Polyamine Content and Release during the Proliferation of Burkitt's Lymphoma Cells in Vitro

Kwang B. Woo, Fulvio Perini, Joan Sadow, Carole Sullivan, and William Funkhouser

ABSTRACT

The cellular content and excretion of polyamines in relation to the cell cycle and proliferation kinetics of Burkitt's lymphoma cells in vitro were investigated. Quantitative relationships were established between the cellular content of polyamines and the growth kinetic parameters, including specific growth rate, labeling index, and cell viability. The intracellular content of putrescine, spermidine, and spermine was significantly correlated with the specific growth rate, suggesting that all the polyamines actively participate in the process of Burkitt's lymphoma cell proliferation. A negative correlation was found between the labeling index and the intracellular content of putrescine ($r = -0.893$); a positive correlation was observed between the labeling index and the ratios of spermidine to putrescine (0.888) and spermine to putrescine (0.855). In addition, the extracellular content of putrescine showed a positive correlation with the labeling index (0.613). The percentage of dead cells determined by trypan blue exclusion exhibited a high positive correlation with the intracellular content of putrescine (0.912).

Examination of the distribution pattern of polyamines with respect to the fraction of cells in each cell cycle stage during a 10-day growth period revealed that the accumulation of cells in $G_1$ was associated with a reduction of intracellular levels of spermidine and spermine.

In cultures synchronized by double thymidine blockade, maximal levels of intracellular spermidine and spermine contents occurred in $S$ phase. Levels of intracellular putrescine were found to be lowest when the DNA content was lowest in early and mid-$S$ phase, and to be highest when the DNA content was highest in late $S$ and early $G_2 + M$.

INTRODUCTION

Burkitt's lymphoma is a rapidly growing malignant tumor characterized by its high rate of proliferation, combined with a high growth fraction (15, 39), and, for the majority of patients, its remarkable sensitivity to cytotoxic agents (38, 39). Toxic effects associated with rapid tumor cell kill due to treatment and a secondary rise in blood levels of cellular constituents have been reported. A biological marker for Burkitt's lymphoma cells would be expected to undergo similar changes in level, the degree of which should be related to the number of malignant cells destroyed. The urinary excretion of polyamines, nucleosides, and $\beta$-aminoisobutyric acid (33, 34) was elevated in patients with Burkitt's lymphoma prior to chemotherapy, and changes in the urinary excretion of these markers correlated with disease response of patients to treatment.

Polyamines are intimately involved in cell growth, and the most marked synthesis and accumulation of polyamines occur in rapidly growing tissues (27). The urinary excretion of polyamines in multiple myeloma patients receiving chemotherapy was correlated with the tritiated thymidine labeling index of tumor cells in a subgroup of these patients. The occurrence of extracellular polyamines in Ehrlich ascites tumor-bearing mice during tumor growth was related to a continuous release from dead or dying tumor cells (8). In the MTW9 mammary tumor, intracellular spermidine levels, which increase during tumor growth, were lowered during tumor regression, and spermidine levels in the serum were considered to reflect tumor cell death (29). These results led to the concept that spermidine can serve as a marker of tumor cell kill, whereas putrescine appears to reflect the growth fraction of the tumor (4, 28).

In a synchronous population of Chinese hamster cells obtained by selective detachment of mitotic cells, increases in spermidine and spermine occurred during $S$, and they were correlated with passage through $G_2$ and entry into $S$ (11, 30). The content of polyamines in AKR leukemia cells in mice increased progressively as the cells traversed the cell cycle from $G_1$ to $M$ (14).

We have studied the cellular content and excretion of polyamines in a Burkitt's lymphoma cell line grown in vitro to establish how the cellular polyamine content changes in relation to the growth kinetics of Burkitt's lymphoma. The growth kinetics of Burkitt's lymphoma cells in vitro has been characterized and described in detail elsewhere (36). In the present study, the kinetic parameters of tumor growth, including the specific growth rate calculated from the growth curve, the labeling index, and the dead cell number, determined by trypan blue exclusion, as well as the cell loss rate derived from cell cycle analysis, were correlated with the intra- and extracellular content of the polyamines.

Analysis of the relationship between polyamines and cell cycle stages was carried out using the time sequence of the fraction of cells in each cycle stage during a 10-day growth period. The fraction of cells in each stage was obtained by analyzing DNA content distributions utilizing the discrete-time kinetic model (36). A cell population synchronized by means of a double thymidine blockade was also studied to further evaluate the polyamine content in relation to the cell cycle.

MATERIALS AND METHODS

Burkitt's Lymphoma Cell Culture. A line of American Burkitt's lymphoma designated CONCEPTION (obtained from Dr. Ian Magrath, National Cancer Institute, Bethesda, Md.) was used in the experiments. The line has been fully characterized immunologically, morphologically, and cytogenetically. It is an
of the total cell number. The samples were then filtered through each sample so that they represented approximately 5 to 20% out for cell samples harvested daily during the growth of 10

Chicken erythrocytes were added to Mithramycin staining solution (0.85% NaCl solution containing a 62-@tmnylon mesh filter (Small Parts, Inc., Miami, Fla.).

adjacent areas of comparable cell size. A cell was considered open at 15°for 4 mm. Background was estimated from 3 accomplished by resuspending the cells in normal medium.

The perturbed cell population revealed the accumulation of the cells near the G,:S boundary, and release from the block was accomplished by resuspending the cells in an hemocytometer.

Cultures were also perturbed by means of double thymidine blockade (7) involving treatment of an exponential culture with 3 mM thymidine for 48 hr, followed by suspension in normal Roswell Park Memorial Institute medium 1640 for 12 hr, at which time 3 mM thymidine was added for an additional 24 hr. The perturbed cell population revealed the accumulation of the cells near the G,:S boundary, and release from the block was accomplished by resuspending the cells in normal medium.

Autoradiography. Cultures were pulse labeled for 30 min with [3H]dThd (specific activity, 2 Ci/mmol; New England Nu
clear, Boston, Mass.) at a final concentration of 0.05 μCi/ml. The cells were then centrifuged at 350 x g for 10 min. The cell pellet was resuspended in 2.5 ml fresh medium containing 10^-9 M thymidine and centrifuged for 5 min. The cell pellet was incubated in 0.075 M KCl at 37° for 15 mm, fixed, and washed 3 times in cold methanol:acetatic acid (3:1), spread on cleaned glass slides, air dried, and stained with acetor earce. Autoradiograms were prepared by dipping in Kodak NTB2 emulsion diluted with an equal part of distilled water. Slides were air dried, exposed to H2O2 vapor for 3 hr, sealed in light-tight boxes containing Drierite, and stored in the dark for 1 week at 4°. The autoradiographs were developed in Kodak D19 developer at 15° for 4 min. Background was estimated from 3 adjacent areas of comparable cell size. A cell was considered labeled if it had 5 or more grains over the nucleus. However, most of the labeled cells showed greater than 20 grains/ nucleus. The labeling index was determined by counting a minimum of 1000 cells/slide. Each experiment was repeated 3 times.

FCM. FCM analysis of DNA content distributions was carried out for cell samples harvested daily during the growth of 10 days. The cells were fixed in 70% ethanol and kept at 4° until stained for DNA analysis. Chicken erythrocytes were added to each sample so that they represented approximately 5 to 20% of the total cell number. The samples were then filtered through a 62-μm nylon mesh filter (Small Parts, Inc., Miami, Fla.).

Mithramycin staining solution (0.85% NaCl solution containing 15 mg MgCl2 and mithramycin (100 μg/ml); Pfizer, Inc., New York, N. Y.) containing 1 x 10^6 cells/ml, after staining for a minimum of 20 min, was analyzed with an EPIC II flow cytometer and cell sorter (Particle Technology, Inc., Los Alamos, N. M.).

Polyamine Analysis. Intracellular polyamine levels were determined by using a modification of the method previously outlined (8, 19). In the present study, the cells (2.5 x 10^6 cells) were sedimented by centrifugation at 1000 x g for 10 min at 4°, and the cell pellet was sonicated with 4% 5-sulfosalicylic acid (approximately 500 μl/10^6 cells) containing 2 nmol/ml of the internal standard, 3,3'-diaminodipropylamine. The homog
enate was kept at 4° for 1 hr and then centrifuged at 2000 x g for 10 min. A 50-μl aliquot of the supernatant was analyzed for its polyamine content, utilizing a Durrum D-500 amino acid analyzer (Durrum Instrument Corp., Sunnyvale, Calif.).

For extracellular polyamines, 3 ml of media were drawn off from the media-cell suspension after centrifuging at 1000 x g for 10 min, and 0.3 ml of 40% 5-sulfosalicylic acid containing internal standard was added. Samples were then centrifuged at 2000 x g for 10 min at 4°. Deproteinized supernatant (0.5 ml) was added to 0.5 ml of 5 n HCl and then hydrolyzed at 110° for 16 hr in screw-capped tubes with Teflon liners. The hydrolysate was then evaporated under a stream of a purified nitrogen gas flow and reconstituted to the desired volume with 0.1 n HCl; 50-μl aliquots of the resulting sample were injected on the analyzer.

A Durrum D-500 amino acid analyzer (Durrum Instrument Corp.) which is equipped with a PDP8 minicomputer with 16K word random access memory, 18-bit single analog-to-digital converter, and a fluorescence detection accessory, was used.

3,3'-Diaminodipropylamine (Aldrich Chemical Co., Milwaukee, Wis.) was used as internal standard. Buffers were mixed with phthalic anhydride (1 g/liter of buffer) for 1 hr at room temperature. After buffers were adjusted for final pH (5.85) with concentrated HCl and were brought to the desired volume, they were passed through a 0.45-μm Millipore filter. The o-

phthalaldehyde solution was made as described by Marton and Lee (19). Durrum potassium-form high-performance cation-exchange resin with 16% cross-linkage was packed to a height of 11.5 cm in a stainless steel, 1.75-mm internal diameter column. Flow rates were 0.45 ml/min for both buffers and reagent. Two computer programs were used. Program A was used for intracellular polyamines, and it used a single buffer at a concentration of 2.4 n in K+ (0.4 n K+ citrate and 2.0 n KCl). The program takes 25 min. Program B was used for extracellu
lar polyamines. It was a 3-buffer system: Buffer 1 (0.4 n K+ citrate and 0.2 n KCl) was pumped for 26 min; Buffer 2 (0.4 n K+ citrate and 0.88 n KCl) was pumped for 18 min; and Buffer 3 (0.4 n K+ citrate and 2.0 n KCl) was pumped for 16 min. All buffers were adjusted to pH 5.85. The total time was 60 min.

Methods of Data Analysis. Analysis of the growth kinetics of Burkitt’s lymphoma cells has been carried out utilizing the discrete-time kinetic model (17), which enables one to characterize the growth curve incorporating cell kinetic parameters, namely cell cycle times, cell loss rate, and growth fraction, and to relate them to DNA content distributions (35). The specific growth rate expressed as [dN(t)/dt]/N(t), where N(t) is the number of cells at Time t, was calculated from the growth curve. The time sequence of DNA content distributions for cultures harvested at various points during a 10-day growth period and also for synchronous cell populations were analyzed by the discrete-time kinetic model, in which the fraction of cells
in each phase of the cell cycle was expressed as a function of time.

The levels of the individual polyamines, as well as the polyamine ratios, were analyzed and correlated with the growth kinetic parameters of Burkitt’s lymphoma cells. The polyamine ratios were determined in such a way as to follow a sequence of synthetic pathways for 3 polyamines.

RESULTS

Growth Kinetics. The growth curve indicated by open circles (Chart 1) represents results from 3 separate experiments, each with the initial cell inoculum of $0.5 \times 10^6$ cells/ml. The solid line was generated by the discrete-time kinetic model, incorporating other key cell kinetic parameters (35). The doubling time during exponential growth was 31.2 hr. The number of dead cells amounted to approximately 1 to 2% of the total cell number throughout the entire period of growth. The decreasing growth rate that is apparent after Day 5 was mainly due to an elongation of the cell cycle. The maximal growth rate, i.e., the peak of the derivative curve, occurred approximately 2 days after plating. The pulse-labeling index was 32.5% on Day 1 and ranged from 40 to 46% (mean, 41.8%) during the exponential growth phase but suddenly fell to 27.5% by Day 5. Over the next 5 days, there was a gradual decrease, falling to less than 10% by Day 10. The mitotic index (not shown) ranged from 1.0 to 2.7% (mean, 1.17%) for exponentially growing cells and fell to 0.5% by Day 10.

Chart 2 shows the time course of the fraction of cells in each cycle stage. The fraction of cells in S phase compared favorably with the labeling index. After 5 days of growth, the fraction of cells in G1 increased significantly and reached about 80% of the total cell number at Day 9. The G2 + M fraction ranged from 15 to 20% throughout the entire growth period.

Polyamines and Growth Kinetics. Chart 3 shows the intracellular content of the polyamines putrescine, spermidine, and spermine during the growth of Burkitt’s lymphoma cells. In the lag and early exponential growth phases (Days 1 to 3), there was a high amount of spermidine and spermine (1.5 to 1.8 nmol/10^6 cells), whereas the amount of putrescine was approximately an order of magnitude lower. As the culture entered
late exponential growth phase (Day 4) and early plateau growth phase (Day 5), the spermidine and spermine content decreased to approximately one-half, whereas the putrescine content increased from 0.1 to 0.35 nmol/10⁶ cells. Cellular DNA content showed a slight alteration during the 10-day growth period.

The putrescine-to-spermidine-to-spermine metabolic pathway, using product:precursor ratios, was assessed during proliferation (Chart 4). The ratios of spermidine:putrescine and spermine:putrescine show a sudden decrease during late exponential and early plateau phases. No significant variation in the spermidine:spermine ratio was observed throughout the entire growth period.

Changes in cellular polyamine content in relation to the growth kinetics of Burkitt’s lymphoma cells have been investigated by correlating the levels of polyamines and polyamine ratios with the growth curve or the specific growth rate, the labeling index, and the cell cycle stage. The relationship of the number of dead cells to the extracellular level putrescine was also examined. Table 1 summarizes the relationships between the polyamines, DNA, polyamine:DNA ratios, and polyamine ratios, and the labeling index and specific growth rate. The correlation coefficients for the relationships to the labeling index were −0.893, 0.565, and 0.593 for putrescine, spermidine, and spermine, respectively. The scattergram illustrating the relationship between the intracellular putrescine content and the labeling index is shown in Chart 5. The correlation coefficients for the relationship between the polyamines and the specific growth rate were −0.812, 0.838, and 0.837 for putrescine, spermidine, and spermine, respectively.

Table 1

<table>
<thead>
<tr>
<th>Polyamine, DNA, polyamine:DNA, and polyamine ratio</th>
<th>Correlation coefficienta</th>
<th>Labeling index</th>
<th>Specific growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine/DNA</td>
<td>−0.893 (&lt;0.001)</td>
<td>−0.812 (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Spermidine/DNA</td>
<td>0.565 (&lt;0.01)</td>
<td>0.838 (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Spermine/DNA</td>
<td>0.593 (&lt;0.01)</td>
<td>0.837 (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Putrescine:DNA</td>
<td>−0.257 (NS)</td>
<td>−0.182 (NS)</td>
<td></td>
</tr>
<tr>
<td>Spermidine:DNA</td>
<td>0.821 (&lt;0.001)</td>
<td>0.575 (&lt;0.100)</td>
<td></td>
</tr>
<tr>
<td>Spermine:DNA</td>
<td>0.784 (&lt;0.001)</td>
<td>0.675 (&lt;0.050)</td>
<td></td>
</tr>
<tr>
<td>Spermidine:putrescine</td>
<td>0.868 (&lt;0.001)</td>
<td>0.896 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Spermine:putrescine</td>
<td>0.855 (&lt;0.001)</td>
<td>0.880 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Spermine:spermidine</td>
<td>0.164 (NS)</td>
<td>0.364 (NS)</td>
<td></td>
</tr>
</tbody>
</table>

a Correlation coefficient between labeling index and specific growth rate, r = −0.858 (p < 0.01).

Numbers in parentheses, p.

NS, Not significant.

To assess the cell death or the rate of cell loss in culture in terms of the polyamine levels, the extracellular medium was assayed for polyamines. Chart 6 illustrates the extracellular content as well as the intracellular content of putrescine at various times after plating, and it shows a negative correlation between the extracellular and intracellular content of putrescine. The extracellular content of spermidine and spermine was too low to be quantified. Correlations of the extracellular and intracellular content of putrescine to the number of dead cells were calculated as −0.240 and 0.912, respectively. A positive correlation of the extracellular putrescine to the labeling index was obtained [0.613, (p < 0.01)]. Also shown in Chart 6 is the intracellular and extracellular content of DNA during the 10-day growth period. The number of dead cells was reflected well by the extracellular DNA content with a correlation coefficient to 0.854. In addition, the extracellular content...
DNA content showed a high positive correlation with the intracellular putrescine content (0.921), but no significant correlation with the intracellular spermidine and spermine content.

**Polyamines and Cell Cycle.** Changes in the cellular polyamine content in the cell cycle during the 10-day growth period have been examined with respect to the fraction of cells in each cell cycle stage which was obtained from FCM measurement of DNA content distributions (Table 2). The cell fraction in G₁ shows a significantly high positive correlation to the intracellular content of putrescine, while negative correlations were obtained for spermidine and spermine. The cell fraction in G₂ + M showed no significant correlation with either intracellular or extracellular polyamine content.

Cultures perturbed by means of double thymidine blockade involving exponentially growing cells resulted in a synchronized population of cells, as revealed by the time course of DNA content distributions (Chart 7). The population of cells perturbed by excess thymidine returned to the preblockade distribution of cells in the cell cycle approximately 14 hr after release from thymidine blockade, which is considerably shorter than the cycle time of the unperturbed cell population of 23 hr (36). It was reported that synchronization by excess thymidine can result in a shortened cell cycle following release from the block (7). In addition, there is apparently a population not undergoing cell cycle traverse. This was demonstrated by an increase in the cell number not doubled after the cohort of cells has passed through mitosis, with the number increasing from $1.6 \times 10^6$ cells to $2.2 \times 10^6$ cells. The DNA content distribution at 0.5 hr shown in Chart 7 also revealed the fraction of the noncycle population in the G₁ population.

Chart 8 shows the intracellular and extracellular polyamine contents in Burkitt’s lymphoma cells after release from the thymidine block. The intracellular putrescine content was considerably lower than the spermidine and spermine content, whereas the extracellular putrescine content ranged from 147 to 399 pmol/3 ml of medium, and the extracellular spermidine and spermine contents were approximately 100 pmol/3 ml of medium throughout the cell cycle. When the majority of cells were in late S and early G₂ + M, the intracellular polyamine contents exhibited maximal levels. Just after release, the majority of the cell fraction was in either early S or mid-S phase, and the level of the intracellular putrescine content was approximately one-third of the late S level, whereas the spermidine and spermine contents were the same as those in the late S or early G₂ + M level, whereas the spermidine and spermine contents were the same as those in the late S or early G₂ + M level, whereas the spermidine and spermine contents were the same as those in the late S or early G₂ + M level. At 10 hr after release, i.e., when the majority of cells traversed into the G₂ + M phase, the intracellular putrescine content decreased to a value half of the late S level, and the intracellular spermidine and spermine contents also decreased from 1.5 nmol/10⁶ cells to 1.0 nmol/10⁶ cells. This fact may be attributable to the possible role of all polyamines being played in preparation of mitosis. As also depicted in Chart 8, there is a negative correlation between the intracellular and extracellular contents of putrescine.

**Correlations of the polyamines to the cell fraction in cell cycle stage**

<table>
<thead>
<tr>
<th>Polyamines</th>
<th>G. fraction</th>
<th>S fraction</th>
<th>G₂ + M fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.929 (&lt;0.0001)⁶</td>
<td>-0.893 (&lt;0.001)</td>
<td>0.057 (NS)⁵</td>
</tr>
<tr>
<td>Spermidine</td>
<td>-0.524 (&lt;0.1)</td>
<td>0.565 (&lt;0.01)</td>
<td>-0.158 (NS)</td>
</tr>
<tr>
<td>Spermine</td>
<td>-0.617 (&lt;0.05)</td>
<td>0.593 (&lt;0.1)</td>
<td>-0.329 (NS)</td>
</tr>
<tr>
<td>Extracellular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Putrescine</td>
<td>-0.373 (NS)</td>
<td>0.613 (&lt;0.01)</td>
<td>-0.241 (NS)</td>
</tr>
</tbody>
</table>

⁶ Numbers in parentheses, p.

⁵ NS, not significant.
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**DISCUSSION**

The utility of polyamines as biological markers of tumor growth kinetics and drug-induced tumor cell kill has been investigated in experimental tumor systems (1, 2, 10, 12, 16, 22-24, 29, 37), and in patients with malignant diseases (4, 18, 27, 33). The metabolism of the polyamines in cultured cells has also been studied in relation to the proliferation of cells (3, 5, 6, 9, 12, 13, 20, 21, 26, 31).

In the present study, the intracellular and extracellular contents of polyamines were evaluated with respect to the growth kinetics of Burkitt's lymphoma cells in culture. Polyamine levels were correlated to the labeling index and the proportion of dead cells, as well as the specific growth rate. The cellular content of polyamines was also examined with respect to the fraction of cells in each cycle stage obtained by FCM measurement of DNA content distributions for both unperturbed and perturbed cell populations.

The labeling index has a high negative correlation (-0.893) with the intracellular putrescine content, and a positive correlation (0.613) with the extracellular putrescine content. It was demonstrated in the double thymidine blockade that, when the majority of the cell fraction was in either early S or mid-S, the level of the intracellular putrescine content was approximately one-third of that when in late S or early G2 + M phases. It was observed from FCM measurements of the DNA content distribution of unperturbed Burkitt's lymphoma cells in vitro that significant numbers of cells accumulated in the G1-S boundary. In addition, the slower rate of DNA synthesis in early S phase was predicted by the discrete-time kinetic model (37). The negative correlation between the labeling index and the intracellular putrescine content is therefore due to the accumulation of cells synthesizing DNA in the early S phase. The positive correlation between the labeling index and the excretion of putrescine, as demonstrated in Burkitt's lymphoma cells, was previously reported with urinary polyamine determination in patients with multiple myeloma (4, 28).

The low correlation between the labeling index and the levels of spermidine and spermine content was largely caused by a delay of approximately 1 day in a decrease in the labeling index at Day 5 (Chart 1), as compared to a decrease in the spermidine and spermine levels at Day 4 (Chart 3). Chart 3 showed that at Day 4 the labeling index remains high (43.6%), while the specific growth rate decreases. This decrease in the specific growth rate was in parallel with variation in the levels of spermidine and spermine content, and it was mainly associated with an elongation of cell cycle time and a reduction in cell production rate (36). However, change in cell cycle time did not significantly alter the fraction of cells in each cycle stage, so that the labeling index remained constant.

Polyamines as predictors of tumor cell kill in response to treatment have been investigated (29, 33, 34), and subsequently a hypothesis has been proposed that spermidine can serve as a marker of tumor cell kill (27, 28). The observed accumulation of extracellular putrescine and spermidine in Ehrlich ascites tumor-bearing mice was a result of a continuous release from dead or dying tumor cells (10). In Burkitt's lymphoma cells in culture, the proportion of dead cells determined by trypan blue exclusion was approximately 1% of the total cell number, and the extracellular content of spermidine and spermine was not sufficient to be quantitated. Only the extracellular putrescine content was detectable and was not significantly correlated with the dead cell number.

Since the majority of cell death occurred in the G1 phase of the cell cycle of Burkitt's lymphoma cells in vitro (36), efforts to correlate the intracellular content of polyamines with the fraction of cells in G1 were made. The intracellular putrescine content showed a high positive correlation with the fraction of cells in G1, whereas the intracellular contents of spermidine and spermine had a negative correlation, which suggests that the extracellular contents of spermidine and spermine will have a positive correlation with either the fraction of cells in G1 or the number of dead cells in culture. In fact, when pronounced increase in dead cells in Burkitt's lymphoma cell culture was obtained as a result of antitumor treatment, we found that the number of dead cells was reflected with considerable accuracy in terms of the extracellular spermidine content.5

The relationships between polyamines and the rate of tumor growth indicate that there is varied kinetic behavior of polyamines associated with the growth rate of different tumor systems. In a rat brain tumor study, the intracellular spermidine content showed a high positive correlation with the specific growth rate and the intracellular putrescine content exhibited a low positive correlation (12), whereas, during Ehrlich ascites

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tumor growth, a high positive correlation was found between polyamine synthesis (ornithine decarboxylase activity) and the specific growth rate (2). In the proliferation of Burkitt’s lymphoma cells, the intracellular contents of spermidine and spermine showed significant positive correlations with the specific growth rate. The intracellular putrescine content revealed a high negative correlation, whereas the extracellular putrescine content exhibited a positive correlation (0.722; \( p < 0.01 \)) with the specific growth rate as the culture entered into exponential growth phase.

The polyamine levels were correlated to each stage of the cell cycle during the 10-day growth period. As the culture entered into the plateau growth phase, the fraction of cells in G1 increased, whereas the fraction of cells in S decreased (Chart 2). The accumulation of the cells in G1 was correlated to a decrease in the spermidine and spermine contents. This observation supports a view on the activity of polyamines in rat embryo fibroblast that low levels of spermidine and spermine arrest this cell type at a restricting point in G1 at which it is decided whether the intracellular level of these polyamines is sufficiently high to enable a cell to enter into and complete a new cell cycle (25).

In summary, both intra- and extracellular contents of putrescine serve as a marker for the labeling index, and intracellular contents of all polyamines correlate significantly to the specific growth rate. It appears that the ratios of spermidine to putrescine or spermine may be useful in quantitating the amount of tumor cell kill in response to treatment. Investigation dealing with the rapid and sensitive determination of the kinetic behavior of Burkitt’s lymphoma cells in vitro and in vivo, utilizing the cellular determination of polyamines in Burkitt’s lymphoma cells during treatment is presently under way.

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REFERENCES


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