Fluctuations in Nucleoside Uptake and Binding of the Inhibitor of Nucleoside Transport, Nitrobenzylthioinosine, during the Replication Cycle of HeLa Cells

Carol E. Cass, Ewa Dahlig, Eda Y. Lau, Thomas P. Lynch, and Alan R. P. Paterson

ABSTRACT

Binding of the potent nucleoside transport inhibitor 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (NBMPR) and rates of uptake of several nucleosides were examined at 4-hr intervals during the replication cycle of HeLa S3 cells. Monolayer cultures of synchronous cells, obtained by mitotic detachment, were assayed for high-affinity binding of NBMPR and for rates of uptake of thymidine, uridine, cytidine, adenosine, inosine, and guanosine. The number of NBMPR binding sites per cell doubled between 4 and 16 hr after detachment (late G1 and S phase); during this interval, V_max's for uptake of cytidine and adenosine doubled, and for uridine and thymidine uptake increased about 4- and 8-fold, respectively. Rates of inosine and guanosine uptake at extracellular concentrations below saturation increased 2-fold between G1 and S phase of the cell cycle. K_m's for cellular uptake of thymidine, uridine, cytidine, and adenosine did not change with progress through the cycle. The results presented suggest that changes in nucleoside uptake during the HeLa cell cycle were due, in part, to changes in the activity of NBMPR-sensitive transport elements in the membrane.

INTRODUCTION

The entry of nucleosides into animal cells is mediated by nucleoside-specific “transporter” elements of the plasma membrane (1). Earlier studies showed that uridine and thymidine permeation in human erythrocytes did not involve the metabolism of either permeant and were mediated by a nucleoside transport mechanism which accepted both purine and pyrimidine nucleosides (6, 7, 22). Taube and Berlin (34) have shown that rabbit polymorphonuclear leukocytes possess a nucleoside transport mechanism with low specificity for the base moiety of permeants. The existence in cultured Novikoff hepatoma cells of a nucleoside transport mechanism with low specificity has been demonstrated in ATP-depleted cells (28, 37); it appears to be more complex than in erythrocytes. In the presence of 5 nM NBMPR, the binding sites are almost fully occupied by KB at about 1 nM) to specific sites in the plasma membrane (5, 8). These sites, about 1.0 to 1.5 x 10^4/cell, are apparently associated with the nucleoside transport mechanism since inhibition of uridine transport by erythrocytes was proportional to the number of sites occupied by NBMPR (5). The potent inhibition by NBMPR of nucleoside uptake in a variety of cell types, including HeLa cells, evidently occurs at the transport step of the uptake process (9, 12, 14, 24, 25, 30).

Although HeLa cells also possess binding sites with high affinity for NBMPR (K_B about 0.1 nM) (18), the relationship between NBMPR binding and transport inhibition appears to be more complex than in erythrocytes. In the presence of 5 μM NBMPR, 50% of uridine sites are occupied by NBMPR, yet a substantial transport capacity (about 25%) for uridine remains functional; in the presence of 5 μM NBMPR, uridine transport is eliminated.

As cells progress through the replication cycle, marked changes occur in uptake rates of uridine (33) and thymidine (15, 29, 32). To determine whether cycle-related changes in properties of the nucleoside transport mechanism could also be perceived, the binding of NBMPR and the uptake kinetics of several nucleosides were assayed at various points in the replication cycle of HeLa cells synchronized by mitotic detachment (35). A rapid sampling technique was used to measure initial rates of nucleoside uptake; for nucleosides other than thymidine, initial uptake rates were rather than transport, is probably the rate-determining event in the thymidine uptake process (21, 28). The initial rate kinetics of nucleoside uptake by HeLa cells indicated that the uptake processes for adenosine (24), uridine-cytidine (25), and inosine-guanosine are saturable and kinetically distinct, suggesting that these processes are independent or have unique rate-limiting steps.

In studies with human erythrocytes, NBMPR and related compounds were recognized as potent inhibitors of nucleoside transport (6, 7, 26, 27) which bind with high affinity (K_B about 1 nM) to specific sites in the plasma membrane (5, 8). These sites, about 1.0 to 1.5 x 10^4/cell, are apparently associated with the nucleoside transport mechanism since inhibition of uridine transport by erythrocytes was proportional to the number of sites occupied by NBMPR (5). The potent inhibition by NBMPR of nucleoside uptake in a variety of cell types, including HeLa cells, evidently occurs at the transport step of the uptake process (9, 12, 14, 24, 25, 30).

Although HeLa cells also possess binding sites with high affinity for NBMPR (K_B about 0.1 nM) (18), the relationship between NBMPR binding and transport inhibition appears to be more complex than in erythrocytes. In the presence of 5 μM NBMPR, the binding sites are almost fully occupied by NBMPR, yet a substantial transport capacity (about 25%) for uridine remains functional; in the presence of 5 μM NBMPR, uridine transport is eliminated.

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constant during the assay intervals and, accordingly, have been taken as a measure of transport rates.

MATERIALS AND METHODS

Cell Culture. Stock cultures of HeLa S3 cells were maintained as monolayers in antibiotic-free MEM-M with subculture at weekly intervals. After 7 or 8 subculture generations, such cultures were restarted from Mycoplasma-free frozen stocks. Trypsinized monolayer cultures provided inocula for suspension cultures in MEM-S medium. Suspension cultures were initiated in spinner flasks, expanded into 5-liter round-bottomed flasks and, in the latter, kept under continuous agitation with vibrating mixers to prevent cell clumping (Vibro-Mixer, Model E1; Chematec, Hoboken, N. J.). In the suspension cultures, cell concentrations were kept below 5 x 10^6 cells/ml, and cell proliferation was exponential with doubling times of about 20 hr.

Mitotic Selection Procedure for Synchronization. Synchronous HeLa cells were obtained by selective detachment of mitotic cells from logarithmically growing monolayer cultures in roller bottles (growth area, 1330 sq cm; Bellico Glass, Vineland, N. J.). In the procedure described below, all operations were performed at 37°. Roller bottles were “conditioned” by exposure to MEM-M for 48 hr at 0.4 rpm, and then each was inoculated with 2 x 10^6 cells (Vibro-Mixer culture) in 600 ml of MEM-S to which were added calcium salts and calf serum to obtain concentrations of 0.9 mM and 7.5%, respectively. The cultures were gassed (10% CO2 in air) and incubated at 0.4 rpm; after 24 hr, when the mitotic selection procedure began, such cultures were almost confluent and had doubling times of about 20 hr.

Mitotic cells were selectively detached from the roller bottle monolayers by liquid shearing forces applied by subjecting the cultures to abrupt rotations. As a first step in this procedure, roller cultures with their original growth medium were subjected to 20 abrupt notations without clumping (Vibro-Mixer culture) in 600 ml of MEM-S with final concentrations of calf serum and calcium salts adjusted to 10% and 1.8 mM, respectively, and then incubated at 37° in air with 5% CO2. The medium in each bottle was replaced 3 hr after plating to remove unattached cells (25 to 40% of the inoculum). Progress through the cell cycle in each set of replicate cultures was evaluated by determination of some or all of the following parameters: (a) cell concentrations and volume, using an electronic particle counter with a multichannel size analyzer (Coulter Electronics, Hialeah, Fla.); (b) cellular protein content (16); and (c) rate of incorporation of [3H]thymidine into acid-insoluble material. For the latter, cultures were incubated for 15 min in MEM-M containing [methyl-3H]thymidine (time courses of thymidine incorporation were linear), washed once with ice-cold 0.15 M NaCl containing 100 µM thymidine, and then extracted for 10 min with cold 5% perchloric acid. After digestion in NCS tissue solubilizer (Amersham/Searle, Arlington Heights, Ill.), the acid-insoluble residues were dissolved in Bray’s counting solution (2) for assay of 3H by liquid scintillation counting.

Nucleoside Uptake. At various intervals after mitotic selection, sets of replicate cultures (each from a particular mitotic harvest) were used to evaluate kinetic parameters of nucleoside uptake. The following procedure was used to measure rates of nucleoside uptake at 37° from MEM-T medium. Each culture (2 assay) was processed individually. Growth medium was aspirated, and 4.0 ml of MEM-T containing 3H-labeled nucleoside were added to the cell-free side of the bottle; to initiate the uptake interval, the cell sheet was immersed by rotating the bottle 180° about its long axis. MEM-T was removed by rapid suction 5 sec before ending the uptake interval by flooding the cell sheet with 60 ml of warmed 0.15 M NaCl containing 5 µM NBMPR, and after 30 sec the bottle was thoroughly drained. NCS digests of cell sheets were dissolved in Bray’s counting solution for assay of 3H content.

Initial rates of nucleoside uptake, interpreted as transport rates for nucleosides other than thymidine, were determined from cellular uptake of 3H-labeled nucleosides during intervals of 10 to 60 sec; under the assay conditions, time courses of uptake were linear and extrapolated through time. Time courses of thymidine uptake were not linear during the 0-to-10-sec interval; because the interval of uptake in the assay procedure used could not be less than 10 sec, initial rates were not measured. The thymidine uptake rates here presented were measured during intervals of linear uptake (10 to 60 sec) and have been interpreted as a measure of the rate-limiting step in thymidine metabolism. 

* Plating efficiencies of 60 to 75% were not due to decreased viability resulting from the synchrony procedure because (a) asynchronous HeLa S3 cells exhibited similar plating efficiencies and (b) synchronous cells, when harvested as above and cultured in spinner flasks, increased in number almost 2-fold at the next mitosis.

* In previous work (8), the rapid accumulation of thymidine during the first 10 sec was attributed to nonspecific absorption; however, recent results suggest that thymidine transport is so rapid that uptake techniques such as those described here cannot be used to measure rates of thymidine transport (28, 37).
NBMPR Binding. Each assay of [35S]NBMPR binding used 3 replicate monolayer cultures in the following way. To initiate an interval of binding, monolayers were immersed in 4 ml of MEM-T containing [35S]NBMPR at 37°; 10 sec before ending such intervals, medium was removed, and a portion was retained for determination of 35S content. Assay intervals were terminated by flooding cell sheets with cold 0.15 mM NaCl. After thorough draining, cell sheets were dissolved in 2.0 ml of 0.5 N KOH, a single portion of which was passed through each of the 3 bottles used per assay condition (this "cascade" procedure was used to increase the sensitivity of the binding assay, which was limited by the specific activity of [35S]NBMPR). The bottles were rinsed with detergent-fluor solution (23) prior to determination of the 35S content of the KOH digests by liquid scintillation counting. Assays of [G-3H]NBMPR binding were similar except that single cultures were used and each assay was conducted in duplicate.

Kinase Activities. Extracts prepared from monolayer cultures in the following way were assayed for pyrimidine nucleoside kinase activities. Cultures were first washed with cold 0.15 M NaCl, 1 ml of extraction buffer [10 mM NaF: 0.01 M KCl: 2 mM dithioerythritol: 25 mM 6-aminopropionic acid: 0.01 M Tris-HCl, pH 7.5:10% glycerol (19)] was added to each bottle, and the cells were lysed by 4 cycles of rapid freezing and thawing. After centrifugation (for 4 min at 4°, 13,000 x g), supernatant fractions were reserved for determination of protein content (16) and kinase activities (19). In the latter assay, incubation mixtures contained 30 μM [methyl-3H]thymidine, [5-3H]uridine, or [5-3H]cytidine and were incubated at 37° for 30 min prior to isolation of nucleoside phosphates on squares of DEAE-cellulose paper (17), the 3H content of which was determined by liquid scintillation counting after oxidation in a Packard Model 306 sample oxidizer.

Materials. Dr. S. R. Naik prepared NBMPR from 6-thiinoisine provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. The preparation of [35S]NBMPR has been described previously (18). [G-3H]NBMPR was obtained from Moravek Biochemicals, City of Industry, Calif., and other radioisotopic materials were obtained from Amersham/Searle. [2-3H]Adenosine was deaminated with crystalline adenosine deaminase (Sigma, St. Louis, Mo.), and the product, [2-3H]inosine, was purified by chromatography on cellulose thin layers developed in ethyl acetate:isopropyl alcohol:water (65:22.5:12.5, v/v). Radiochemical purity of labeled compounds was routinely verified by chromatography before use. Cell material materials were purchased from Grand Island Biological Co., Calgary, Alta.

RESULTS

The synchrony achieved in the replicate monolayer cultures established with mitotic cells is apparent in Chart 1, which depicts typical results. Under these conditions, the HeLa cell cycle time was about 21 hr, with G1, S, and G2 + M phases of approximately 7, 12, and 2 hr, respectively.

Rates of nucleoside uptake by the synchronous cultures at 37° were measured at various times during the HeLa replication cell. Entire synchrony experiments were committed to determination of the uptake kinetics for individual nucleosides. Six to 10 successive harvests from 8 to 10 roller cultures were used. Sets of replicate monolayer cultures, each derived from a single harvest of mitotic cells obtained by selective detachment; midway through one harvest, the mitotic index was 95%. A, cultures from 2 replicate sets (A, O), established from successive mitotic collections, were assayed at various times after detachment for [3H]thymidine incorporation (pmol/15 min/106 cells) into acid-insoluble material. B, cultures from a single replicate set were assayed for the other parameters specified. Each point represents the average of 2 to 5 samples.

Changes in Vmax's for uptake of thymidine and uridine during the HeLa cell cycle are illustrated in Chart 2, where Vmax's are plotted as a function of time after mitotic selection. As the monolayer cultures progressed from G1 to S phase of the cell cycle, Vmax's for uridine uptake were determined 12 hr after harvest; the values obtained with cultures from >4 successive harvests were similar (within experimental error).

Changes in Km's for uptake of thymidine and uridine during the HeLa cell cycle are illustrated in Chart 2, where Vmax's and Km's for thymidine uptake were determined 12 hr after harvest; values obtained with cultures from >4 successive harvests were similar (with similar experimental error).

The data of Chart 3 illustrate the influence of cell cycle stage on initial uptake rates of several other nucleosides, each at a particular concentration for which it was known that uptake rates were constant during the interval of the uptake assay. It is apparent from the results of Chart 3A that the uptake rates of inosine and guanosine, which share the same uptake mechanism in HeLa cells, increased sharply as cells progressed through the replication cycle. The uptake rates of adenosine (Chart 3B) and cytidine (Chart 3C) also increased as cells moved from G1 through S phase of the cycle.

Kinetic constants determined at 4 and 16 hr after mitotic selection (G1, and late S phase, respectively) for the uptake
of adenosine, cytidine, thymidine, and uridine are listed in Table 1. $K_m$'s for these uptake processes did not change significantly through the cell cycle. Thus, cellular capacity for phosphorylation of thymidine, but not of cytidine and uridine, increased substantially as cells moved through the DNA-synthetic phase of the replication cycle.

The time course of $[^{35}S]$NBMPR binding to asynchronous monolayer cultures is shown in Chart 5; at low NBMPR concentrations, about 20 min were required to establish equilibrium between bound and free NBMPR. This interval was used in binding assays with synchronous cells. The amount of NBMPR bound per cell was independent of cell density over the range used for experiments reported here (Chart 5A, inset).

Saturable binding (Chart 5B) of $[^{35}S]$NBMPR was prevented by the presence of 5 μM NBTGR, an inhibitor of nucleoside transport with potency comparable to NBMPR.
Kinetic constants for nucleoside uptake during G₁ and S phases of the HeLa cell cycle

Using graded concentrations of the nucleosides listed, rates of uptake at 37° were determined at 4 and 16 hr after mitotic selection with replicate monolayer cultures as described in Chart 2 and the text, and \( K_m \)'s and \( V_{max} \)'s were estimated from linear plots of reciprocals of uptake rates and substrate concentrations. Results are from 7 separate synchrony experiments, and each pair of \( K_m \)-\( V_{max} \) values was obtained from a set of replicate monolayer cultures prepared from a single mitotic harvest. In the last column are presented the ratios of \( V_{max} \)'s in S phase to those in G₁ phase for each of the 7 experiments.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( K_m (\mu M) )</th>
<th>( V_{max} ) (pmol/min/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 hr</td>
<td>16 hr</td>
</tr>
<tr>
<td>Thymidine</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Uridine</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Cytidine</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

\( a \) \( V_{max} \)'s from the experiment of Chart 2A.
\( b \) Uptake rates were determined at 0.08, 0.10, 0.13, 0.20, 0.33, and 1.0 \( \mu M \).
\( c \) \( V_{max} \)'s from the experiment of Chart 2B.
\( d \) Uptake rates were determined at 0.57, 0.67, 1.0, 2.0, 10, and 20 \( \mu M \).
\( e \) Uptake rates were determined at 0.67, 0.8, 1.0, 1.33, 2, and 4 \( \mu M \).

The cell content of NBMPR in the presence of NBTGR (Chart 5B) was proportional to NBMPR concentration and evidently represented nonspecific association of NBMPR with cells. A similar result was obtained previously in studies with asynchronous HeLa cells in suspension culture (18) and with human erythrocytes (5, 8).

Scatchard plots (not shown) of the NBTGR-sensitive component of binding in the experiment of Chart 5B were linear, suggesting that NBMPR binding sites were of a single type. Values obtained from mass law analysis of binding data for total occupancy of the saturable sites and the apparent \( K_d \) for site-bound NBMPR were, respectively, 0.3 pmol/10⁶ cells (about 2 \( \times 10^{10} \) sites/cell) and 0.7 \( \times 10^{10} \) M. The cell content of NBMPR in the presence of NBMPR with cells. A similar result was obtained previously in studies with asynchronous HeLa cells in suspension culture (18) and with human erythrocytes (5, 8).

The site-specific binding of NBMPR (NBTGR-displaceable) increased as cells progressed from G₁ through S phase (Chart 6), with an apparent doubling in the number of NBMPR-binding sites per cell between 4 and 16 hr after mitotic selection (Chart 6C). \( K_d \) values, determined from results of Chart 6D, did not change significantly, suggesting little change in affinity for NBMPR during the cell cycle. A similar result was obtained in experiments with the \([35S]arabinosyl homolog (6-[(4-nitrobenzyl)thio]-9-β-D-ara-

\[ \text{DISCUSSION} \]

This work was undertaken to assess variation in transport

\[ \text{11} \] Studies of binding of the \([35S]arabinosyl homolog of NBMPR to synchronous HeLa monolayers provided \( K_d \) values of 2 to 3 nm at 4 and 16 hr after mitotic detachment; the number of high-affinity sites increased about 2-fold from 2.3 \( \times 10^{10} \) to 4.2 \( \times 10^{10} \) sites/cell (A. R. P. Paterson and C. E. Cass, unpublished results).

\[ \text{12} \] The results cited in Table 2 are not comparable to the results cited in Table 1 and Chart 2; in the experiment of Table 2, freshly harvested mitotic cells were plated at room temperature, and the uptake measurements were conducted at 20°, whereas in the experiments of Table 1 and Chart 2 all manipulations (mitotic detachment, plating, and uptake measurements) were conducted at 37°.
of nucleosides during the replication cycle of HeLa cells. With the exception of thymidine, initial uptake rates have been taken as a measure of nucleoside transport rates. Because the binding sites with high affinity for NBMPR are evidently closely associated with, or are part of, the nucleoside transporter(s), cycle-dependent changes in NBMPR binding provided additional evidence of changes in transporter activity during the cell cycle.

The rate of utilization of exogenous nucleosides is clearly influenced by position in the cell cycle, with the maximum uptake activities exhibited during mid to late S phase. Since K_m's for uptake did not change during the cell cycle, increased uptake rates were apparently due to increased activity of the various components of the uptake processes. Assuming that initial uptake rates measure transport rates for adenosine, cytidine, guanosine, inosine, and uridine, the activity of transporters for these nucleosides increased about 2-fold during the cell cycle, with the exception of the uridine transporter which experienced an apparent 4-fold increase in activity. The S phase increase in thymidine uptake rates reported here cannot be attributed to changes in transport activity since initial rates of thymidine uptake were not measurable with the assay procedure used.

The number of NBMPR-binding sites per HeLa cell was estimated by quantitation of cell-bound [35S]NBMPR under saturating conditions and by mass law analysis of data measuring the influence of NBMPR concentration on binding. Asynchronous, G1, and S-phase cells possessed between 2 and 7 x 10^5 sites/cell. Somewhat lower values were obtained previously for [35S]NBMPR binding at 37° to asynchronous HeLa cells from spinner cultures (18), possibly because such cells are smaller and have a lower protein content than do HeLa monolayer cells.13

The number of NBMPR-binding sites per cell increased about 2-fold between 4 and 16 h after mitotic selection without significant change in the apparent K_m's for NBMPR; similar results were obtained with the arabinosyl homolog of NBMPR. It is not known whether such increases are due to activation of preexisting sites or to production of new sites as membrane biosynthesis proceeds in preparation for cell division. Assuming that cellular capacity for high-affinity binding of NBMPR is related to the number of active nucleoside transporters present, the observed S-phase doubling of the cellular content of binding sites (without change in their affinity for NBMPR) indicates that the number of transport elements was doubled in S phase and that transport rates increased correspondingly. Since the cycle-dependent increase in thymidine uptake rates was abolished by excess NBMPR, the mechanism by which thymidine is transported also underwent an increase in activity comparable to that of the other nucleoside transporter(s).

Thymidine kinase activity in HeLa monolayer cultures increased significantly, as others have noted (3, 4, 36), as cells moved through S phase. Cytidine and uridine are thought to be phosphorylated by the same enzyme (20), and, consistent with this view, the specific activity of cell extracts for phosphorylation of both uridine and cytidine changed little during the HeLa cell cycle. While it is not meaningful to compare directly data obtained in studies with intact cells and cell extracts, changes in cellular content of nucleoside kinases also must contribute importantly to cycle-dependent fluctuations in nucleoside uptake. Increased activity of thymidine kinase has long been considered to be important to the utilization of exogenous thymidine as cells enter the DNA-replicative phase of the cell cycle.

The observed 4-fold increase in uridine uptake capacity during the HeLa cell cycle, which we suggest may result from an increase in transport activity, appears to be inconsistent with the apparent doubling of the cell content of NBMPR-sensitive sites when a simple proportionality between the latter and transporter activity is assumed. How-

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14 A similar result was obtained in a separate study with synchronous HeLa cells in suspension cultures (Y-C. Cheng, C. Chang, M. V. Williams, C. E. Cass, and A. R. P. Paterson, unpublished results).
ever, relationships between the nucleoside transporter(s) and the NBMPR-inhibitory sites have not been defined, and it is not known whether HeLa cells possess a single class of NBMPR-sensitive nucleoside transporters of low specificity or several types of NBMPR-sensitive transporters with different permeant specificities.

Increases in rates of uridine uptake were observed when growth-inhibited hamster or mouse fibroblasts were stimulated to proliferate by serum or insulin (12-14, 30, 31); within minutes of serum addition, uridine uptake rates were increased severalfold by a mechanism which was not sensitive to inhibitors of protein synthesis. These increased rates have been attributed to (a) changes in the activity of the membrane transport mechanism without change in the number of transporters (12) or (b) changes in the rate of intracellular phosphorylation of uridine without significant change in transporter activity (30, 31). These alternate interpretations assumed, respectively, that the rate-limiting step in uptake of uridine is (a) transport (12) or (b) phosphorylation (30, 31).

In conclusion, our results suggest that the increase in cellular uptake of nucleosides as HeLa cells progress through S phase was achieved through increase in number, perhaps through activation of latent preexisting transporters, or synthesis of new transporters and, for thymidine, through increase in cellular kinase activity.
Table 2

Inhibition of the increase in thymidine uptake during the HeLa cell cycle by NBMPR

<table>
<thead>
<tr>
<th>Time after mitotic selection</th>
<th>Thymidine uptake (pmol/10^6 cells)</th>
<th>-NBMPR</th>
<th>+NBMPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.9</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.4</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>4.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>7.1</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>5.1</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

Whether the mechanisms responsible for changes in nucleoside uptake rates during the cell cycle are similar to those in serum stimulation of quiescent cells is not known; in the latter, changes in uptake rates were rapid, occurring within minutes of the proliferative stimulus. Cell lines such as HeLa S3 do not exhibit density-dependent inhibition of growth characteristic of the mouse and hamster fibroblasts used in the studies of Rozengurt and Stein (30), Rozengurt et al. (31), and Eilam and Bibi (12).

REFERENCES


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