The Relationship between Polyamine Accumulation and DNA Replication in Synchronized Chinese Hamster Ovary Cells after Heat Shock

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SUMMARY

The relationship of polyamine accumulation and semiconservative DNA replication was studied in synchronous Chinese hamster ovary cultures, progressing through the cell cycle either normally at 37° or after hyperthermic exposure (43° for 1 hr) during G1 or S phase. In control cultures, intracellular polyamine levels decreased as cells divided and then reaccumulated as cells exited G1 and proceeded through the S and G2 phases. Immediately after cultures were exposed to 43° heat for 1 hr in G1 phase, intracellular levels of spermidine and spermine were reduced compared to controls. Coordinate with the depletion of the intracellular levels of these polyamines following exposure at 43°, extracellular levels of spermidine and spermine were increased. The ratio of intracellular to extracellular amounts of both these polyamines changed from 1 to 1.5 to approximately 0.2 to 0.3 after hyperthermic exposure. These cultures exposed to 43° heat during G1 initially showed depressed levels of replicated DNA, but near-control rates of DNA replication were attained in a temporally related manner with the reaccumulation of intracellular spermidine and spermine levels. When cultures were exposed to 43° heat in S phase, intracellular amounts of spermidine and spermine were again reduced, and increased extracellular levels of these polyamines were observed. In these S-phase-treated cultures, cells were able to continue replicating their DNA but at a much reduced rate compared to controls. These results and others show that: (a) exposure of cells at 43° causes a depletion of intracellular levels of spermidine and spermine, suggesting that an immediate aspect of thermal damage is a membrane defect that markedly affects the transport of these molecules across cell membranes; (b) exposure of either G1- or S-phase cultures to 43° heat causes a depression of bulk DNA-synthetic rates resulting in a prolongation of S phase; and (c) the intracellular reaccumulation of spermidine and spermine following exposure of G1 cells to a 43° heat shock is temporally related to the recovery of near-normal DNA synthetic rates in these cells.

INTRODUCTION

The potential clinical importance of hyperthermia as an anticancer agent, used either alone or as an adjuvant to radiation or chemotherapeutic drugs, derives from an extensive number of reports in both the scientific and medical literature dating back to the last century (see Refs. 7, 17, and 27 for current reviews). It is now well documented that exposure of cells in culture to temperatures above 41° results in a loss of proliferative capacity (6, 10, 30). An increasing number of reports suggest that cancer cells may be more sensitive to thermal killing than normal cells (2, 13). However, the biochemical nature of hyperthermia-induced loss of cellular proliferative capacity is still poorly understood. We have chosen to study polyamine biosynthesis after heat shock because of previous work which has presented strong evidence demonstrating a close correlation between intracellular polyamine levels and cell growth in vivo and in vitro (3, 11, 22, 23) and because the specific steps in polyamine biosynthesis are fairly well understood. Intracellular spermidine levels have been shown to correlate well with RNA content (3, 4, 19), while spermine seems to parallel DNA amounts (24). Thus, the observation that hyperthermic exposure inhibits RNA (26) and DNA synthesis (18, 18) lends further rationale to the study of polyamines in order to understand the mechanisms of heat-shock-induced loss of cellular proliferative potential.

In this study, the effects of hyperthermia on polyamine biosynthesis and accumulation are compared with parallel studies measuring rates of semiconservative DNA replication, which we have used as a measure of cell cycle progression. Our results suggest that the major immediate effects of hyperthermia on cell progression may derive from a cell membrane defect resulting in the loss of molecules, including the polyamines, which are necessary for normal cell growth rates.

MATERIALS AND METHODS

Cell Culture Procedures. CHO cells were grown as monolayer cultures in McCoy’s 5A Medium supplemented with 20% fetal calf serum (both from Grand Island Biological Co., Grand Island, N. Y.). The growth medium also contained,

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3 The abbreviation used is: CHO, Chinese hamster ovary.
per ml, 100 units penicillin, 0.25 μg Funginone, and 100 μg streptomycin. Cultures were maintained in exponential
growth at 37° in humidified 5% CO₂-95% air incubators. Cell
doubling times were 13 to 15 hr under these conditions. Cell
numbers were determined using an electronic particle
counter (Coulter Electronics, Hialeah, Fla.). Cells were syn-
chronized by the selective detachment of cells in mitosis
(28) which were then cold-accumulated to obtain a large
number of cells at the beginning of each experiment (29).
These cells (mitotic index greater than 85%) were then
plated into T-25 flasks (Falcon Plastics, Oxnard, Calif.) con-
taining medium at 37° and allowed to progress through the
cell cycle. In cultures used to measure intra- and extracellu-
ar amounts of polyamines, the medium contained 10⁻⁸ M
amoguanidine bicarbonate (Sigma Chemical Co., St.
Louis, Mo.) to inhibit polyamine degradation by diamine
oxidase (25) present in the serum. This treatment did not
alter intracellular polyamine amounts or culture doubling
time for at least 48 hr after addition of amoguanidine.
Cultures were heated to 43° in the T-25 flasks by immer-
sion of the sealed flasks in a temperature-controlled water
bath (temperature constant to ±0.1°) as previously de-
scribed (6). In all experiments, cells were harvested from
these flasks by scraping with a rubber policeman.

Measurement of DNA Replication Kinetics. This proce-
dure has been described in detail in previous reports (8, 15).
Prior to synchronization, cultures were grown for 20 to 24 hr
in medium containing [¹⁴C]thymidine, 0.15 μCi/ml (53 mCi/ mmole; Schwartz/Mann, Orangeburg, N. Y.). After synchro-
nization and hyperthermic treatments, the culture medium
was adjusted to 50 μg/ml for 5-bromodeoxyuridine and 0.1
μg/ml for 5-fluorodeoxyuridine. Sample plates were then
harvested at various times after treatment with the cell
pellets stored at −15° in buffer containing 0.15 M NaCl:0.01 M
EDTA:0.01 M Tris, pH 8. Deproteinized DNA was obtained
by extraction in chloroform:i-soamyl alcohol (24:1). The per-
centage of DNA replicating semiconservatively at any time
after treatment was determined by measuring the propor-
tion of [¹⁴C]DNA that acquired hybrid density in CsCl gra-
dients [4.0 ml of 62.8% (w/w) CsCl in 0.01 M Tris at pH 8.1
and 0.7 ml of deproteinized DNA per sample]. Centrifuga-
tion was for 45 hr at a SW 50.1 rotor at 33,000 rpm (20°) in
a preparative ultracentrifuge. Density information was ob-
tained by measuring the refractive indices of samples, and
radioactivity was assayed on a Searle Mark II liquid scintilla-
tion spectrometer.

Determination of Polyamine Concentrations. This
method has also been previously outlined in detail (14, 21).
Briefly, intracellular polyamine levels were determined as
follows. Cells (5 x 10⁶/sample) were disrupted by sonica-
tion with an E/MC Corporation ultrasonic cell disruptor
(4.5-inch probe) in 400 μl of 10% trichloroacetic acid at 0.2°.
The homogenates were centrifuged at 10⁴ x g for 15 min. A
75-μl aliquot was then placed on a Durrum Model D-500
automatic amino acid analyzer for assessment of putres-
cine, spermidine, and spermine concentrations (14, 21).

To assay for extracellular polyamines, 3 ml of medium
were washed with 2 ml of cold 10% trichloroacetic acid and
vortexed. Samples were centrifuged at 20,000 rpm in a
Sorvall SS-34 rotor for 15 min. The resulting pellet was
resuspended in 1 ml of 10% trichloroacetic acid and centri-
fuged as before, and the supernatants from both spins were
combined. Following 2 washes of these supernatants with
15 ml of anhydrous ether, an equal volume of 12 n HCl was
added. This solution was then sealed in an extraction tube,
hydrolyzed for 16 hr at 110°, and then dried to powder form.
Samples were redissolved in 200 μl of 0.1 n HCl with 75-μl
aliquots assayed as stated before. [¹⁴C]Spermidine was
used to monitor recovery rates.

RESULTS

DNA Replication Kinetics following Hyperthermic Expos-
sures during G₁ or S Phase. Exposure of exponentially
growing CHO cells to 43° heat for 1 hr reduces single-cell
survival (as measured by colony-forming ability) to approxi-
mately 20% under the conditions reported here (see Ref. 30
for complete hyperthermic survival data for this cell line).
While 80% of the cells exposed to this heat shock may
ultimately die, Chart 1 demonstrates that exposure at 43°
either during G₁ (3 to 4 hr after mitosis) or S phase (9 to 10 hr
after mitosis) does not result in immediate cell lysis, since
there is no appreciable decrease in cell numbers in the
treated cultures compared to controls. In both instances,
cell division is inhibited. By 20 to 25 hr after mitosis, a small
number of treated cells have begun to divide, in contrast to
cells growing normally at 37°, which have completed 1
division by 16 hr after mitosis. Cell numbers in the treated
cultures continue to increase after 20 to 25 hr, finally reach-
ing twice the original number by 48 hr after mitosis. During
this time, from 20 to 50 hr after mitosis, the cell number
increase in the treated cultures probably reflects both lysis
of dead or dying cells as well as division of viable cells.

Chart 2 shows the [¹⁴C]DNA profiles after centrifugation
on CsCl gradients which are used to determine amounts of
DNA replicated. Normal-density DNA bands around Fraction
19 (density 1.70 g/ml), while newly replicated DNA, which
includes 5-bromodeoxyuridine in place of thymidine, bands
around Fraction 12 (density 1.75 g/ml). Chart 2, A and B,
shows DNA distributions from untreated cultures that were
harvested at 12 and 24 hr after mitosis, respectively. Chart

Chart 1. Relative cell number increase for normally progressing control
cells (A) and cultures exposed to 43° heat in mig-G₁ phase at 3 to 4 hr after
mitosis (B) or in S phase at 9 to 10 hr after mitosis (C). Values represent
the average of 3 separate determinations.
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2, C and D shows similar data from cultures exposed from 9 to 10 hr after mitosis to 43° heat and then harvested at 12 and 24 hr, respectively. As seen in Chart 1, there is only minimal cell lysis or cell division occurring at these times in the treated cultures. It is apparent at both times (comparing Chart 2A with Chart 2C and Chart 2B with Chart 2D) that the 43°-treated cultures show a lower proportion of their [14C]DNA in the hybrid-density or newly replicated region of the gradient as compared to controls. This indicates a suppression of DNA synthesis.

This heat-shock-induced suppression of bulk DNA synthesis is described in more detail in Charts 3 and 4. Chart 3 shows the kinetics of semiconservative DNA replication for control cultures and for cells exposed to 43° during G1 at 3 to 4 hr after mitosis. Control cultures begin to show measurable newly replicated DNA by 6 hr after mitosis and have completed 1 round of replication by 18 hr after mitosis. In contrast, cultures exposed during G1 to 43° heat have not replicated measurable amounts of DNA until 9 hr after mitosis and have replicated less than 75% of their DNA by 25 hr after mitosis. While the rate of DNA replication is initially reduced after treatment of these G1 cultures, near-normal synthetic rates are recovered by 15 to 25 hr. During this time (Chart 1), cell lysis appears to be minimal, so that this recovery of DNA replication rates is not an artifact of impending cell death.

The results in Chart 4 describe the effects of heat exposure at 43°, 9 to 10 hr after mitosis, which is during S phase of the CHO cell cycle, on DNA replication kinetics. By 10 hr after mitosis, approximately 15 to 20% of the population’s DNA has already replicated (see Charts 2 and 3). Since 5-bromodeoxyuridine and 5-fluorodeoxyuridine were added to the cultures at 10 hr after mitosis, this amount of DNA replication will not be measured in these experiments. This explains why maximum percentage of DNA replication attained in the control cultures is only 80% as determined by CsCl gradient analysis. Similar to the results for cells treated in mid-G1, the rate of semiconservative DNA replication initially was reduced after exposure at 43°. In contrast, however, the rate of replication did not recover to control rates and remained reduced in these S-phase-treated cultures through 45 hr after mitosis (35 hr after the heat shock). Also, the final percentage of DNA replicated was only 12.5% lower in the 43°-treated culture (67% at 45 hr) than in the control culture (79.5% at 24 hr). This indicated that cultures exposed to 43° heat in S phase are able to complete nearly a full round of replication.

Polyamine Levels in Synchronized Cells after 43° Exposure in G1 or S Phase. Chart 5 shows the intracellular levels of the 3 polyamines, putrescine (Chart 5A), spermidine (Chart 5B), and spermine (Chart 5C), at different times after...
synchronization in mitosis. The closed symbols represent values from control cultures progressing normally through the cell cycle at 37°, while the open symbols illustrate polyamine levels for cells exposed to 43° heat during G1 from 3 to 4 hr after mitosis. Intracellular levels of all 3 polyamines decrease as cell division occurs. Intracellular spermidine and spermine amounts then reaccumulate nearly 2-fold by 10 to 12 hr after mitosis as these CHO cells progress through S phase. When these synchronized cells are exposed in mid-G1 phase to 43° heat, an immediate depletion of intracellular spermidine and spermine levels is observed, while putrescine amounts seem unaffected. When the 43°-treated cultures are returned to 37° at 4 hr, spermidine and spermine levels begin to increase and reach control values by approximately 15 hr after mitosis. Here the results in Chart 3 should be noted. The maximum rate of DNA replication in control cultures is attained between 8 and 10 hr after mitosis and by 15 hr after mitosis in cultures exposed to 43° heat in G1 phase. This closely corresponds to the attainment of high levels of spermidine, especially as shown in Chart 5 for both controls and cultures treated during G1 at 43°.

In order to determine the fate of the intracellular polyamines that are lost following heat exposure at 43° and to determine whether the increases in spermidine and spermine observed in control and treated cultures are due to new synthesis or simply transport from outside the cell, the extracellular medium was assayed for polyamines. Tables 1 to 3 summarize intracellular and extracellular amounts of putrescine, spermidine, and spermine, respectively, at various times after mitosis for control cultures and cultures exposed to 43° heat in G1 (3 to 4 hr after mitosis) and S (9 to 10 hr after mitosis) phase. These tables also express total culture levels (intra- plus extracellular) and give the ratio of intracellular to extracellular amounts. The intracellular data (except for cultures treated from 9 to 10 hr) are taken from Chart 5, and all values are expressed as nmols/10⁶ cells. Table 1 shows little variation in putrescine amounts, either as a function of cell cycle position or after heat exposure at 43° with the exception of S-phase-treated cultures. In these cells, there is an increase in intracellular...
Table 2
Spermidine levels (nmols/10^6 cells) in synchronized CHO cells

<table>
<thead>
<tr>
<th>Hrs after mitosis</th>
<th>Intracellular</th>
<th>Exteracellular</th>
<th>Total culture</th>
<th>Ratio (intracellular:extracellular)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>43° at 3-4 hr</td>
<td>43° at 9-10 hr</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>1.50±0.29</td>
<td>1.21±0.21</td>
<td>2.71±0.36</td>
<td>1.24±0.32</td>
</tr>
<tr>
<td>4</td>
<td>1.49±0.35</td>
<td>1.04±0.20</td>
<td>2.53±0.40</td>
<td>1.43±0.43</td>
</tr>
<tr>
<td>6</td>
<td>1.55±0.22</td>
<td>1.12±0.20</td>
<td>2.67±0.30</td>
<td>1.38±0.32</td>
</tr>
<tr>
<td>8</td>
<td>1.44±0.19</td>
<td>1.50±0.28</td>
<td>2.94±0.34</td>
<td>0.96±0.22</td>
</tr>
<tr>
<td>10</td>
<td>2.37±0.13</td>
<td>1.73±0.26</td>
<td>4.10±0.29</td>
<td>1.37±0.22</td>
</tr>
<tr>
<td>12</td>
<td>2.61±0.16</td>
<td>1.69±0.17</td>
<td>4.30±0.23</td>
<td>1.54±0.16</td>
</tr>
<tr>
<td>15</td>
<td>2.43±0.42</td>
<td>1.04±0.39</td>
<td>3.47±0.57</td>
<td>2.34±0.97</td>
</tr>
<tr>
<td>20</td>
<td>2.01±0.42</td>
<td>0.79±0.38</td>
<td>2.80±0.57</td>
<td>2.54±1.33</td>
</tr>
</tbody>
</table>

Table 3
Spermine levels (nmols/10^6 cells) in synchronized CHO cells

<table>
<thead>
<tr>
<th>Hrs after mitosis</th>
<th>Intracellular</th>
<th>Extracellular</th>
<th>Total culture</th>
<th>Ratio (intracellular:extracellular)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>43° at 3-4 hr</td>
<td>43° at 9-10 hr</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>0.85±0.12</td>
<td>0.94±0.17</td>
<td>1.79±0.21</td>
<td>0.90±0.21</td>
</tr>
<tr>
<td>4</td>
<td>0.68±0.13</td>
<td>0.85±0.07</td>
<td>1.53±0.15</td>
<td>0.80±0.17</td>
</tr>
<tr>
<td>6</td>
<td>0.95±0.11</td>
<td>0.95±0.21</td>
<td>1.90±0.24</td>
<td>1.00±0.25</td>
</tr>
<tr>
<td>8</td>
<td>0.95±0.12</td>
<td>0.96±0.14</td>
<td>1.91±0.18</td>
<td>0.99±0.19</td>
</tr>
<tr>
<td>10</td>
<td>1.41±0.08</td>
<td>0.90±0.19</td>
<td>2.31±0.21</td>
<td>1.57±0.34</td>
</tr>
<tr>
<td>12</td>
<td>1.60±0.07</td>
<td>1.06±0.11</td>
<td>2.66±0.13</td>
<td>1.51±0.17</td>
</tr>
<tr>
<td>15</td>
<td>1.39±0.01</td>
<td>0.96±0.16</td>
<td>2.35±0.16</td>
<td>1.45±0.24</td>
</tr>
<tr>
<td>20</td>
<td>1.30±0.18</td>
<td>0.72±0.13</td>
<td>2.02±0.22</td>
<td>1.81±0.41</td>
</tr>
</tbody>
</table>
putrescine from approximately 0.5 nmole/10^6 cells determined immediately after 43° exposure to amounts greater than 1 nmole/10^6 during the following 10 hr (through 20 hr after mitosis). During this same time there is a decrease in extracellular putrescine. The major variation in total culture putrescine occurs in the S-phase-treated cultures where a decrease in total putrescine is observed at 20 hr (10 hr after exposure at 43°). Here also, an increase in the ratio of intracellular to extracellular putrescine is observed. For all values listed, this ratio is on the order of 0.15 to 0.3 except in the cultures exposed to 43° heat at 9 to 10 hr, where the ratio increases from 0.24 at 10 hr, to 0.79 at 15 hr and finally to 1.30 at 20 hr after mitosis.

In contrast to the minor changes observed in putrescine, spermidine levels undergo large changes, both as a function of cell cycle phase and in response to heat shock. Table 2 lists the intra- and extracellular amounts of spermidine, showing the near doubling in intracellular levels as cells move through the division cycle, while extracellular values remain essentially unchanged. Note here that while there may be small fluctuations in extracellular levels of spermidine during the cell cycle, the variations are statistically questionable and certainly smaller than the statistically significant increase in intracellular values that occurs between 8 and 10 hr after mitosis. When cultures are exposed to 43° heat during G1, or S phase, intracellular levels are decreased 50% or more with a coordinate increase in extracellular amounts. Table 2 also demonstrates that, while intra- and extracellular levels of spermidine change following heat shock, total culture values remain similar through 8 hr after mitosis. In control cultures, a significant increase in total culture spermidine is observed at 10 hr, representing new synthesis of spermidine in the culture. Cultures exposed to 43° heat in G1 phase show an increase in total culture spermidine beginning at 12 to 15 hr after mitosis. Cultures treated during S phase at 43° do not show any major increase in total spermidine through 20 hr after mitosis. In contrast to putrescine, which showed intracellular to extracellular ratios of 0.15 to 0.30, spermidine ratios are greater than 1 at all times for control cultures. After heat exposure at 43° either in G1, or S phase, this ratio decreases as low as 0.12. Combined with the observation that total spermidine levels do not change (for example, see 4 hr after mitosis), this means a loss of intracellular spermidine with an increase in extracellular spermidine. Near normal S-phase ratios are attained by 15 to 20 hr after mitosis for G1-phase-treated cultures, while S-phase-treated cultures have not attained control values even by 20 hr after mitosis.

Table 3 shows intracellular and extracellular spermidine data. Similar to spermidine, although not as marked, 43° exposure causes a decrease in intracellular amounts of this molecule with a subsequent increase in extracellular levels. Intracellular spermine levels seem to accumulate to near-normal S-phase levels following 43° exposure either in G1, or S phase.

It should be noted here that cell numbers for these cultures were always determined after the hyperthermic treatment when samples were harvested at the times shown in the charts and tables. This fact plus the observation (Chart 1) that appreciable cell lysis does not occur through 20 to 25 hr after mitosis in either G1- or S-phase-treated cultures suggests that the observations reported here are not simply an artifact of lysing cells.

**DISCUSSION**

The data reported here for synchronous cultures proceeding through the cell cycle at 37° confirm previous findings that intracellular polyamine concentrations increase as cells leave G1 phase and progress through S and G2 phases (11, 23). This relationship is conserved in cultures exposed to 43° heat in G1 phase. An immediate result of this treatment is a depletion of normal intracellular spermidine and spermine amounts, followed by a delay in DNA replication kinetics. As spermidine and spermine reaccumulate, DNA replication proceeds at a reduced rate until near normal S-phase levels of these polyamines are attained. At this time, the rate of DNA replication increases for a time to approximately that of control cultures. These results are in agreement with those of others, who showed that heat shock inhibited DNA synthesis (16, 18) and that some cells treated during G1 were able to enter S phase but were then blocked in that cell cycle compartment (12).

An important implication of our results is that 43° exposure in either G1, or S phase causes an immediate change in the transport properties of the cytoplasmic membrane which leaves it porous to the outward flow of spermidine and spermine into the extracellular medium. This conclusion is in agreement with the results of others who have also observed hyperthermic treatment-induced changes in cell membranes (1, 9).

Our data are also of interest in comparison with the work of others who have studied the effects of heat shock on RNA and DNA synthesis. Intracellular spermidine and spermine levels seem to parallel RNA and DNA accumulation (3, 4, 19, 24) leading to the postulate that polyamine biosynthesis is involved in the control of both RNA and DNA synthesis. Thus the inhibition of RNA synthesis (26) and DNA synthesis (16, 18) caused by exposure to high temperatures may be due to the depletion of normal intracellular polyamine concentrations. Our results show that, as spermine and spermidine levels reaccumulate to normal values after 43° exposure in mid-G1, DNA replication proceeds at a near-normal rate for a time. In contrast, when cells are exposed to 43° heat during S phase, spermidine levels are reduced and do not reaccumulate over the next 10 hr. In these cells, DNA replication proceeds but at a much reduced rate compared to controls.

In terms of the effects of 43° exposure for 1 hr on cell kinetics, our data suggest that cells heated in mid-G1 (3 to 4 hr after mitosis) are inhibited from entering S phase (i.e., replicating their DNA) for a few hr and are then able to replicate DNA, but at a reduced rate for a period of time before attaining normal rates. In these mid-G1-treated cultures, only 75 to 80% of the DNA is replicated by 50 hr after mitosis, while control cultures have replicated 1 round by 18 to 20 hr. Since cell division is recommencing by 25 hr after mitosis in the 43°-treated cultures and about 20% of the cells will remain viable after this treatment, it seems likely that while all the cells are initially inhibited from replicating...
their DNA, some cells are able to complete at least 1 full round of DNA replication during this time. Since nearly a full round of DNA replication occurs after 43° exposure in S phase, our results would suggest that hyperthermia causes a large extension of the time S-phase cells will remain in S phase, in agreement with the conclusions of others using different techniques (12).

When comparing the ratio of intracellular to extracellular levels of each of the 3 polyamines, putrescine ratios are on the order of 0.2 for control cultures. Spermine ratios are nearly 1, and spermidine ratios are even higher, with values ranging from 1 to 1.5. This must indicate specific cell membrane transport mechanisms for each polyamine. Putrescine, with its low intracellular-to-extracellular ratio, seems not to be affected by exposure to thermal stress (except at later times after S-phase cultures are exposed to heat shock), while both spermidine and spermine, which have higher ratios, are greatly affected. The observation that the intracellular accumulation of high levels of spermidine and spermine, in particular, is temporally related to maximum bulk DNA-synthetic rates suggests a possible causal relationship between these 2 molecular events. Results to be published elsewhere show that the polyamine-biosynthetic enzymes, ornithine decarboxylase and the S-adenosylmethionine decarboxylases, are also temporally related to bulk DNA-synthetic rates in a manner similar to that of intracellular spermidine and spermine levels shown here. The fact that the ratio of intracellular to extracellular spermidine and spermine must reapproach controls for DNA replication to attain maximum rates further suggests that the transport of these molecules across cell membranes, as well as their biosynthesis, may be involved in the DNA synthetic process. That these 2 processes, biosynthesis and transport across cell membranes, both may be involved in DNA synthesis is further suggested by the results in Table 2 which show that extracellular spermidine levels may begin to increase between 6 and 8 hr after mitosis, while intracellular levels increase between 8 and 10 hr after mitosis in control cultures. These increases occur along with maximum S-adenosylmethionine decarboxylase activity being expressed at about 6 hr after mitosis (unpublished results). Evidence that polyamine accumulation is necessary for optimal DNA synthesis has recently been reported by others using procedures different from those reported here (5). In this regard, it is necessary to consider the results of exposing S-phase cells to 43° heat. These cells replicate their DNA at reduced rates and do not recover S-phase intracellular levels of spermidine or normal S-phase ratios for at least 10 hr after the heat shock. Thus, while 43° exposure kills both G1- and S-phase cells, although not to the same degree (30), these observations on the relationship between intracellular polyamine levels and DNA-synthetic rates in 43°-treated cultures do corroborate the temporal relationship of these 2 biochemical processes observed in control cultures.

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REFERENCES


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