Polyamines in the Synthesis of Bacteriophage Deoxyribonucleic Acid

II. Requirement for Polyamines in T4 Infection of a Polyamine Auxotroph

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Polyamine depletion produced by exogenous arginine in *Escherichia coli* K-12 cultures defective in agmatine ureohydrolase activity resulted in a marked inhibition of the rates of growth and nucleic acid synthesis. Addition of putrescine or spermidine to such depleted cultures restored the control rate of growth and nucleic acid accumulation. The omission of lysine resulted in a further decrease in the rates of growth and nucleic acid synthesis in polyamine-depleted cells. The addition of exogenous cadaverine increased the rates of growth and ribonucleic acid synthesis to those observed in lysine-supplemented cultures, suggesting that lysine or a derivative of lysine serves a function similar to cadaverine. Addition of lysine to polyamine-depleted cultures at neutral pH results in the synthesis of cadaverine and a new spermidine analogue, both containing lysine carbon. This new metabolite has been isolated and identified as *N*-3-aminopropyl-1,5-diaminopentane. T4D infection of the polyamine-depleted mutant resulted in a very low rate of DNA synthesis and phage maturation. The addition of putrescine or spermidine 15 min before infection restored phage DNA synthesis and phage maturation to control rates, i.e., rates observed in infected cells grown in the absence of arginine.

In the previous paper (9), we reported that the accumulation of putrescine and spermidine after infection was independent of phage deoxyribonucleic acid (DNA) synthesis. The present communication deals with the possible dependence of phage DNA synthesis on the polyamines available to the infected cells.

That Mg^{2+} and polyamines are important in phage morphogenesis is indicated from several reports. Garen (12) demonstrated that high Mg^{2+} concentrations permitted the multiplication of T4rII mutants in *Escherichia coli* K-12 (λ). Ferroluzzi-Ames and Ames (11) reported a similar phenotypic reversal resulted from exogenous putrescine or spermidine. The replication of rII mutants in *E. coli* K-12 (λ) was also shown to be enhanced by a number of diamines (3); 1,7-diaminoheptane was the most effective diamine tested. The mode of action of polyamines in this system remains uncertain; however, two explanations have been proposed: (i) restoration of internal polyamine levels which were depleted by ex-

tensive leakage after infection of the lysogen with the rII mutant (11) and (ii) the polyamines stabilized a leaky cell envelope (4).

That Mg^{2+} or polyamine enhancement of phage synthesis is not confined to the T-even bacteriophages was shown by Tucker (23) in a report concerning ϕR , a small coliphage containing single-stranded DNA. In this system, the above cations are necessary in late events of phage morphogenesis and may also be involved with phage liberation.

Attempts have been made to establish a spermidine deficiency and to study its effects on phage infection; however, a suitable biological system was not available and these studies were inconclusive (7). The availability of a putrescine auxotroph appeared to make such studies feasible.

Two constitutive pathways function in the biosynthesis of putrescine in *E. coli* (19, 20) from arginine and ornithine. In the agmatine ureohydrolase deficient-mutant which we have employed, the synthesis of putrescine from agmatine is almost totally blocked (15), and the addition of arginine inhibits the other pathway of putrescine

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synthesis by inhibiting the synthesis of ornithine from glutamate. Through the use of this polyamine auxotroph, we have investigated the polyamine dependence of nucleic acid synthesis in uninfected cells and a possible polyamine requirement for DNA synthesis in phage-infected cells.

MATERIALS AND METHODS

Lysates of T4r⁺D were prepared by infecting *E. coli* B at an initial multiplicity of infection (MOI) of 0.1. Supplies of purified phage stocks were obtained by two cycles of low ($4,080 \times g$)- and high ($27,300 \times g$)-speed centrifugation of overnight broth lysates.

The properties of *E. coli* K-12 (MA 159), defective in agmatine ureohydrolase and auxotrophic for histidine, leucine, and threonine, have been described (15). This strain is markedly inhibited in growth by addition of arginine in the absence of polyamines. Two methods of polyamine depletion were used in this study. In method 1 (15, 17), an overnight broth culture was chilled for 5 hr at 4 C and diluted 1:20 in AFA medium [AF medium (see below) supplemented with arginine (100 μ g/ml)]. Samples (0.1 ml) of suspension were plated on AFA agar plates in which putrescine production was inhibited. After overnight growth at 37 C, the slight bacterial lawns were scraped from the agar surface with small portions of AFA medium. These harvested bacteria were then sedimented ($4,080 \times g$ for 15 min) and resuspended in Davis-Mingioli (8) medium without glucose. After washing the cells once more, samples of the suspension were added to various AF media (arginine-free medium containing amino acids, vitamins, adenosine, uracil, glucose, and inorganic salts as described in reference 15) containing the designated supplements.

In method 2, an overnight broth culture was treated as above and diluted 1:40 in AF medium containing arginine (100 μ g/ml). After overnight growth at 37 C, the polyamine-depleted cells were washed as in method 1, and samples of a fourfold-concentrated suspension were added to AF media containing the designated supplements.

For polyamine assays of cells and media, a 2.0-ml sample was removed and filtered on membrane filters (0.45 μ m; Millipore Corp.). The filters (cells) were extracted in 1.0 ml of 0.2 N perchloric acid (PCA). PCA (2.0 N) was added to the filtrate (medium) to a final concentration of 0.2 N. Dansylation of acid extracts (0.2 ml) and polyamine estimation by thin-layer separation and in situ scanning of fluorescent dansyl derivatives (9, 10) have been described. For the estimation of agmatine, acid extraction and dansylation were performed as above. Samples were spotted on Avicel thin-layer-chromatography plates (Analtech, Newark, Del.) and subjected to thin-layer electrophoresis in 8% formic acid (pH 2.0) for 120 min at 15 v/cm. After electrophoresis, the plate was carefully dried for approximately 30 min in a cold air stream in the dark to minimize quenching of fluorescence.

Turbidities were measured in a Klett colorimeter with a 420 filter. The mass doubling time is defined as the time necessary for the turbidity of a culture to double.

Infected or uninfected cells were precipitated at 0 C with 5% trichloroacetic acid. After centrifugation, the supernatant fluids were discarded, and the diphenylamine reagent was used to estimate DNA in pellets of infected cells (6). For the estimation of DNA and RNA from uninfected cells, pellets were resuspended and extracted two times with cold 5% trichloroacetic acid. The washed pellets were then hydrolyzed in 5% trichloroacetic acid at 80 C for 15 min. The hydrolysate was assayed for DNA and RNA by the method of Burton (5) and by the orcinol procedure (18), respectively.

Putrescine and spermidine hydrochloride salts and dansyl chloride were obtained from Calbiochem (Los Angeles, Calif.). The hydrochloride salt of cadaverine was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio). The free base of *N*-3-aminopropyl-1,5-diaminopentane was obtained from E. F. Elslager (Parke-Davis, Ann Arbor, Mich.), converted to the hydrochloride salt, and recrystallized from absolute ethanol. All of the above compounds were homogeneous (greater than 99% purity) after chromatography and ninhydrin staining or when chromatographed as dansyl derivatives.

RESULTS

Identification of cadaverine and a new spermidine analogue in putrescine-depleted cells. As shown in Fig. 1 (analysis 2), dansylated PCA extracts of polyamine-depleted cells, grown on lysine, contained two fluorescent derivatives which are chromatographically distinct from the known polyamines of *E. coli*, i.e., putrescine and spermidine. Further chromatographic comparisons of these dansyl derivatives with a number of authentic dansyl polyamines were performed on Silica Gel G plates in two solvents, namely, ethyl acetate-cyclohexane and benzene-triethylamine. Each thin-layer plate was chromatographed three times in the same dimension with a short drying step between each repeated run. The results of these analyses are shown in Table 1. In ethyl acetate-cyclohexane, one of the unknowns had a slightly higher relative R_F value than dansyl spermidine; however, in benzene-triethylamine, this unknown was clearly distinct from spermidine and coincided in both solvent systems with authentic dansyl *N*-3-aminopropyl-1,5-diaminopentane. The other unknown corresponded chromatographically to dansyl cadaverine in both solvents and is clearly distinguishable from all other authentic dansyl derivatives.

Larger quantities of the unknowns were isolated from similarly grown cells. A pellet of 6.8×10^{11} polyamine-depleted cells was extracted twice with 20 ml of cold 5% trichloroacetic acid each time at 4 C. After removal of trichloroacetic acid with ether, the solution was made strongly alkaline and extracted with *n*-butanol (21). After acidification with HCl, butanol was removed in

TABLE 1. Relative R_F values^a of various dansylated polyamines

Dansyl polyamine ^b	Solvent 1 ^c	Solvent 2 ^d
1,3-Diaminopropane	0.75	0.75
1,4-Diaminobutane (putrescine)	0.76	0.74
1,5-Diaminopentane (cadaverine)	0.84	0.88
R-1,3-Diaminopropane	0.58	0.95
R-1,4-Diaminobutane (spermidine)	0.59	1.01
R-1,5-Diaminopentane	0.62	1.09
R-1,6-Diaminohexane	0.70	1.21
R-1,4-Diaminobutane-R (spermine)	0.42	1.14
Unknowns	0.62 0.85	0.88 1.08

^a Relative R_F value = distance from origin to dansyl compound/distance from origin to dansyl ammonia.

^b R = N-3-aminopropyl.

^c Solvent 1: ethyl acetate-cyclohexane (30:60; v/v).

^d Solvent 2: benzene-triethylamine (80:16; v/v).

TABLE 2. Melting point data from various polyamine picrates

Polyamine picrate ^a	Found (C)	Reported
1,4-Diaminobutane (putrescine)	250 (d) ^b	250 (d) ^c
1,5-Diaminopentane (cadaverine)	215–217	221 ^c
R-1,3-Diaminopropane	228–229	230 ^d
R-1,4-Diaminobutane (spermidine)	208–209 (d)	210–212 (d) ^d
R-1,5-Diaminopentane	182–183	182 ^d
R-1,4-Diaminobutane-R (spermine)	238–240 (d)	248–250 (d) ^c
Unknowns	215–217 182–183	

^a R = N-3-aminopropyl.

^b (d), Decomposes.

^c Guggenheim, M. 1940. Die biogenen amine, p. 235–237. S. Karger, New York.

^d von Braun, J., and W. Pinkernelle. 1937. Ber. Deut. Chem. Ges. 70:1230–1240.

vacuo. The dried residue, containing approximately 20 μ moles of polyamine hydrochlorides, was dissolved in 2.0 ml of water and applied to a Dowex-50 (H^+) column (0.8 by 8.0 cm). The polyamines were eluted from the column with an HCl gradient (reference 22; 300 ml of water in the mixing vessel and 300 ml of 2.5 N HCl in the reservoir) at a flow rate of 30 to 40 ml per hr, and approximately 6-ml fractions were collected.

The polyamines in the collected fractions were monitored by dansylation, and material eluting between 156 to 186 ml (peak 1) behaved on chromatography in the above solvents as authentic dansyl cadaverine. Dansylation of material eluting between 256 and 300 ml (peak 2) yielded a derivative which chromatographed as dansyl N-3-aminopropyl-1,5-diaminopentane. Pooled fractions from each peak were dried in vacuo, dissolved in 0.01 M HCl, and converted to crystalline picrates by the method of Beer and Kosuge (2). As shown in Table 2, the melting points (uncorrected, Fisher-Johns apparatus) of the picrates of peak 1 and peak 2 were 215 to 217 C and 182 to 183 C, respectively, corresponding to the melting points of authentic picrates of cadaverine and N-3-aminopropyl-1,5-diaminopentane.

Figure 1 (analysis 6) demonstrates that the removal of lysine from arginine-inhibited cultures reduced the level of the two unknowns. To ascertain whether lysine was a precursor for one or both of these compounds, arginine-inhibited polyamine-depleted cells were grown in the presence of uniformly labeled ^{14}C -lysine (1.1 μ moles/ml; 3.25×10^5 counts per min per μ mole). The cells were extracted in cold 5% trichloroacetic acid

FIG. 1. Thin-layer chromatogram of dansyl polyamine derivatives of PCA cell extracts of cultures at $T = 180$ chromatographed twice in ethyl acetate-cyclohexane (30:60, v/v). 1 + 5, Arginine-free; 2 + 6, arginine added; 3 + 7, arginine and putrescine added; 4 + 8, arginine and cadaverine added. Designations as in legend to Fig. 2. 1 to 4, With lysine; 5 to 8, without lysine. 9 to 11, Dansyl putrescine and spermidine standards at concentrations of 7.5×10^{-11} , 15.0×10^{-11} , and 30.0×10^{-11} moles/spot for dansyl putrescine and 2.5×10^{-11} , 5.0×10^{-11} , and 10.0×10^{-11} moles/spot for dansyl spermidine. P, putrescine; C, cadaverine; SPD, spermidine. The dansyl spermidine analogue has a slightly higher R_F value than dansyl spermidine in this solvent system.

and the polyamines were extracted into *n*-butanol and fractionated on Dowex-50 (H^+) as described above. Two peaks of radioactivity were obtained. Dansylation of fractions from the first peak (144 to 186 ml) revealed the presence of a derivative which co-chromatographed with dansyl cadaverine. However, since the specific activity was not constant throughout the peak, a further fractionation was attempted by thin-layer electrophoresis on an Avicel cellulose plate in 0.1 M citrate buffer (pH 3.5) for 2 hr at 17.5 v/cm. The latter procedure cleanly separated the unknown from a trace of lysine which amounted to approximately 12% of the radioactivity. (This purified diamine had a specific activity of 2.48×10^5 counts per min per μ mole).

Dansylated material from the second peak (264 to 300 ml) co-chromatographed with dansyl *N*-3-aminopropyl-1,5-diaminopentane. The specific activity throughout the peak was essentially constant and slightly less (2.24×10^5 counts per min per μ mole) than five-sixths of the specific activity of the exogenous uniformly labeled ^{14}C -lysine. This small dilution of the labeled lysine was not unexpected, since the cells had been depleted in the presence of unlabeled lysine. From these data, we conclude that the two unknowns are identical to cadaverine and *N*-3-aminopropyl-1,5-diaminopentane and are both derived in largest part from lysine. The appearance of these compounds led us to examine the effect of lysine supplementation.

Effect of polyamines on growth rates. Polyamine-depleted cells, grown in the absence of arginine, exhibited similar mass doubling times in the presence or absence of lysine, i.e., 68 and 64 min, respectively. However, the growth rates of similarly treated cells grown in the presence of arginine were prolonged to 135 min in the presence of lysine and 183 min in cultures grown without lysine. The addition of putrescine to arginine-inhibited cells, with or without lysine, enhanced the growth rates to control values, i.e., 66 min in both instances. The addition of cadaverine to arginine-inhibited cells supplemented with lysine decreased the mass doubling time only slightly (from 135 to 115 min). In contrast, the mass doubling time of arginine-inhibited cultures lacking lysine was reduced, more significantly, by the addition of cadaverine, namely, from 183 to 105 min; however, in no instance was cadaverine as effective as putrescine.

Polyamines and nucleic acid synthesis. Concurrent with the above growth rate studies, samples were withdrawn from each culture at identical cell densities of approximately 1.5×10^8 , 3.0×10^8 , and 4.5×10^8 cells/ml, corresponding to turbidities of 60, 120, and 180 Klett units at 420 nm, for analyses of DNA, RNA, and polyamine

accumulation. As shown in Fig. 2, increases in nucleic acid, especially RNA, paralleled turbidity changes. Omission of arginine, allowing the synthesis of putrescine and spermidine from ornithine (Fig. 1, analyses 1 and 5), resulted in the control rate of RNA and DNA synthesis, with or without lysine. In contrast, the addition of arginine inhibited nucleic acid synthesis which was further inhibited by the omission of lysine. As shown in Fig. 1, no detectable levels of putrescine were found in arginine-inhibited (analysis 2) cultures grown in the presence of lysine; however, the presence of cadaverine and a new spermidine analogue was apparent. A similarly treated culture (analysis 6), grown in the absence of lysine, revealed no detectable levels of putrescine or cadaverine but apparently low levels of the spermidine analogue. The latter culture exhibited the

FIG. 2. Effect of polyamine depletion and supplementation on RNA and DNA synthesis in the agmatine ureohydrolase mutant of *E. coli* K-12. The mutant was depleted of polyamines as described as method 2. Washed cells were added to the designated media at an initial turbidity of 40. Samples for nucleic acid analyses were withdrawn from each culture at identical turbidities, i.e., $T = 60, 120, \text{ and } 180$. AF, arginine-free; AFA, arginine added; AFAP, arginine and putrescine added; AFAC, arginine and cadaverine added. Arginine and lysine dihydrochlorides added at concentrations of 100 and 200 $\mu\text{g/ml}$, respectively. Putrescine and cadaverine dihydrochlorides added to a final concentration of 1.0 $\mu\text{moles/ml}$.

longest mass doubling time and the lowest rate of RNA synthesis.

Putrescine addition to arginine-inhibited cells enhanced both the rate of RNA and DNA synthesis to control levels. Polyamine analyses of these cultures (analyses 3 and 7) revealed increases in intracellular putrescine and spermidine but no detectable levels of cadaverine or the spermidine analogue, whether grown with or without lysine, indicating the possible inhibition of lysine decarboxylase by putrescine or spermidine, or by both. Although no effect on the synthesis of RNA and DNA in lysine-supplemented cultures was apparent after cadaverine addition, exogenous cadaverine stimulated the rate of RNA synthesis, but not DNA synthesis, in arginine-inhibited cultures grown in the absence of lysine. These cultures (Fig. 1, analyses 4 and 8) exhibited increased intracellular concentrations of both cadaverine and the spermidine analogue but no detectable putrescine or spermidine.

We have consistently observed that polyamine addition to arginine-inhibited cells effects almost an immediate stimulation of growth rate and RNA synthesis; however, DNA synthesis is affected only after a pronounced lag. No detectable levels of agmatine were observed in any of the above cultures except for trace levels in arginine-

inhibited cultures unsupplemented with polyamines.

Polyamines and synthesis of phage DNA. Figure 3 illustrates the effect of polyamines on phage DNA synthesis in the T4D-infected wild strain and the agmatine ureohydrolase mutant of *E. coli* K-12 grown in the complex medium employed in the above study and described by Hirshfield et al. (15). Infection of polyamine-depleted cells, released from arginine inhibition, resulted in the initiation of DNA synthesis at approximately 15 min after infection, resulting in a threefold increase in DNA accumulation by 120 min. Maintenance of arginine inhibition not only effected a later time of DNA initiation but also a slower rate of DNA accumulation. The addition of putrescine or spermidine to similarly treated cultures 15 min before infection restored both the time of onset of DNA synthesis and the rate of DNA accumulation to control levels. No differences were observed when the wild parent was infected under this range of conditions.

Polyamines and phage maturation. We have also performed one-step growth experiments at low multiplicity (MOI = 0.1). Under the same growth conditions as above, we observed a 90% loss of infectious centers upon infection (Fig. 4). Phage release, without the use of chloroform to

FIG. 3. Effect of polyamines on DNA synthesis in T4D-infected *E. coli* K-12 (wild) and *E. coli* K-12 (agmatine ureohydrolase-negative). Experimental conditions for polyamine depletion are as described in method 1. Washed cells were added to AF and AFA media at an initial turbidity of 40 Klett units. When the turbidity increased to 80, putrescine (AFAP) or spermidine (AFASPD) was added, and infection with T4D ensued 15 min later at an MOI of 5. Final concentrations of arginine, putrescine, and spermidine were 100 μ g and 1.0 and 0.5 μ moles/ml, respectively.

FIG. 4. Effect of polyamines on one-step growth of T4D-infected *E. coli* K-12 (agmatine ureohydrolase-negative). Conditions for polyamine depletion and growth before infection were exactly as described in Fig. 3. After infection with T4D at an MOI of 0.1, each culture was diluted 10^{-4} . Assays for infectious centers on *E. coli* B and chloroform lysis were performed according to Adams (1).

lyse the infected cells, was observed only in the infected culture grown in the absence of arginine and only after a rather extended period of time after the initiation of DNA synthesis. However, when infected cultures were lysed with chloroform, we noted the release of infectious particles, indicating that these mature phage are maintained in an inactive state, perhaps through association with a membrane component. It is apparent that inhibition by arginine produces a protracted period of phage maturation compared to maturation in cultures infected in the absence of arginine. Thus, we find that exogenous polyamines, when added to arginine-inhibited cells, not only facilitate DNA synthesis but also enhance the rate of phage maturation to control levels, although the time of onset of maturation remains unaffected.

An electron microscopic study, kindly carried out by Margit Nass, of multiply infected (MOI = 5) cultures under these conditions have corroborated our conclusions concerning the stimulatory effects of exogenous polyamines on phage

synthesis. In addition, we have noted that the mutant under all of the above conditions appears extremely fragile after infection.

Synthesis of phage DNA in minimal medium. In an effort to maximize the stimulatory effects of added polyamines on the synthesis of phage DNA, a simplified medium was employed which contained only supplements necessary for growth, i.e. histidine, leucine, and threonine. Under these conditions (Fig. 5), DNA synthesis began at 15 min, resulting in a two- to threefold accumulation of DNA in T4D-infected cultures not containing arginine. Addition of arginine delayed the inception of DNA synthesis for 1 hr, and only a 60%

FIG. 5. Effect of polyamines on DNA synthesis in T4D-infected *E. coli* K-12 (agmatine ureohydrolase-negative) in minimal medium (MIN). A 6-hr culture in MIN was chilled overnight at 4°C; the cells were washed twice and resuspended in MIN, and a sample was added to MIN containing arginine (100 μg/ml). This culture was depleted of polyamines by growth for 6 hr and chilled overnight at 4°C. Samples of washed cells were added to either MIN or MIN + A at a cell density of approximately 10^8 /ml ($T = 40$). At $T = 80$ or approximately 2×10^8 cells/ml, putrescine or spermidine was added to MIN + A cultures at final concentrations of 1.0 and 0.5 μmoles/ml. After 15 min, cultures were infected with T4D at an MOI of 5 after the addition of L-tryptophan (25 μg/ml). MIN, minimal salts medium of Davis and Mingoli supplemented with glucose, L-histidine, L-leucine, and L-threonine at final concentrations of 2 mg and 30, 50, and 50 μg/ml, respectively. A, arginine; P, putrescine; SPD, spermidine.

increase was formed after 3 hr. Exogenous putrescine or spermidine added 15 min before infection decreased the time necessary for the initiation of DNA synthesis and almost restored the control level of DNA accumulation.

DISCUSSION

The studies of Hirschfield et al. (15) implied that arginine inhibition of growth in the agmatine ureohydrolase-negative mutant was the result of a polyamine depletion; however, no polyamine assays were performed to support this conclusion. The present paper clearly demonstrates that exogenous arginine depletes polyamine levels in the mutant which we have studied.

We have presented evidence that exogenous polyamines supplied to polyamine-depleted cells permits rates of nucleic acid synthesis and growth rates similar to these activities in the same cells which do not have inhibited endogenous polyamine synthesis. We have also noted the presence of a backup system resulting in the synthesis of cadaverine and a new spermidine analogue in polyamine-depleted cells grown in the presence of lysine. Cadaverine, at least, appears to possess some of the biological activity of putrescine and spermidine, perhaps via the formation of the spermidine analogue, *N*-3-aminopropyl-1,5-diaminopentane. To extend these results, studies should be repeated in a system in which the decarboxylation of lysine is blocked. In addition the biological effects of *N*-3-aminopropyl-1,5-diaminopentane should be examined. It is interesting to note in this regard that, although cadaverine has little or no growth-promoting activity, *N*-3-aminopropyl-1,5-diaminopentane had growth factor activity for *Lactobacillus casei* (13) and the polyamine auxotroph *Haemophilus parainfluenzae* (14). That cadaverine was not a growth factor for these organisms may be the result of the inability to convert cadaverine to the spermidine analogue. Conversion of cadaverine to the new spermidine analogue observed in the present study requires either that cadaverine can act as a propylamine acceptor, utilizing the wild-type transferase, or that a new propylamine transferase is induced.

Evidence purporting to demonstrate the non-essentiality of putrescine in T-even phage synthesis was reported by Kim (16). In the latter study, a mutant of *E. coli* B, containing a constitutive α -ketoglutarate transaminase, utilized putrescine as the sole source of carbon and nitrogen, resulting in putrescine-depleted cells upon nitrogen starvation. T2 infection of these depleted cells, when compared to infected, nondepleted cells, resulted in similar plating efficiencies and plaque morphology. However, as noted by Kim, the

putrescine-depleted cells contained substantial quantities of acetyl putrescine, acetyl spermidine, and spermidine. In addition, the infected putrescine-depleted cells were maintained for only 20 to 25 min after infection in a nitrogen-starved condition. Therefore, no information concerning the effect of polyamines on rates of phage synthesis was obtained as in the present study.

In conclusion, we have found that exogenous polyamines added 15 min before infection of polyamine-depleted cells effected a marked stimulation of phage DNA synthesis and maturation, approaching rates observed in nondepleted cells.

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