nucleotide in the thymidine triphosphate pool remains unchanged in infected cells. The experiments in Table 2 and Fig. 3 provide strong evidence that pool dilution does not occur in infected cells.

**Phosphorylation of thymidine**

Another aspect of thymidine metabolism that can be examined in infected cells is the phosphorylation of thymidine to thymidine triphosphate. A block in the formation of thymidine triphosphate could cause a decrease in the size of the thymidine triphosphate pool and lead to decreased DNA synthesis. We examined this in cells-labelled for 5 min with 3H-thymidine at 8 h after infection. The distribution of radioactivity among thymidine and its nucleotides in acid-soluble extracts was determined by paper chromatography. Over 90 % of the exogenous label is in the form of thymidine triphosphate in control and infected cells, despite a 50 % decrease in the amount of label in the extract (Fig. 4). This shows that phosphorylation of thymidine proceeds to the same extent in control and infected cells.

**Uptake of exogenous thymidine**

A block in the uptake of exogenous thymidine in infected cells could lead to a decrease in incorporation of thymidine into DNA. The incorporation of 3H-thymidine into total cellular material at several different concentrations was measured in infected cells during a short pulse at 8 h after infection. At all concentrations tested, incorporation was reduced (Fig. 5a). A double-reciprocal plot of the data (Fig. 5b) shows that this inhibition is apparently competitive. There is a more than fourfold increase in the apparent $K_m$ with little alteration in the $V_{\text{max}}$ in infected cells (Table 3).

**DISCUSSION**

We have found a decrease in DNA and histone synthesis in infected cells. This is accompanied by a reduction in the size of the thymidine triphosphate pool and in the uptake of thymidine into the cell. The elongation of nascent DNA chains, the phosphorylation of thymidine to thymidine triphosphate, and the amount of exogenous nucleotide in the thymidine triphosphate pool are normal.
These findings show that several aspects of thymidine metabolism and DNA synthesis are altered in infected cells. The question arises as to which of these alterations is primary. We feel it is the inhibition of DNA synthesis. The block to DNA synthesis is at the level of initiation, since chain elongation, as measured by replication fork progression and maturation to full size DNA, is normal (Ensminger & Tamm, 1969b; Hand & Tamm, 1972; present report). If the primary alteration in infected cells were at the level of precursor synthesis or histone metabolism then it is most likely that all the steps in DNA replication would be inhibited, not initiation alone. The reductions in histone synthesis, thymidine triphosphate pool size and thymidine uptake, therefore, are probably secondary to the block in DNA synthesis.

Because of the long pulse time (5 min) in the experiment on thymidine uptake, we cannot distinguish between a block in transport and an inhibition of thymidine kinase. Equilibrium of free thymidine occurs within 20 to 40 s in mammalian cells and the rate-limiting step for incorporation into the nucleotide pool is phosphorylation (Wohlheuter et al. 1976). Infection does not block phosphorylation during a 5 min pulse and the kinase activity in infected cells is normal (Ensminger, 1969). Although these observations suggest that thymidine kinase activity is unaffected by reovirus, they do not exclude the possibilities that infected cells contain an altered form of the kinase with reduced activity of a competitive inhibitor of kinase activity. Regardless of the mechanism, the reduced uptake of thymidine into the cell is almost certainly a secondary reaction since the size of the triphosphate pool is reduced in the absence of exogenous thymidine and incorporation into DNA is reduced even at concentrations of exogenous thymidine that are one to two orders of magnitude greater than the apparent $K_m$ in infected cells.

It has been suggested that the reduction in DNA synthesis in infected cells is secondary to the inhibition of protein synthesis (Joklik, 1974). A number of reports (Ensminger & Tamm, 1969a, b, 1970; Cox & Shaw, 1970; Hand & Tamm, 1972, 1974; Shaw & Cox, 1973; Cox & Clinkscales, 1976) have shown a decrease in DNA synthesis of at least 50% at 8 h post infection or earlier. Cellular protein synthesis is not reduced 50% until 10-5 h after infection (Zweerink & Joklik, 1970). Thus the changes in DNA synthesis in infected cells preceded the inhibition of protein synthesis by several hours. In addition, the inhibition of protein synthesis causes an immediate decrease in the rate of replication fork progression (Weintraub & Holtzer, 1972; Gautschi & Kern, 1973), an effect not found in reovirus-infected cells (Hand & Tamm, 1972). The evidence favours the view that the virus-induced inhibition of DNA synthesis is independent of the inhibition of cellular protein synthesis.

The changes in DNA metabolism and histone synthesis described here suggest that the nucleus is an early target for reovirus-induced cytopathology. Although the presence of a nucleus is not absolutely required for reovirus synthesis (Follett et al. 1975), there is evidence that it is involved in virus replication. High doses of actinomycin D, which presumably inhibit DNA-dependent RNA synthesis, inhibit virus RNA synthesis (Shatkin, 1965; Silverstein et al. 1974). The inhibition of histone synthesis may also involve nuclear function since it occurs before the inhibition of overall protein synthesis. The block therefore could be prior to translation of histone messenger, perhaps at some step associated with the synthesis of histone messenger. The reduction in DNA synthesis and the thymidine triphosphate pool size are most probably also associated with nuclear pathology. Morphological changes in nuclei have been found in brain cells from suckling rats inoculated intracerebrally with reovirus (Margolis et al. 1975); granular aggregates have been observed within these nuclei. These are not virus particles but rather nuclear bodies, structures found in a number of pathological conditions and in particular, virus infections. These structures are not observed
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in reovirus-infected cells in culture. But our present findings suggest that there may be extensive biochemical damage to the nucleus early in infection.

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