POLYAMINE SYNTHESIS AND LEVELS DURING THE GROWTH AND REPLICATION OF *LEISHMANIA TROPICA MINOR* AND *LEISHMANIA AETHIOPICA*

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1. Introduction

The polyamines putrescine, spermidine and spermine, which occur widely in nature [1–5], are involved in cellular growth processes [6]. At physiological levels, they stimulate DNA [7], RNA [8] and protein [9,10] synthesis, and reach their peak levels during periods of maximal growth [1]. They are required for optimal growth in bacteria, where their limitation leads to reduced cellular growth [11] and their concentration is markedly increased in rapidly growing mammalian cells [1], with this growth being arrested on inhibition of polyamine synthesis [12]. It was shown that polyamines also occur in various trypanosomatid protozoa, where they are at their maximal levels during the logarithmic phase of growth [13–16]. We have demonstrated that some antileishmanial drugs and polyamine analogues, i.e., pentamidine isethionate, ethidium bromide and methyglyoxal-bis (guanyl hydrazone) (MGBG), impair leishmanial growth by interfering with polyamine biosynthesis [16]. In order to demonstrate the causal relationship between leishmanial growth and polyamine biosynthesis, a correlation between growth rate and putrescine to spermidine ratio must be established. In this study we demonstrate such a correlation.

2. Materials and methods

The two strains studied were obtained from the culture collection of the World Health Organisation's International Reference Centre for Leishmaniasis (WHO-LRC), housed in the Department of Protozoology of the Hebrew University-Hadassah Medical School, Jerusalem. Their origins and some of their intrinsic taxonomic characters are given in table 1.

These strains were maintained at 28°C by biweekly passage on semisolid Locke-blood–agar [19] or NNN diphasic medium [20]. For experimental studies, the strains were grown in a fully-liquid, Panmede (Paines and Byrne Ltd., Greenford, Middlesex, England) based medium, containing 7.5% normal rabbit blood [21].

Number counts of living promastigotes were done in a haemocytometer. Experimental cultures were seeded to give 4×10^6 promastigotes/ml at the start of growth. Following daily counting, i.e., twice per day, the aliquots removed were adjusted to give $\sim 50 \times 10^6$ promastigotes/pellet.

Polyamines were extracted from pellets of promastigotes washed with saline, using 3% perchloric acid. Their dansyl derivatives, prepared according to [22], were separated and identified by thin-layer chromatography on 300 μm thick silica gel G plates, using ethyl acetate : cyclohexane (2:3) as the solvent. Quantification of the various polyamines was done fluorometrically. Once identified, dansyl-polyamines were scraped off plates, extracted with 10 ml aliquots of toluene and assayed in a Turner Model III Fluorometer ($>$ exc. –365 nm, emission –505 nm), the fluorescence corresponding to the scraped spots being compared to that of known standards.

Table 1
Biochemical and serological characters of the Leishmanial strains

WHO-LRC	Other designations	Name	Place of origin	Source		Biochemical and serological characterisation						
				Host	Condition	DNA buoyant density		Enzyme variants				EF serotype
						Nuclear	Kinetoplast	MDH	GPI	G6PDH	6PGDH	
LRC-L32	LV142	*L.tropica minor*	Iraq	man	LR	1.719	1.707	IV	III	I	VII	A_2
LRC-L147	LV546, L100	*L.aethiopica*	Ethiopia	man	DCL	1.719	1.706	V	II	I	II	B_1

Abbreviations: MDH, malate dehydrogenase; GPI, glucose phosphate isomerase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; EF, excreted factor; LR, *Leishmaniasis recidivans;* DCL, diffuse cutaneous Leishmaniasis

Data were taken from [17,18] and Schnur and Chance (unpublished data)

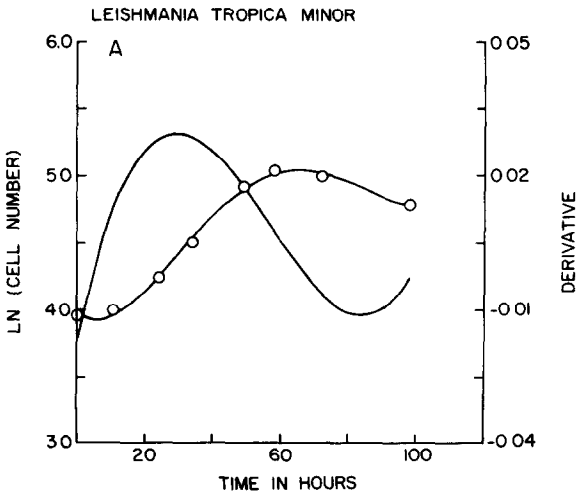
3. Results

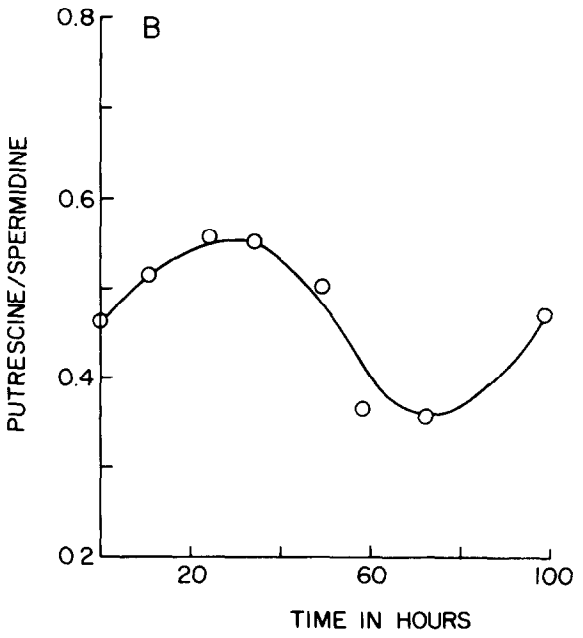
Putrescine, spermidine and traces of spermine demonstrated [15] in the promastigotes of 5 leishmanial strains, where identification of these polyamines was further confirmed by mass-spectrometry. They also showed that these polyamines are closely related to the growth of the leishmanial parasites. Figures 1 and 2 present the data on growth and polyamine contents of the two strains studied here, as they varied over a 4-day period.

The growth curves obtained (fig.1A,2A) were essentially the same, exhibiting S-shaped patterns with a lag phase, an exponential growth phase and a short stationary phase, before a fairly rapid decline in cell numbers. About 2 days after seeding, inflection points are seen in these curves, delineating between the phases of increasing and decreasing growth rates, also shown by the plot of the derivatives of the cell numbers, which cross the growth curves at these points.

The ratios of putrescine to spermidine varied during the various phases of growth (fig.1B,2B) and correlated well with growth rates (cf. fig.1A,2A), being maximal when exponential growth was maximal, i.e., 30 h for *L.t.minor* LRC-L32 and 19 h for *L.aethiopica* LRC-L147.

On plotting the putrescine : spermidine ratios against the derivatives of the growth rates, a linear relationship was demonstrated (fig.1C,2C), indicating that leishmanial growth is directly related to and dependent on polyamine synthesis.





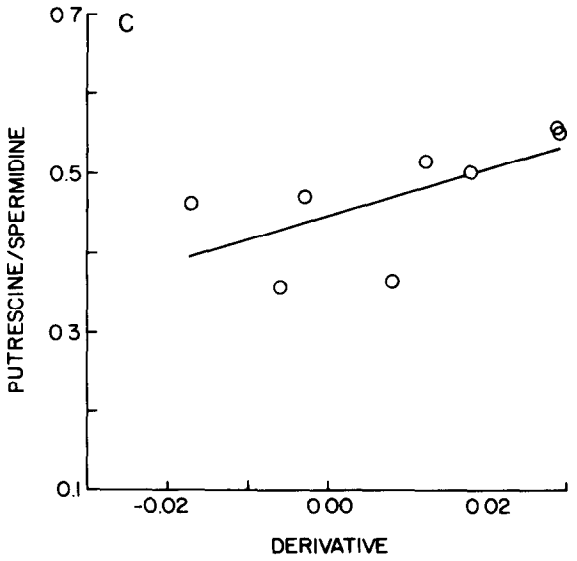
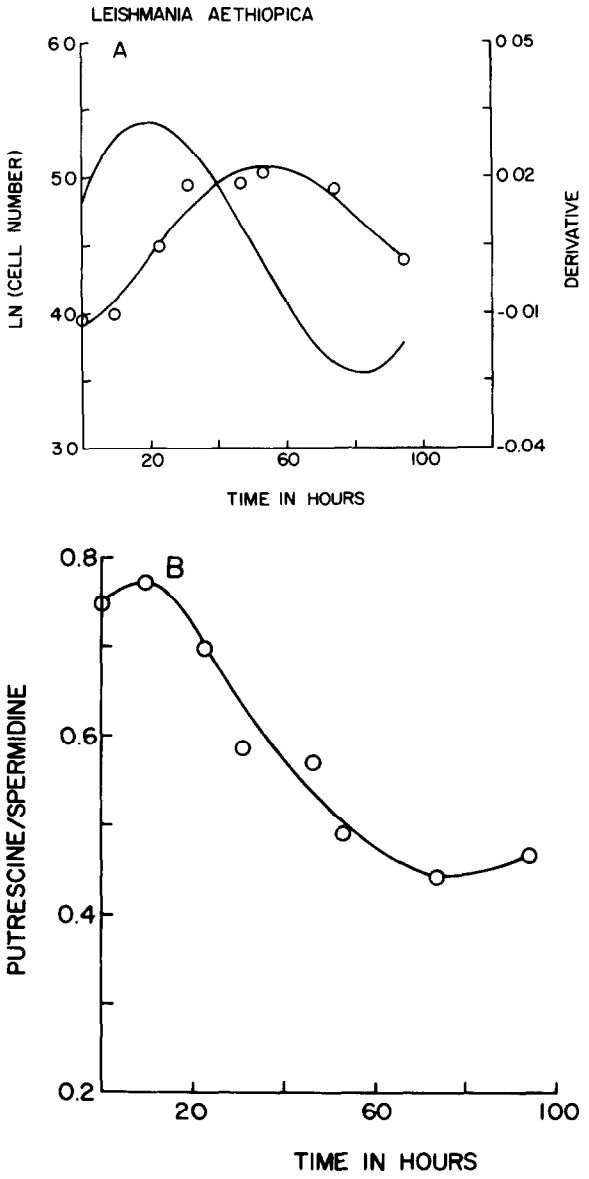


Fig.1. Growth and polyamine content of *Leishmania tropica minor* (LRC-L32) promastigotes: (A) growth rate; (B) putrescine/spermidine ratios; (C) their interrelationship. Curves are computer drawn and weighted to include the experimental errors.



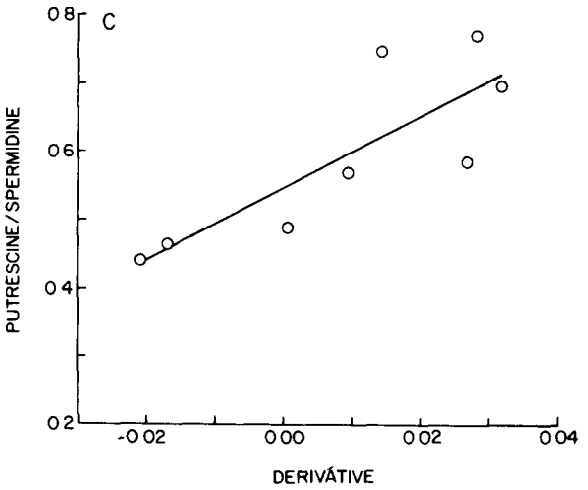


Fig.2. Growth and polyamine content of *Leishmania aethiopica* (LRC-L147) promastigotes: (a) growth rate; (B) putrescine ratios; (C) their interrelationship. Curves are computer drawn and weighted to include the experimental errors.

4. Discussion

The experiments described in [15] clearly showed that leishmanial strains of distinct character follow a similar pattern of polyamine synthesis. The strains studied [15] all contained polyamines and were able to convert putrescine to spermidine. This was also the case in the Iraqi *L.t.minor* LRC-L32 and Ethiopian *L.aethiopica* LRC-L147 strains studied here. Similarly, the polyamine levels in these two strains fluctuated during growth. The growth characteristics and polyamine levels differed between the two strains, and it has been suggested that such differences may have phylogenetic significance [13–14].

A linear correlation has been demonstrated between the polyamine synthesis and the specific rate of growth of rat brain tumour cells. A similar linear correlation was obtained with the leishmanial promastigotes, on plotting their putrescine to spermidine ratios against the derivatives of their growth rates, indicating that leishmanial growth is directly related to and, apparently, dependent on polyamine synthesis.

Knowledge of the polyamine biochemistry of leishmanial parasites may aid in evaluating the efficacy and mode of action of existing and potential anti-leishmanial drugs [16].

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