Relationship of Spermidine and Nucleic Acids to Growth in *Lactobacillus casei* and *Escherichia coli** (33814)

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Spermidine, α-(γ-aminopropylamino)-δ-aminobutane, is widely distributed in biological material but its metabolic role is unknown (1). Several studies (2–4) relating spermidine to ribonucleic (RNA) synthesis and its stabilization have implicated spermidine in the growth process. In the rat, its accumulation in regenerating liver parallels the increased rate of RNA synthesis (3). A high percentage of spermidine in rat liver is associated with the microsomal RNA (5).

In *E. coli* Raina and Cohen (6) found that the polyamines, putrescine and spermidine, are involved in the control of RNA synthesis. However, Ezekiel and Brockman (7) have recently shown that spermidine inhibits the synthesis of protein and stimulates degradation of protein and so leads to the formation of an amino acid pool large enough to lead to charging of the RNA. They suggested that RNA synthesis is influenced by spermidine through this mechanism. Others (8, 9) have shown that spermidine, or related polyamines, or both, serve as growth factors for a variety of bacteria including *L. casei*. Furthermore, Kihara and Snell (9) found that growth cultures of *L. casei* have a shorter lag phase and steeper slope of logarithmic growth when the medium is enriched with spermidine. Although such characterization of growth *per se* is meaningful, there is incomplete information regarding intracellular spermidine during the various phases of the growth cycle.[2]

All the above studies of the cellular content of spermidine relied on colorimetric methods for its determination. The limited sensitivity of the colorimetric method makes it difficult to measure the very small amounts of spermidine present in bacteria throughout the lag phase, and during early logarithmic growth. Fluorometric analysis (11, 12) is the most sensitive physical method available for the determination of spermidine, and a modification of this method (13) measures spermidine in tissue samples containing as little as 0.8 nmoles of this compound. This sensitivity makes feasible the analysis of multiple samples from all phases of the growth cycle of either *E. coli* or *L. casei*.

The present communication reports our findings on *de novo* synthesis and concentration of spermidine in these organisms during the various phases of the growth cycle. In studies with *E. coli* the DNA and RNA content was also analyzed.

Methods. The *L. casei* ATCC no. 7469 was grown in the chemically defined, spermidine-free medium described by Birnbaum and Lichstein (14) with biotin (10^{-2} μg/ml) added. Inoculum grown for 48 hr was centrifuged and the cells were thrice washed in saline. The turbidity of the final saline suspension was measured with a Klett-Summerson photoelectric colorimeter (blue filter, 420 mμ); dry weight in mg (mgDW) was calculated as 0.129 × Klett units.

Two liters of growth medium, inoculated with 8.0 mgDW of *L. casei* in 2 ml of saline, was incubated at 30° without shaking. Growth was followed by turbidimetric measurement at 660 mμ and by viable counts. Samples (190 ml in the lag phase containing 1 mgDW down to 70 ml in the stationary

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[2] During the preparation of this manuscript Raina *et al.* (10) published their independent findings on polyamines and the accumulation of ribonucleic acid in polyauxotrophic strains of *E. coli*.

phase containing 46 mgDW) were centrifuged at 3020*g* for 5 min in the cold. The pellets were thrice washed with cold saline and then analyzed for spermidine.

The *E. coli* ATCC no. 8739 was grown on the chemically defined, spermidine-free medium used by Birnbaum *et al.* (15). Inoculum grown for 18 hr was treated as above. Dry weight was calculated as 0.139 × Klett units. Five liters of medium inoculated with 30 mgDW in 5 ml of saline was incubated at 38° under aerobic conditions. Samples (1210 ml in the lag phase containing 5.5 mgDW down to 68 ml in the stationary phase containing 51 mgDW) were treated as above and analyzed for spermidine, RNA, and DNA.

Chemical analysis. Analysis for spermidine was done after extraction of the pellet into 0.4 *N* $HClO_4$, by the method of Elliott and Michaelson (13). The first extraction removed 85% of the recoverable spermidine. Nucleic acids were extracted as described by Munro and Fleck (6). The RNA was measured by its adsorption at 260 mμ, and DNA by the colorimetric indole method (17). RNA and DNA values were calculated from standard curves. Recovery of spermidine added to pellets was 75%. The data presented are not corrected for incomplete recovery.

Results. The relationship of growth and spermidine content in *L. casei* is illustrated in Fig. 1; those of spermidine to growth, RNA, and DNA for *E. coli* are in Fig. 2. By converting the concentration of spermidine to numbers of molecules and relating this to the viable count, one can determine the numbers of molecules in each cell at a given time during the growth cycle.

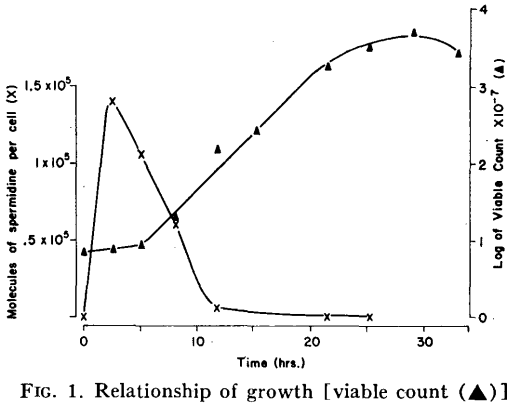

FIG. 1. Relationship of growth [viable count (▲)] and spermidine content per cell (X) in *L. casei*; log viable count per ml.

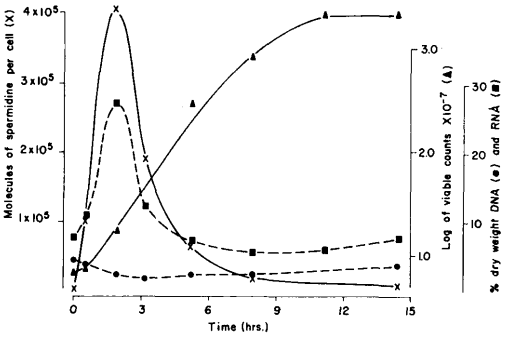

FIG. 2. Relationship of growth [viable count (▲)] to spermidine (X), RNA (■) and DNA (●) in *E. coli*; log viable count per ml.

In *L. casei*, spermidine rose from 200 molecules/bacterium at inoculation to 140,000 at 2.6 hr, and later diminished to 1400 molecules at 21 hr and 123 at 25 hr. In *E. coli*, spermidine rose from 1400 molecules/bacterium at inoculation to 423,000 at 2 hr and fell to 14,000 at 7.9 hr and 8000 at 14 hr. Net spermidine synthesis ceased shortly after the start of logarithmic growth. By 3 criteria (viable counts, dry weight, and DNA concentration) spermidine concentration per cell reached a peak before 2% of maximal growth had occurred (Figs. 1 and 2). The RNA concentration/cell rose and fell with the same time course as spermidine. In *E. coli* grown in a minimal salt–glucose medium, spermidine concentration had the same relation to growth (mgDW) as described above. In both bacteria spermidine increased 180-fold in the early hours of growth (Table I). In *E. coli* DNA and RNA increased 109- and 127-fold.

Discussion. Since all media were chemically defined and free of spermidine, the increase of spermidine content represents synthesis *de novo*. Spermidine increased mainly in the lag phase and the early log phase of growth, and rose and fell with RNA content. Raina and Cohen (6) showed that spermidine can stimulate RNA synthesis in *E. coli*. Others (1) have suggested that spermidine is

TABLE I. Values Determined at Stages in the Growth Cycle of *L. casei* and *E. coli*.[a]

Time (hr)	Spermidine (pmoles)	RNA (μg)	DNA (μg)	Dry wt. (μg)	Viable count 10^{-7} of colonies
L. casei					
0.	0.12			3.9	7.0
2.6	16.7			5.2	7.2
5.1	16.0			7.3	8.8
8.2	20.0			14.4	19.8
11.9	17.6			77	160
15.2	—			108	275
21.4	8.3			525	1290
25.2	5.6			665	3080
29.0	5.0			695	4700
E. coli					
0.	1.7	0.44	0.24	5.9	7.0
0.6	13.3	0.81	0.15	4.5	7.5
1.9	141	4.8	0.33	17.5	20.0
3.2	328	15.0	1.88	125	105
5.2	294	37	11.5	522	280
7.9	201	46	15.5	861	830
11.2	—	59	—	1040	2630
14.3	328	55	26.2	750	2240

[a] Spermidine, RNA, DNA, and dry weight were determined in the pellet after centrifugation; viable count, in samples of culture medium. All values are expressed per milliliter of culture medium.

a "physiological stablilizer" of RNA. Tabor (18) showed that spermidine stabilizes nucleic acids *in vitro*, and suggested that this was accomplished by neutralizing the mutually repelling negatively charged inorganic phosphate groups.

Recently Raina *et al.* (10) reported that there is parallelism between the intracellular accumulation of unconjugated spermidine and RNA in a variety of growth conditions in *E. coli*: (a) normally growing cells, (b) cultures inhibited by amino acid or thymine deprivation, and (c) cultures grown in the presence of streptomycin or chloramphenicol. These authors (10) analyzed cells taken from the exponential growth phase as well as during various periods up to 180 min after reinoculation. The present study makes it clear that there is a striking parallism between spermidine and RNA during all stages of growth, especially during the lag phase. This parallelism further supports the hypothesis that a considerable portion of cellular spermidine is associated with RNA *in vivo*. Raina *et al.* (10) discussed the possible significance of spermidine relative to RNA.

Summary. *L. casei* and *E. coli*, growing in spermidine-free medium, synthesize considerable spermidine during the early phases of the growth cycle. As the cultures grow older the spermidine concentration in the cells falls again. In *E. coli*, the changes in spermidine concentration are parallel to changes in RNA concentration.

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Effect of *in Vitro* Glucocorticoid Treatment on Acid Ribonuclease Activity in P1798 Lymphosarcoma Cells (33815)

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Lymphosarcoma P1798 has been shown to regress rapidly following subcutaneous injection of glucocorticoids (1, 2) with a concomitant rise in tumor acid ribonuclease activity (3, 4). Burton *et al.* (5) showed that newly suspended P1798 cells are sensitive to corticoids *in vitro*. The present experiments show that the effect of corticoid treatment on the acid ribonuclease of the tumor is caused by a direct action of the drug on the tumor cell rather than by an indirect action mediated through some other part of the host body. Cells from the corticoid sensitive strain of lymphosarcoma P1798 were grown in tissue culture and shown to be sensitive to *in vitro* treatment with 9α-fluoroprednisolone (9FP) and dexamethasone phosphate. Gabourel and Aronow (6) demonstrated similar sensitivity of cultured ML-388 cells *in vitro*. The P1798 cells from our established cultures have now been shown to exhibit an increase in acid ribonuclease activity following *in vitro* treatment with 9FP.

Methods. Cultures of lymphosarcoma P1798 have been quite difficult to establish. Repeated culturing similar to the method of Sato and Buonassisi (7) was used. Solid tumors were minced in medium A (MEM which had BME vitamins substituted for MEM vitamins and supplemented with 100 μmoles/liter each pyruvate and asparagine as well as 10% fetal calf serum), filtered through cheese cloth, and incubated at 37° for 24 hr. The cell suspension was then injected subcutaneously into female BALB/c mice and after the resultant tumor grew to approximately 10-mm diameter (14–18 days) it was again put into culture. The second culture was incubated for 2–4 days before it was injected into another host mouse while the third culture generation was kept for 5–7 days and the fourth was kept in culture 1–4 weeks. Corticoid sensitive, strain 1 (CS-1) reported here was established in the fifth culture generation and has now been maintained for 2 years while strain 2 (CS-2) was established in the fourth culture generation and has been maintained for 1 year. These cells grow in suspension so that trypsin is not necessary for passage and they have not been subcultured more than once every 4–6 weeks. The stock cultures were maintained in a volume of approximately 50 ml and twice a week 40 ml of the culture was replaced with fresh medium B (1:1 mixture of Fischer's media and media A described above).

Established cultures from the corticoid resistant strain (CR-1) of the P1798 lym-

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