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Cadaverine in Bacteriophage T4

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Cadaverine was found in bacteriophage T4 when the host cells of *Escherichia coli* K-12 were grown in complex media and aerated by agitation. Only traces of cadaverine were found if the host was grown and agitated in synthetic medium or was aerated by vigorous bubbling in a complex medium. When the host cells were grown anaerobically in a complex medium, cadaverine became the major polyamine in the progeny phage. The polyamine content comprised 80% cadaverine, 14% spermidine (or its recently discovered homologue, *N*-3-aminopropyl-1,5-diaminopentane), and the remainder putrescine. The conditions that favored appearance of cadaverine are known to be required for induction of lysine decarboxylase. It was shown that lysine was the sole source of bacterial cadaverine.

Putrescine and spermidine are the usual polyamines of bacteriophage T4 (1, 2) and, as such, reflect the polyamines of the host bacteria (1). If the polyamine content of host bacteria is changed by incorporation of a foreign polyamine from an appropriately supplemented medium, the added polyamine can also be found in the progeny phage (1). During the course of some comparative analyses of the polyamine content of T4r$^+$ and T4rII mutant bacteriophages we unexpectedly found what we eventually interpreted as a further example of the same phenomenon of polyamine replacement. At first it appeared that T4rII contained significant quantities of a polyamine not found in T4r$^+$ phage. The difference was soon found to be independent of phage genotype and was caused by a consistent difference in the preparation of the phages. The T4rII were replicated within cells aerated by agitation on a rotary shaker, whereas the paired host for T4r$^+$ phage was aerated by vigorous air bubbling. Identification of the new compound as cadaverine suggested that the conditions we used for growth of T4rII phage permitted induction of lysine decarboxylase. This enzyme, which converts lysine to cadaverine, is maximally induced when cells of *Escherichia coli* are grown anaerobically in a complex medium (12), and presumably the less vigorous aeration achieved with shaken cultures allowed some induction of the enzyme. In a sense, activity of lysine decarboxylase would be a natural way to provide a different polyamine to cells. When, in fact, cells were grown under optimal conditions for induction of the enzyme anaerobically in complex medium, cadaverine was present in the host cells and became the principal polyamine of progeny phage.

MATERIALS AND METHODS

Media. Tryptone broth contained 1% tryptone. Tryptone-Na$^+$ and tryptone-Mg^{2+} were supplemented with 0.1 M NaCl or 0.08 M $MgCl_2$, respectively. FJ medium was a modified 3XD medium (10) and contained 74 mM Na_2HPO_4, 33 mM KH_2PO_4, 37.4 mM NH_4Cl, 1.2 mM $MgSO_4$, 0.15 mM $CaCl_2$, 0.2 to 0.4% glucose, 0.25% Casamino Acids, and 1 ml of 1% gelatin in 0.5% NaCl per liter. Minimal medium E (28) was supplemented with 0.1 mM $CaCl_2$, 5 μM $FeCl_3$, and 1.1 mM lysine. When cells were grown anaerobically, all media contained 0.5% glucose and, when noted, 25 ml of filter-sterilized Gramercy universal indicator (Fisher Scientific Co.) per liter. The indicator allowed an easy monitoring of pH. Phage were plated on tryptone-Na$^+$ plus 1.2% agar with an overlay of tryptone-Na$^+$ plus 0.7% agar. Phage dilution fluid contained 100 mM NaCl, 1 mM $MgCl_2$, and 0.1 mM $CaCl_2$.

Phages and bacteria. The sources and descriptions of our phage and bacteria have been presented (3). T4r$^+$ phage or T4rII phage (T4r1993), grown phenotypically as r$^+$ on either *E. coli* BB or *E. coli* K-12, were prepared by growing the host in FJ medium to a cell density of 2×10^8 to 3×10^8 cells per ml before infecting with 10^6 phage per ml. The mixture was shaken 4 h at 37 C and refrigerated overnight. Routinely, more than 50% of the phage could be centrifuged down with the bacterial pellet and then released by shaking the pellet resuspended in phage dilution fluid with 1/10 volume of chloroform for 10 min. This was a convenient way to concentrate most of the phage from a large volume. Phage were also prepared by one-step infection cycles of cells that were either shaken, aerated, or grown anaerobically. For anaerobic growth, the deaerated medium, usually

in a volumetric flask, was covered with mineral oil and stirred gently by a magnetic stirrer. The pH was adjusted by additions of 1 M Na_2CO_3. All phage preparations were purified by DNase treatment and 2 to 3 cycles of differential centrifugation (14). The criterion for an acceptable preparation was that the ratio of phage titer to absorbance at 400 nm have a value greater than 2×10^{12} (14).

Radioactivity. Uniformly labeled ^{14}C-lysine was purchased from New England Nuclear Corp. Samples were counted in a scintillation mixture containing 874 ml of dioxane, 60 ml of methanol, 112.5 g of naphthalene, 6.75 g of 2,5-diphenyloxazole, and 0.657 g of 1,4-bis-2-(5-phenyloxazolyl)benzene.

Polyamine preparation. In most analyses of phage polyamines, the phage preparation was suspended in phage dilution fluid and hydrolyzed with 4 N HCl in sealed tubes at 105 C for 24 h. After removal of HCl in a vacuum desiccator over NaOH and $CaCl_2$, the dried residue was dissolved in water. The amino acids from phage protein served as a confirmatory measure of the amount of phage assayed. Polyamines were also prepared by extraction with 0.3 M trichloracetic acid (9).

Column chromatography of polyamines. With the exception of the choice of eluting solvents, our method is very similar to the ion-exchange procedure for separation and analysis of basic amino acids (18, 24). Polyamines, on a 15-cm by 0.9-cm bed of 8% cross-linked sulfonated polystyrene in the Na^+ form (Beckman/Spinco type 15A ion exchange resin), were eluted at 55 C with 1.5 M sodium acetate, flowing at a rate of 30 to 34 ml per h. When 50 fractions, each containing 2 ml, were collected, elution was continued with 2.5 M sodium acetate containing 0.01 M sodium tetraborate. To obtain the correct pH for ninhydrin assay of amines, 0.2 ml of 6.6 N acetic acid was added to each 1.5 M acetate fraction, and 0.2 ml of 11 N acetic acid was added to each 2.5 M acetate fraction. The assay procedure used was that of Moore and Stein (19) except that 1 ml of ninhydrin reagent (24) was added to each fraction. Standard solutions of leucine and the individual polyamines were assayed for nitrogen content by the Kjeldahl procedure (15) so that relative ninhydrin color yields could be determined (19). In the solvents in which the polyamines were eluted, the relative molar color yields were: leucine, 1.0; putrescine, 1.1; cadaverine, 1.1; and spermidine, 1.64. One micromole of leucine yielded an absorbancy of 1.1 to 1.9, varying with the age of the ninhydrin reagent. Figure 1 shows the separation and quantitative analysis of polyamines. When an acid hydrolysate of whole phage was chromatographed, the first 70 ml of effluent was collected in one batch and assayed as a measure of phage protein.

The same separation and quantitation of polyamines was obtained when sodium citrate buffer (pH 5.28; reference 18) was used to separate basic amino acids prior to elution with 1.5 M sodium acetate. In comparison with an automated analytic procedure (20), our method is slower and has the additional disadvantage of high salt concentration in the effluent. However, prior purification of polyamines from salt-rich media is not required by our method.

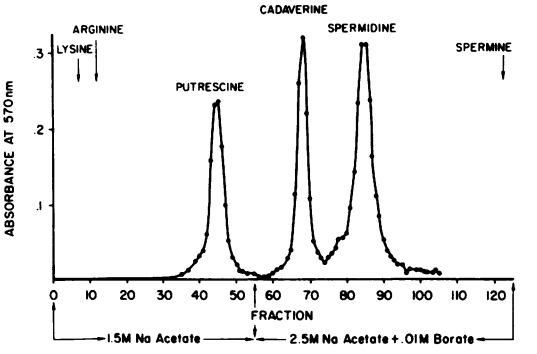

FIG. 1. *Separation and analysis of polyamines. An aqueous solution containing 0.98 μmol of putrescine, 0.83 μmol of cadaverine, and 0.99 μmol of spermidine was chromatographed on a 0.9- by 16-cm column of cation exchanger. The second elution solvent was started after fraction 50 was collected and first appeared in tube 56 of the effluent. All 2-ml fractions were analyzed by the ninhydrin method. The summed absorbancies in the peaks indicated the presence of 0.95 μmol of putrescine, 0.83 μmol of cadaverine, and 0.97 μmol of spermidine, or recoveries of 97 to 98%. The arrows indicate where arginine, lysine, and spermine would be eluted. Neutral and acidic amino acids preceded lysine.*

Identification of cadaverine. Cadaverine from phage cochromatographed with authentic cadaverine in the system described above. For further identification, it was desalted on a 1- by 15-cm column of weakly acidic carboxylic (polymethacrylic) cation exchanger, Amberlite CG-50 in the pyridinium form. The polyamine in 30 ml of 2.5 M sodium acetate was diluted to 1 liter and passed through the column. The residual retained salt was eluted with 500 ml of 0.1 M pyridinium acetate (pH 5.6), and the polyamine was eluted with 100 ml of 0.5 M pyridinium acetate (pH 5.0). After flash evaporation, the salt-free residue cochromatographed with cadaverine in four systems. The first three utilized solvents I, II, and III of Dubin and Rosenthal (9) on, respectively, Whatman papers No. 1, 20, and 20. The fourth utilized chromatography on Reeve-Angel paper SA-2, loaded with Amberlite cation exchange resin IR-120-Na^+ form. After application of the sample, the ion-exchange paper was wet with 2.5 M sodium acetate (pH 5.3 with acetic acid) from both ends toward the origin, blotted, and eluted with the same solvent for 7 h. Putrescine, cadaverine, and spermidine were well separated, migrating, respectively, 13.1, 8.2, and 5.8 cm. Amines were detected by dipping the paper in a ninhydrin solution (4).

Dipicryl derivatives of authentic cadaverine and of the "unknown" were prepared (21) and crystallized from ethyl acetate containing sufficient 70% aqueous ethanol to make the mixture a poor solvent at room temperature. The crystals were recrystallized from a similarly made mixture of benzene and 95% ethanol. The mp for authentic dipicrylcadaverine is 164 C; unknown, 164 to 165 C; mixed, 165 C.

RESULTS

Data in a prior publication (3) showed that the polyamine content of bacteriophage T4rII mutants is similar to that of T4r$^+$ phage. The host cells were grown in tryptone-Mg^{2+} (a medium which permits replication of T4rII phage within *E. coli* K-12 [λ]; reference 13) and were vigorously aerated during growth and infection. The progeny phage contained putrescine and spermidine in amounts that did not vary significantly between T4r$^+$ and T4rII phage; nor were the amounts varied when the phage were replicated within *E. coli* K-12, K-12 (λ), or K-12 (λi^{434}). Only a trace of ninhydrin reactive material was observed in addition to putrescine and spermidine. The average content of putrescine and spermidine in these phages is presented in the first line of Table 1.

Cadaverine in T4 bacteriophage. In other experiments, the host cells were aerated by agitation on a rotary shaker rather than by the previous procedure of bubbling air through a sintered glass sparger. A significant amount of polyamine was now observed in fractions which previously had contained only traces of material. The new compound's elution profile was identical to that of cadaverine. The compound was identified as cadaverine by cochromatography in four separate systems and by formation of a dipicrylcadaverine derivative that did not depress the melting point of authentic dipicrylcadaverine.

Cadaverine has not previously been reported to be present in T4 bacteriophage. It is a minor component and may not have been clearly discernible in assays performed on smaller samples of phage. Possibly, a more important factor is that much less cadaverine is found in phage if the host cells are grown in a synthetic medium. The data of Table 1 show that the cadaverine content of phage was much lower when the host cells were shaken in FJ medium rather than tryptone-Mg^{2+}. Two preparations of phage replicated in tryptone-Na$^+$ were also assayed. Although less cadaverine was found than when the medium was tryptone-Mg^{2+}, the difference was not statistically significant.

Anaerobically replicated bacteriophage. Since more cadaverine was observed in phage from less vigorously aerated host cells, phage were obtained next by infection of anaerobic cells. The data in Table 2 show that cadaverine is the major polyamine in phage from anaerobic cells grown in tryptone-Mg^{2+} or tryptone-Na$^+$. In comparison with the data in Table 1, it appears that cadaverine primarily replaced putrescine, although the content of "spermidine" was also diminished. Spermidine may have contained some proportion of the recently discovered cadaverine analogue of spermidine (7, 8, 27; see Discussion). In contrast to these results, there was no marked increase in cadaverine when the host cells were grown anaerobically in synthetic medium. Several differences between the conditions of growth in complex and synthetic media were examined as possible factors responsible for the great disparity in phage cadaverine content. The synthetic medium contained 0.25% (wt/vol) acid-hydrolyzed casein (Difco Casamino Acids) and therefore did not contain acid-labile amino acids and amides that would be present in tryptone. However, these acid-labile compounds cannot by themselves play a significant role because replacement of Casamino Acids by the same amount of vitamin-free enzymatically hydrolyzed casein (Nutritional Biochemicals Corp.) resulted in only a small increase of cadaverine. Another difference in growth conditions was that the pH in complex media was monitored by

TABLE 1. *Effect of growth conditions on phage polyamine content*[a]

Growth conditions of host cells		Amount per 10^{12} phage (nmol)				Cadaverine nitrogen per polyamine nitrogen
Medium	Aeration	Putrescine	Spermidine	Cadaverine	Polyamine nitrogen	
Tryptone-Mg^{2+}	Vigorous bubbling (8)	109 ± 16	35 ± 4.5	0.8 ± 0.35	325	0.5%
Tryptone-Mg^{2+}	Shaker (8)	100 ± 25	35 ± 5.6	10 ± 4.8	325	6.2%
FJ (synthetic)	Shaker (8)	95 ± 19	19 ± 4.5	0.5 ± 0.15	248	0.4%
Tryptone-Na$^+$	Shaker (2)	102 ± 18	32 ± 9	3.1 ± 0.8	306	2.0%

[a] Cells were grown and infected with T4 bacteriophage under the listed conditions. The phage were purified, hydrolyzed, and assayed for polyamines by Dowex 50-Na$^+$ chromatography. The table records means and standard deviations of the data. The numbers in parentheses indicate how many phage preparations were purified and assayed. The preparations included: line 1, T4r$^+$ and T4rII on K-12 and on K-12 (λ); line 2, T4r$^+$ on K-12, T4r$^+$ and T4rII on K-12 (λ); line 3, T4r$^+$ and T4rII on K-12, T4r$^+$ or K-12 (λ), and T4rII on K-12 (λ$_i^{434}$); line 4, T4r$^+$ on K-12 and K-12 (λ).

TABLE 2. *Polyamines in anaerobically grown bacteriophage T4*[a]

Medium	Amount per 10^{12} phage (nmol)				Cadaverine nitrogen per polyamine nitrogen
	Putrescine	Spermidine	Cadaverine	Polyamine nitrogen	
Tryptone-Na^+	16 ± 6	14 ± 0.7	122 ± 29	318	77%
Tryptone-Mg^{2+}	9.1	19	165	405	82%
FJ	88	22	1.9	246	1.5%
FJ (−) CAA + 0.25% Enz. Cas. Hyd.	51	27	7.9	199	7.9%
FJ (−) CAA + 0.25% tryptone	59	9.7	25	197	25%
FJ + 0.25% tryptone	28	28	61	262	47%

[a] Cells of *E. coli* K-12 were grown anaerobically in different media and infected with T4r$^+$. All media contained 0.5% glucose, and the first two listed media contained Gramercy universal indicator. The medium FJ (−) CAA + 0.25% Enz. Cas. Hyd. contained the ingredients of FJ medium except that the 0.25% (wt/vol) of casamino acids (CAA) was replaced by the same amount of enzymatically hydrolyzed vitamin-free casein (Enz. Cas. Hyd.). In FJ (−) CAA + 0.25% tryptone, the Casamino Acids were replaced by tryptone, whereas FJ + 0.25% tryptone contained all the ingredients of FJ plus 0.25% (wt/vol) tryptone. Two separate phage preparations from tryptone-Na^+ were analyzed; the means and standard deviations are presented. From all the other media, only one phage preparation was obtained and analyzed.

color changes of Gramercy universal indicator and adjusted by addition of 1 M Na_2CO_3, whereas the pH in synthetic medium was controlled by the presence of phosphate buffer. This factor was of limited importance because replacement of Casamino Acids in synthetic medium by the same amount of Tryptone resulted in the appearance of a large amount of cadaverine; an even greater increase was observed when tryptone and Casamino Acids were both present. The results suggest that some ingredients of tryptone stimulate the formation of cadaverine. It has been reported (17) that the formation of lysine decarboxylase, an enzyme responsible for cadaverine synthesis, is greatly stimulated in synthetic medium by the presence of some peptides from casein in addition to neutral amino acids.

Cadaverine in anaerobic cells. It is known that a greater induction of lysine decarboxylase in *E. coli* occurs in complex media than in synthetic media (12) and that induction is inhibited by aeration (23). This exactly parallels our observations on appearance of cadaverine in bacteriophage and suggests that the cadaverine is formed after induction of lysine decarboxylase. If so, there should be a stoichiometric relation between lysine disappearance and cadaverine formation. The data in Table 3 show the extent of cadaverine formation by anaerobic cells in tryptone broth and indicate that cadaverine is derived from free lysine in the medium. Aerobically grown cells did not remove significant amounts of lysine from the medium, but in 2 h of anaerobiosis the initial concentration of 2.4 mM free lysine in the medium was reduced to 1.4 mM. At the same time, 1 mM

TABLE 3. *Stoichiometry of lysine disappearance and cadaverine formation*[a]

Sample	Concentration (mM)			
	Free lysine (1)	Total lysine (2)	Peptide lysine (2-1)	Cadaverine
Broth	2.35	4.41	2.06	0
Aerobic supernatant extract[b]	2.30	4.25	1.95	0.01
Broth and aerobic supernatant extracts (3:2)[c]	2.40	4.52	2.12	—
Anaerobic supernatant extracts	1.43	3.38	1.95	0.98

[a] Cells of *E. coli* K-12 in tryptone broth were aerated by a sparger and grown to 1.1 × 10^9 cells/ml. A small sample was centrifuged to provide supernatant medium for analysis. The remaining culture was diluted with broth to 4 × 10^8 cells/ml and glucose was added to a final concentration of 0.5%. A sample of supernatant extract was obtained, and then the culture was incubated anaerobically for 2 h to a final cell density of 9.7 × 10^8 cells per ml. At 30-min intervals the pH was measured with glass electrodes and was adjusted to pH 6.7 by addition of 1 M Na_2CO_3. The lowest pH observed before neutralization was pH 6.2. The supernatant extracts of anaerobic cells and the other supernatant extracts were analyzed for lysine (sodium citrate elution) before and after acid hydrolysis; the content of cadaverine in sterile broth and in the aerobic and anaerobic supernatant extracts was also measured.

[b] Concentrations corrected for evaporation during aeration.

[c] The measured values are high because the aerobic supernatant extract was concentrated ca. 11% by evaporation.

cadaverine, equivalent to the amount of lysine consumed, was found in the medium.

In a second experiment, uniformly labeled ^{14}C-lysine was added to the anaerobic culture. The data in Table 4 show again the balance between lysine disappearance and cadaverine formation and also indicate that lysine in the medium is the only source of cadaverine. When the results are corrected for the number of carbon atoms, the specific activity of the lysine that disappeared was similar to that of newly formed cadaverine found either in the medium or in the cells. A comparison was not made between cadaverine and the initial specific activity of lysine because a small amount of radioactive contaminant appeared to be present in the lysine.

In this and the preceding experiment, approximately 40% of the lysine in the medium was converted to cadaverine in 2 h. If the data is expressed in terms of average number of cells ($[N_2 - N_1]/[\ln N_2 - \ln N_1]$) an average of 16 μmol of cadaverine was produced per 10^{10} cells over a 2-h period. In experiments preliminary to the ones presented, 22 μmol of cadaverine per 10^{10} cells were formed when the medium was tryptone-Na^+, whereas only 1 μmol was formed when the cells were incubated anaerobically in minimal medium E supplemented with 1.1 mM lysine. This gives some quantitative measure to the difference between complex and synthetic media with regard to cadaverine formation.

DISCUSSION

Although anaerobic growth in complex media resulted in a very high content of cadaverine within progeny phage, significant amounts of putrescine and spermidine were also found. Ames and Dubin (1) compared polyamine contents of phage and host bacteria and could

TABLE 4. *Synthesis of radioactive cadaverine*[a]

Anaerobic growth time	Totals per 50 ml of culture									
	Supernatant medium[b]					Bacteria[b]				
	Lysine		Cadaverine		Putrescine	Acid soluble			Lysine in protein	
						Cadaverine		Putrescine		
	μmol	counts/min ($\times 10^{-5}$)	μmol	counts/min ($\times 10^{-5}$)	μmol	μmol	counts/min ($\times 10^{-5}$)	μmol	μmol	counts/min ($\times 10^{-5}$)
0 h	115	105	0.5	—	1.16	0	—	1.14	2.13	—
2 h	70	69	48	30.2	3.0	1.96	1.24	1.18	5.79	2.19[c]
Difference	−45	−36	47.5	30.2	1.84	1.96	1.24	0.04	3.66	2.19[c]

[a] Cells of *E. coli* K-12 in tryptone broth were aerated by a sparger and grown for 2.5 h to a density of 9.1×10^8 cells per ml. Forty milliliters of culture were mixed with 60 ml of broth containing sufficient glucose for a final concentration of 0.5% glucose. One-half of the mixture (50 ml) was put on ice for 15 min and then centrifuged at 4 C for 15 min at 3,000 $\times$ *g* to provide the zero time samples of supernate and cells. To the remaining 50 ml was added 5 μCi of uniformly labeled ^{14}C-lysine, and the mixture was incubated anaerobically for 2 h to a final cell density of 8.9×10^8 cells per ml. A sample was taken for radioactivity measurement immediately after addition of ^{14}C-lysine to give the zero time value for lysine in the supernatant extract. Periodic pH adjustments were done as described in the legend to Table 3. After centrifugation of the anaerobic suspension, the 0 and 2-h supernatant extracts were fractionated on Amberlite CG-50 columns. Lysine appeared in the effluent and in the fraction eluted by 0.1 M pyridinium acetate, whereas diamines were eluted in 0.5 M pyridinium acetate. This was confirmed by paper chromatography (solvent I) and Dowex-50 chromatography. After evaporation, the fractions were dissolved in water and counted, and then the amounts of lysine and of diamines were measured by Dowex-50 chromatography. The specific activity of cadaverine was the same whether counts were measured before Dowex 50 chromatography or on column fractions. Counts of lysine were obtained before Dowex-50 chromatography (see footnote *c*). The bacterial pellets were extracted three times with 0.3 M trichloroacetic acid, the latter was removed by extraction with ether, and the diamines were assayed by Dowex-50 chromatography. The acid-insoluble material was hydrolyzed with 4-N HCl, counted, and assayed for lysine.

[b] Specific activity (counts/min/μmol) of material appearing or disappearing. Supernatant medium: lysine—80,000; cadaverine—63,600, ($\times$ 6/5 = 76,300). Bacteria: cadaverine—63,300, ($\times$ 6/5 = 76,000); lysine in protein (cf. footnote *c*)—60,000. Specific activity of cadaverine is multiplied by 6/5 to compare the activity with that of uniformly labeled lysine.

[c] Values only approximate because acidic sample was not corrected for quenching. Samples for approximate number of counts was taken from hydrolysate with expectation that counts in column fractions would give accurate values. Unfortunately, lysine in the citrate eluant gave inaccurate and rapidly decreasing counts in our scintillation fluid.

conclude that the phage polyamines were determined by the bacterial polyamine content, but the precise amounts of each polyamine were probably regulated by relative affinities for phage DNA. The same interpretation can accommodate the present data if it is also recognized that within the cell are various substances (DNA, ribosomes, membrane phospholipids), each of which has a different affinity for the various polyamines (5). Two curious observations are interpretable by this general concept. We observed that phage from cells grown in synthetic medium have a lower spermidine content than if the host were grown in complex media (Table 1). Since spermidine concentrations are closely linked to cellular ribosome content (6, 22), the slower growing cells in synthetic medium with a lower RNA content (29) could have less spermidine. Although the phage content of spermidine probably is not a direct function of the bacterial spermidine concentration but must be influenced by relative concentration, availability and affinity for phage DNA of other polyamines and competing cations, a decreased amount of spermidine within the cell could be the major cause of a lowered content of spermidine within the progeny phage. A second observation was that the content of putrescine within anaerobically cultured cells remained relatively high in the presence of a great excess of cadaverine in the medium (Table 4). Admittedly, the data can be used only qualitatively because centrifuged and chilled cells excrete putrescine into the medium and acetylate part of the polyamines (26). If it is assumed that putrescine and cadaverine are secreted or acetylated in roughly similar fashion by chilled cells, it appears that the cellular affinity for putrescine is much greater than for cadaverine. On the other hand, cadaverine may have a higher affinity or be more available for binding to bacteriophage DNA. A slightly higher affinity was indicated by experiments which showed that cadaverine is more effective than putrescine for stabilization of DNA (11, 16, 25). In our experiments, the ratio of cadaverine to putrescine within anaerobic uninfected cells was approximately two after the cells were chilled, but progeny phage from anaerobic infected cells exhibited ratios of 10 to 20. If the ratio observed in uninfected cells was not grossly altered during the chilling procedure, the data would indicate that cadaverine is preferentially selected for incorporation into phage. Clearly, however, it would be interesting to determine whether similar proportions of cellular cadaverine and putrescine are excreted or acetylated when cells are chilled.

The presence of putrescine and spermidine, despite the great excess of cadaverine in anaerobically replicated phage, is probably a function of greater affinity for cellular materials, but may also reflect a requirement for these compounds during cell and phage replication. Dion and Cohen's experiments with a polyamine auxotroph (7, 8) demonstrated that (i) cellular DNA synthesis was greatly stimulated by the addition of putrescine or spermidine but not by cadaverine, and that (ii) phage DNA synthesis was dependent on polyamine synthesis or addition. More recent experiments indicated that the primary effect of putrescine addition to putrescine-depleted cells is to stimulate protein synthesis insofar as an increased rate of leucine incorporation preceded an increase in the rates of uracil or thymine incorporation (30). Dion and Cohen (7, 8) found no putrescine or spermidine in the auxotroph, but the starved cells which could replicate slowly contained small amounts of cadaverine and a new spermidine homologue that is apparently derived from cadaverine. (In vitro synthesis of the homologue is catalyzed by purified propylamine transferase when cadaverine rather than putrescine is utilized as the acceptor for the propylamine moiety of decarboxylated *S*-adenosylmethionine [27]). As a further test of a specific requirement for putrescine and spermidine during growth and macromolecule synthesis, we suggest that similar experiments be performed with anaerobic cells of the polyamine auxotroph to stimulate much greater synthesis of cadaverine and perhaps of the new spermidine homologue. In the same context, the sensitive fluorometric method, used for detection of the homologue, could be used to reinvestigate whether any or all of the spermidine found in anaerobically replicated phage is, in fact, a spermidine homologue.

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LITERATURE CITED

1. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. **235**:769-775.
2. Ames, B. N., D. T. Dubin, and S. M. Rosenthal. 1958. Presence of polyamines in certain bacterial viruses. Science **127**:814-815.
3. Buller, C. S., and L. Astrachan. 1968. Replication of T4rII bacteriophage in *Escherichia coli* K-12. (λ). J. Virol. **2**:298-307.
4. Canfield, R. E., and C. B. Anfinsen. 1963. Concepts and

experimental approaches in the determination of the primary structure of proteins, p. 311-378. *In* H. Neurath (ed.), The proteins, vol. 1. 2nd ed. Academic Press Inc., New York.
5. Cohen, S. S. 1971. Introduction to the polyamines. Prentice-Hall, Englewood Cliffs, New Jersey.
6. Cohen, S. S., and J. Lichtenstein. 1960. Polyamines and ribosome structure. J. Biol. Chem. **235**:2112-2116.
7. Dion, A. S., and S. S. Cohen. 1972. Polyamine stimulation of nucleic acid synthesis in an uninfected and phage-infected polyamine auxotroph of Escherichia coli K12. Proc. Nat. Acad. Sci. U.S.A. **69**:213-217.
8. Dion, A. S., and S. S. Cohen. 1972. Polyamines in the synthesis of bacteriophage deoxyribonucleic acid. II. Requirement for polyamines in T4 infection of a polyamine auxotroph. J. Virol. **9**:423-430.
9. Dubin, D. T., and S. M. Rosenthal. 1960. The acetylation of polyamines in Escherichia coli. J. Biol. Chem. **235**:776-781.
10. Fraser, D., and E. A. Jerrel. 1953. The amino acid composition of T3 bacteriophage. J. Biol. Chem. **205**:291-295.
11. Fraser, D., and H. R. Mahler. 1958. Effects of diamines on the protoplast-infecting agent derived from T2 bacteriophage. J. Amer. Chem. Soc. **80**:6456.
12. Gale, E. F. 1940. The production of amines by bacteria. I The decarboxylation of amino acids by strains of *Bacterium coli*. Biochem. J. **34**:392-413.
13. Garen, A. 1961. Physiological effects of rII mutations in bacteriophage T4. Virology **14**:151-163.
14. Herriott, R. M., and J. W. Barlow. 1952. Preparation, purification and properties of *E. coli* virus T2. J. Gen. Physiol. **40**:809-825.
15. Kabat, E. A., and M. M. Mayer. 1961. Experimental immunochemistry, 2nd ed. Charles C. Thomas, Springfield, Illinois.
16. Mahler, H. R., B. D. Mehrotra, and C. W. Sharp. 1961. Effects of diamines on the thermal transition of DNA. Biochem. Biophys. Res. Commun. **4**:79-82.
17. Maretzki, A., and M. F. Mallette. 1962. Nutritional factors stimulating the formation of lysine decarboxylase in *E. coli*. J. Bacteriol. **83**:720-726.
18. Moore, S., D. H. Spackman, and W. H. Stein. 1958. Chromatography of amino acids on sulfonated polystyrene columns. An improved system. Anal. Chem. **30**:1185-1190.
19. Moore, S., and W. H. Stein. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem. **176**:367-388.
20. Morris, D. R., K. L. Koffron, and C. J. Okstein. 1969. An automated method for polyamine analysis. Anal. Biochem. **30**:449-453.
21. Okuyama, T., and K. Satake. 1960. On the preparation and properties of 2,4,6-trinitrophenol amino acids and peptides. J. Biochem. **47**:454-466.
22. Raina, A., M. Jansen, and S. S. Cohen. 1967. Polyamines and the accumulation of ribonucleic acid in some polyauxotrophic strains of *Escherichia coli*. J. Bacteriol. **94**:1684-1696.
23. Sher, I. H., and M. F. Mallette. 1954. Purification and study of L-lysine decarboxylase from *Escherichia coli* B. Arch. Biochem. Biophys. **53**:354-369.
24. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. **30**:1190-1208.
25. Tabor, H. 1962. The protective effect of spermine and other polyamines against heat denaturation of deoxyribonucleic acid. Biochemistry **1**:496-501.
26. Tabor, C. W., and L. G. Dobbs. 1970. Metabolism of 1,4-diaminobutane and spermidine in *Escherichia coli:* the effects of low temperature during storage and harvesting of cultures. J. Biol. Chem. **245**:2086-2091.
27. Tabor, H., and C. W. Tabor. 1972. Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine and related amines, p. 203-268. *In* A. Meister (ed.), Advances in enzymology and related areas of molecular biology, vol. 5. Interscience Publishers, New York.
28. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli:* partial purification and some properties. J. Biol. Chem. **218**:97-106.
29. Volkin, E., and L. Astrachan. 1956. Phosphorus incorporation in *Escherichia coli* ribonucleic acid after infection with bacteriophage T2. Virology **2**:149-161.
30. Young, D. V., and P. R. Srinivasan. 1972. Regulation of macromolecular synthesis by putrescine in a conditional *Escherichia coli* putrescine auxotroph. J. Bacteriol. **112**:30-39.