POLYAMINES: A HIGH CORRELATION WITH CELL REPLICATION

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1. Introduction

Evidence is rapidly accumulating which suggests that the naturally occurring polyamines, putrescine, spermidine, and spermine are linked to cellular growth processes [1]. At physiological concentrations they have been shown to stimulate DNA synthesis [2–5], RNA synthesis [6–12], and protein synthesis [13–20] in cell-free systems, and in bacteria they are required for optimal growth, with their limitation leading to reduced cellular growth rates and decreased rates of DNA, RNA, and protein synthesis [21–26]. In mammalian cells polyamine concentrations are markedly increased during periods of rapid growth [1] and the increases have been related to the fraction of proliferating cells [27]. Indications of a positive correlation between tumor growth rate and polyamine metabolism have been reported [28–31], but no quantitative relationship has thus far been delineated to relate these parameters. To determine whether the changes in cellular polyamine content, which accompany alterations in cell growth patterns, do show a relationship with growth rate, the following experiment was performed.

2. Materials and methods

An *N*-nitrosomethylurea-induced rat brain tumor, provided by W. H. Sweet, P. T. Kornblith, J. R. Messer, and B. O. Whitman of the Massachusetts General Hospital, Boston, Mass., was cultivated in vitro as previously described [32]. Tumor cells, suspended in Eagle's basal medium (BME) with Earle's balanced salt solution supplemented with L-glutamine, fetal calf serum, BME vitamin mixture, BME essential amino acid mixture, and antibiotics, were plated into plastic Falcon flasks (1×10^6 cells/flask) with a 75 cm^2 surface area [33]. The cultures were grown in a 5% CO_2-atmosphere at 37°C and were harvested at 24-hr intervals by trypsinization during the 7-day growth period studied. The trypsinization procedure used did not affect the cellular polyamine content. The cells were suspended in growth medium, an aliquot removed for cell counting, and the remainder centrifuged at 900 *g* for 10 min at 4°C. The cellular pellets obtained were sonicated in 4% 5-sulfosalicyclic acid (approx. 200 $\mu l/10^6$ cells), kept at 0°C for 1 hr, and then centrifuged at 8 000 *g* for 5 min. An aliquot of the supernatant was utilized for polyamine analysis using a Durrum D-500 amino acid analyzer as previously described [34].

3. Results

Table 1 shows the growth data of the rat brain tumor cell line and the cellular polyamine contents as they vary over the 7-day growth period studied. The growth curve (C1, fig.1) exhibits an S-shaped pattern with an initial lag phase, followed by an exponential phase, and finally a stationary phase. Approx. 2 days after plating there is an inflection point that delineates between the phases of increasing and decreasing specific growth rate.

The spermidine content was maximal during exponential growth, when cells were actively dividing, and decreased when the growth curve reached a plateau and cell division ceased (table 1). The maximal growth rate, i.e., the inflection point of the growth curve, and the maximal spermidine content seemed

Table 1
Polyamine content of rat brain tumor cells at various stages of growth

Days after plating	Cells per Falcon flask × 10^{-6}	Putrescine	Spermidine (nmol/10^6 cells)	Spermine
1	1.4 ± 0.2	2.03 ± 0.32	3.56 ± 0.56	1.61 ± 0.20
2	2.8 ± 0.2	2.41 ± 0.13	3.85 ± 0.16	1.51 ± 0.10
3	5.7 ± 0.8	2.50 ± 0.10	3.52 ± 0.11	1.42 ± 0.06
4	8.1 ± 0.7	2.80 ± 0.15	3.54 ± 0.16	1.61 ± 0.07
5	11.5 ± 1.0	1.93 ± 0.41	2.93 ± 0.44	1.57 ± 0.12
6	11.2 ± 0.5	1.84 ± 0.27	2.43 ± 0.14	1.52 ± 0.07
7	11.3 ± 1.7	2.43 ± 0.53	2.56 ± 0.47	1.67 ± 0.25

The number of cells per Falcon flask and the cellular polyamine content was determined at various times after plating 1 × 10^6 cells per flask. Values reported are means ± S.E.M. (n=7).

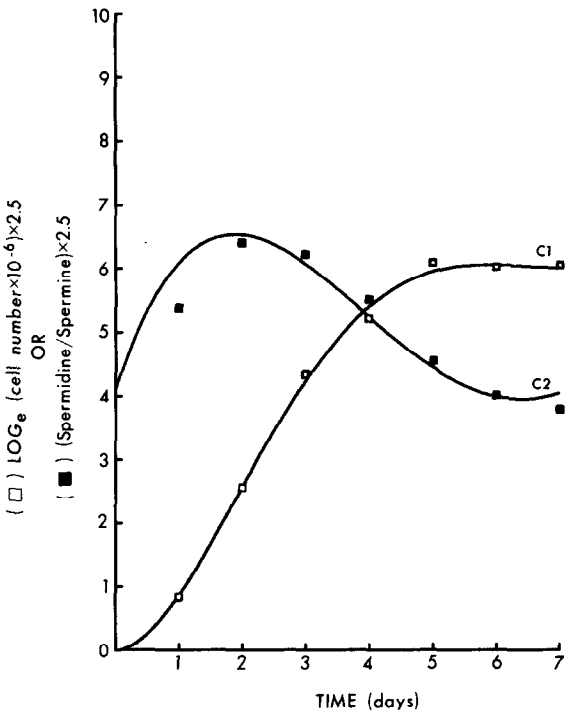

Fig. 1. Time course of population growth and the molar spermidine/spermine ratio. Growth data is represented by (□) as log of cell number and the spermidine/spermine data by (■). The values plotted are means of 7 separate experiments. Curve C1 is a 5th degree polynomial fitted to the growth data and curve C2 is the time derivative of C1 linearly fitted to the spermidine/spermine data. Both curves are computer drawn and weighted to include the experimental errors.

to show a temporal relationship. For the statistical evaluation of this possible relationship the spermidine/spermine ratio, which in essence reflects the cellular content of spermidine (the spermine content was relatively constant throughout the 7-day growth period), was used, since it is not subject to the errors inherent in calculating absolute amounts of the polyamines, i.e., it is independent of the error in cell counting. Furthermore, the standard errors of the means of the spermidine/spermine ratios were less than those of the spermidine content.

A continuous representation, curve C1 (fig.1) of the growth data was obtained by a least squares fit of the data with a 5th degree polynomial, $P(t)$. The derivative curve of this polynomial was taken as a representation of the specific growth rate $[(dN(t)/dt)/N(t)]$ and was linearly fitted to the spermidine/spermine ratios, i.e., it was assumed that spermidine/spermine versus time is of the form $a \cdot dP/dt + b$, and the constants a and b then determined by linear regression. The curve C2 (fig.1) is the resulting linear fit, indicating a close linear relationship between the spermidine/spermine ratio and specific growth rate. This linear relationship is further depicted in fig.2, which shows the spermidine/spermine ratio versus the specific growth rate. The correlation coefficient for this relationship is 0.95 thus showing a significant degree of correlation between the spermidine/spermine ratio and the specific growth rate of the tumor cells. Though the correlation coefficient for the absolute spermidine content versus the specific growth rate was also 0.95 the correlation

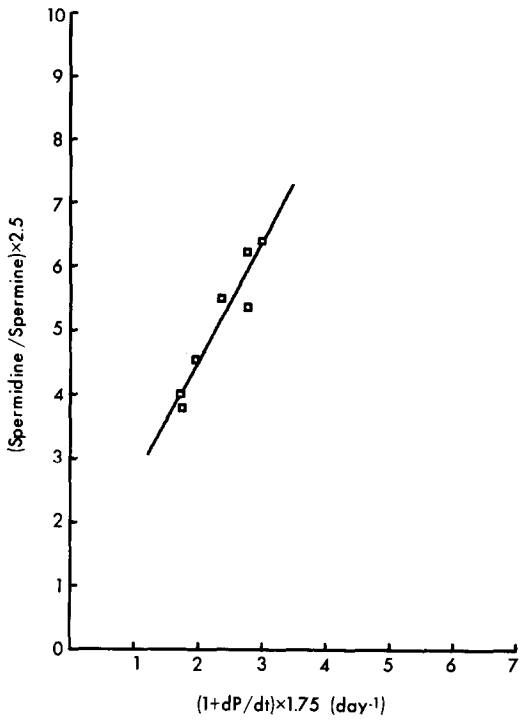

Fig. 2. Spermidine/spermine ratio vs. specific growth rate. The specific growth rate at the corresponding time points is taken as the derivative dP/dt of the polynomial $P(t)$ representing curve C1 of fig.1. Correlation coefficient = 0.951. The curve is computer drawn.

coefficients for putrescine and spermine versus specific growth rate were only 0.36 and −0.43 respectively.

4. Discussion

Defining the precise functions of the polyamines has been an elusive task. Although in in vitro and in vivo systems nucleic acid and protein synthesis are stimulated when polyamine levels are increased [1], it is not known to what extent the rise in cellular polyamine content influences these processes.

Although previous studies have indicated that a relationship may exist between polyamine metabolism and specific growth rate [28–31], the present study represents the first demonstration of a linear correlation between these parameters. The fact that the spermidine/spermine ratio, which reflects the spermidine content of the tumor cells, shows a direct linear correlation with the specific growth rate, indicate that spermidine accumulation is an event primarily associated with the process of cell replication.

In a recent study of cells in partial synchrony we have shown that the spermidine/spermine ratio increases during the G_1 phase, reaches a peak during the S phase, and decreases during the G_2 and M phases of the cell cycle [35]. This fact probably explains why the spermidine/spermine ratio exhibits a maximum at the time of maximal cellular growth rate, inasmuch as the fraction of actively cycling cells is greatest at this time of growth [36–38].

The data presented, showing a direct linear correlation between the cellular spermidine/spermine ratio and the growth rate of the tumor cells, combined with our previous observation, showing that effective chemotherapeutic agents (those causing decreased tumor growth rate) cause decreased spermidine/spermine ratios [29], suggest the possible use of polyamine determinations in evaluating the efficacy of various modes of therapy, not only qualitatively, but quantitatively.

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