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Polyamine Content of Nucleated and Enucleated *Escherichia coli* Cells

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There is currently considerable interest in the physiological function(s) of polyamines. Investigations have shown many in vitro interactions of polyamines with various cellular components, particularly deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosomes; the in vivo role(s) of polyamines is still unclear (H. Tabor and C. W. Tabor, Pharmacol. Rev. **16**:245, 1964). This is owing largely to the uncertainty concerning the in vivo distribution of polyamines within the cell. Extrapolation of in vitro binding studies of the polyamines to subcellular components to in vivo localization of the polyamines is complicated by the redistribution of these highly basic substances after cell disintegration.

Recently, Adler et al. (Proc. Natl. Acad. Sci. U.S. **57**:321, 1967) reported the isolation of a strain of *Escherichia coli* (K-12 P678-54) which produces miniature DNA-deficient cells designated "minicells." This unique property of this strain of *E. coli* provides a system to test whether there exists a pronounced preferential binding of the polyamines, or of a particular polyamine, to the DNA. If such were the case, one might expect the minicells to contain relatively less polyamines. We therefore examined the amounts of DNA, RNA, and polyamines of *E. coli* K-12 P678-54 and its minicells and have found no evidence for preferential binding of the polyamines to DNA.

Growth of the organism (kindly provided to us by Dr. Adler) and the separation of the minicells from normal cells were carried out as described by Adler et al. (Proc. Natl. Acad. Sci. U.S. **57**: 321, 1967). DNA, RNA, and polyamines were determined by standard methods (K. Burton, Biochem. J. **62**:315, 1956; W. Medbaum, Z. Physiol. Chem. **258**:117, 1939; K. Kim, J. Bacteriol. **91**:193, 1966; D. T. Dubin and S. M. Rosenthal, J. Biol. Chem. **235**:776, 1960). The polyamines were also subjected to thin-layer chromatography on silica gel with two solvent systems (methanol-concentrated NH_3, 7:3, v/v, and methanol-concentrated HCl, 8:2, v/v), which further identified the polyamines and eliminated the possibility of ornithine or lysine contaminating the putrescine obtained from column chromatography.

The DNA, RNA, and polyamine contents of the minicells and of normal K-12 P678-54 cells are shown in Table 1. In the normal cells, the polyamines, assuming two positive charges per putrescine and three positive charges per spermidine, can neutralize a total of 12 to 13% of the phosphate groups in the nucleic acids. If the DNA, which is approximately 8 to 9% of the total nucleic acids, were to show a preferential binding of the polyamines to the extent that 50% of the DNA-phosphate groups would be neutralized by the polyamines, this would account for approximately one-third of the total polyamines. The ratio of polyamine to RNA in the minicells should then be reduced to two-thirds of the value in normal cells. Experimentally, the observed polyamine-RNA ratio in the minicells was not significantly different from that in the normal cells. There was also no evidence of a relative enrichment of spermidine over putrescine in the normal cells, as would be expected to occur when there is pronounced preferential binding of spermidine by DNA.

TABLE 1. *RNA, DNA, and polyamine content of normal cells and minicells*

	Total RNA content (mg/g, wet cells)	Total DNA content (mg/g, wet cells)	Moles polyamine/moles ribotide		Moles N^+/moles RNA phosphate[a]
			Putrescine	Spermidine	
E. coli K-12 P678-54					
Normal cells	56.0	5.2	0.06	0.006	0.14
Minicells	52.0	0.15	0.05	0.017	0.15

[a] The moles N^+ in this column refers to the N of the polyamines.

In summary, the molar ratio of polyamine-nitrogen to RNA-phosphate was approximately the same for both the normal K-12 P678-54 cells and the DNA-deficient minicells derived from it. No evidence for pronounced preferential binding of polyamines by DNA was obtained.

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