DEPENDENCE OF Tm OF DNA COMPLEXES WITH PUTRESCINE, HEXANDIAMINE AND SPERMIDINE ON THE IONIC STRENGTH.

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It has been suggested, that osmotic pressure of putrescine and spermidine in the T_2 phage head acts as a motor for the injection of DNA into the bacterial cell (Zárybnický, 1966). Osmotic pressure inside the phage head depends on the ionic strength of the environment. As the concentration of salts in the solution increases it is assumed, that the complex DNA-polyamine dissociates and the internal osmotic pressure hence increases (Zárybnický and Horáček, 1968).

The behavior of this complex in solutions of various ionic strength has not been studied systematically. It has been stated that polyamines of the osmotically resistant strain $T4Bo_1$ can be removed by 1.10^{-2}M Mg^{2+} (Ames and Dubin, 1960), or 5.10^{-2}M Na^+ (Leibo and Mazur, 1966). The stabilization effect of spermine on phage DNA is abolished in 1.10^{-1}M NaCl (Kaiser, Tabor and Tabor, 1963). The increase of Tm of DNA has been observed in the environment exhibiting a lower ionic strength when using lower concentration of polyamines (Tabor, 1962, Mahler and Merothra, 1963).

We wish to report here our results concerning some properties of DNA-polyamine complex at various NaCl concentrations.

Materials and methods.

DNA from calf thymus, prepared by the method of Zamenhof (1958), was

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a kind gift from Dr. V. Pačes, Institute of Organic Chemistry and Biochemistry, Prague. Putrescine.2HCl M.A., cat. No. 2169 was purchased from Mann Research Lab. Spermidine.3 HCl lot 63625 from Calbiochem. Hexandiamine - 1,6.2 HCl was prepared from hexandiamine - 1,6/Lachema, Czechoslovakia/ and recrystalized twice from water-ethanol mixture, m.p. 255-6°C. All other chemicals used were commercially available. Thermal denaturation curves were measured in the spectrophotometer "Spectromom 201", Hungary. A special holder for heating of cells and "Suprasil" fused cells with a ground valve, 1 cm light path were used. The temperature of water leaving the heated holder was

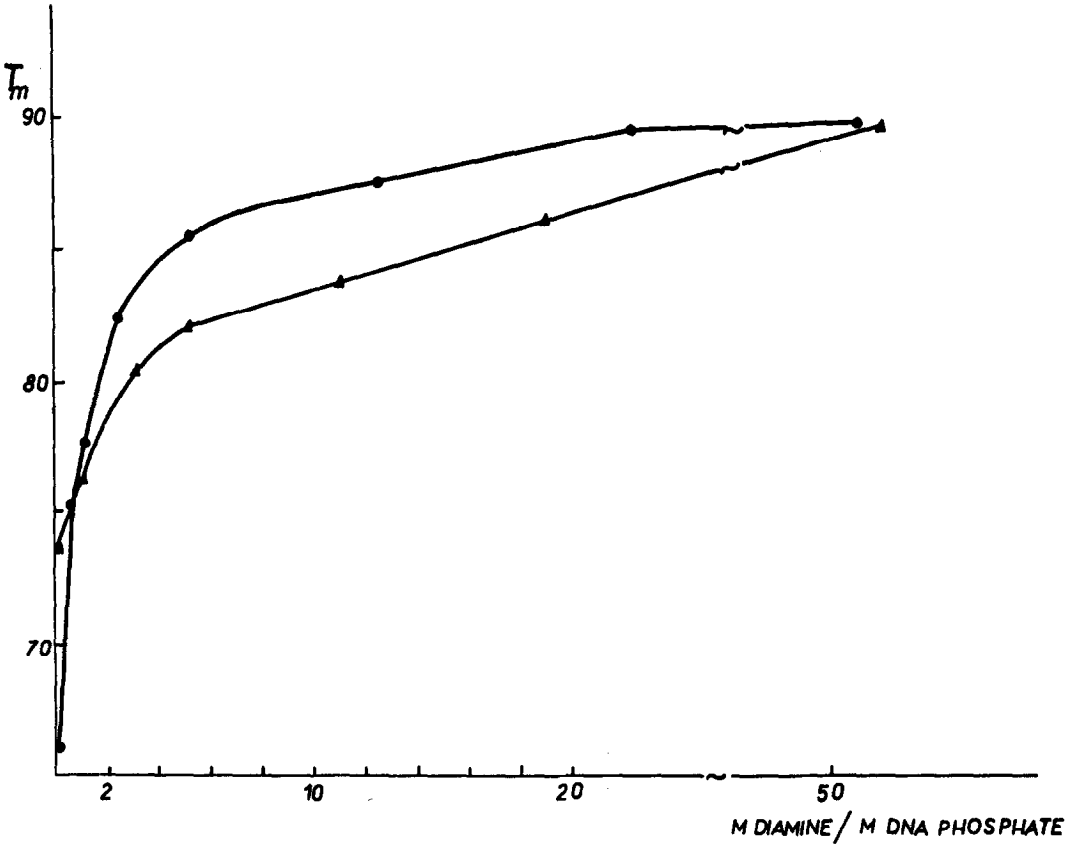

Fig. 1. Dependence of Tm of calf-thymus DNA on the ratio hexandiamine/phosphate DNA in solutions of various ionic strength.

▲ $1{,}9.10^{-2}$M NaCl + $1{,}9.10^{-3}$M Na citrate

● $2{,}7.10^{-3}$M NaCl + $2{,}7.10^{-4}$M Na citrate

measured by Hg-thermometer and was not corrected on the protruding column of Hg. The pH was checked in a pH-meter Beckman H2 and maintained at 6,7 ± 0,2.

The points in the figures represent the arithmetic mean. Two and four measurements were taken into consideration when determining the dependence of Tm on polyamine concentration and ionic strength, respectively. Standard mean error was $\leq 0{,}25^{\circ}C$ in the latter case.

Results

1) The Tm-polyamine concentration curve approaches a saturation value and the curve is steeper when using a lower ionic strength (Fig.1.) This is in agreement with the results of Mahler and Mehrotra (1963), Mehrotra and Mahler (1964).

2) Tm of DNA at the constant polyamine concentration depends on the NaCl concentration in the environment. Tm decreases with increasing NaCl conc. and increases again, after reaching its minimum, when the concentration of salts is further increased. The slope of this curve is lower or maximally equal to that of Tm of DNA alone (Fig. 2).

3) We may express the rate of stabilization of DNA by action of polyamines as Δ Tm = Tm of DNA + polyamine minus Tm of DNA alone. The ionic strength of both samples was the same. The contribution of amine hydrochlorides was taken into consideration when counting the ionic strength. At first, Tm decreases linearly with log $[Na^+]$, then the linear dependence is no longer observed and ΔTm reaches zero (Fig. 3).

4) DNA is stabilized by lower polyamine concentrations, i.e. $2{,}2.10^{-6}$M spermidine and $3{,}6.10^{-6}$M putrescine in the presence of lower salt concentrations as compared with the results of Tabor (1962). Both analytical concentrations of polyamines are equivalent to 0,1M NH_2 of polyamines/1,0 M DNA phosphate.

5) Three forms of the complex were observed in the system DNA + spermidine + + Na^+ (pH 6,8, DNA conc. 25 μg/ml):

a) precipitate: $2{,}35.10^{-4}$M spermidine and $2{,}4.10^{-3}$M Na^+

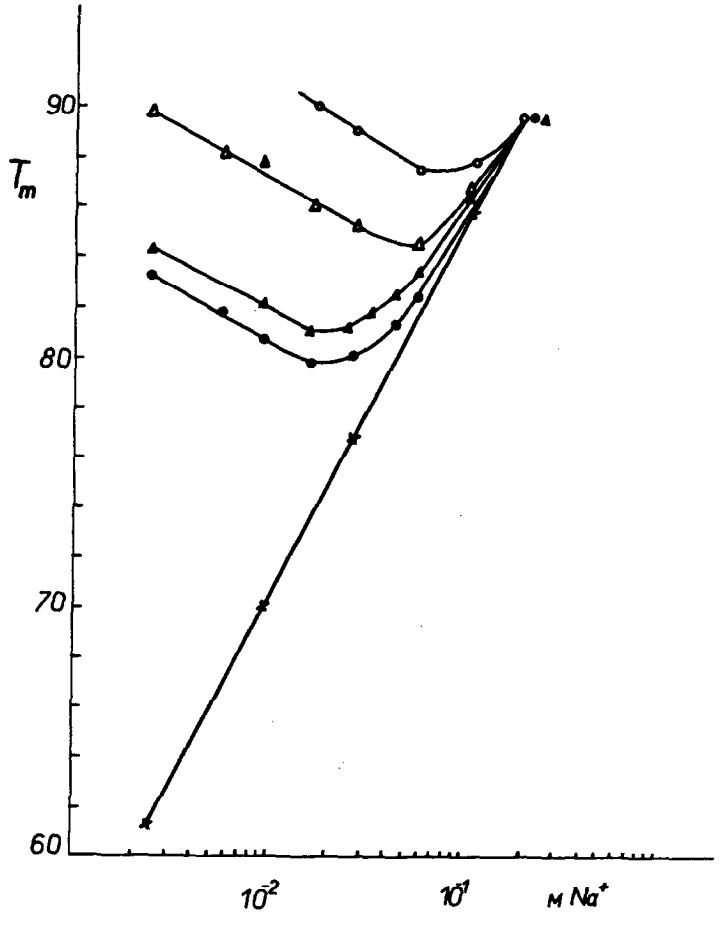

Fig. 2. Dependence of Tm of calf-thymus DNA in the presence of polyamines on Na^+ concentration in $2{,}2.10^{-4}$M citrate. Na^+ was added as NaCl.

- ▲ $8{,}05.10^{-5}$M spermidine.3 HCl
- ○ $2{,}35.10^{-4}$M spermidine.3 HCl
- ▲ $2{,}35.10^{-4}$M hexandiamine - 1,6.2 HCl
- ● $2{,}35.10^{-4}$M putrescine.2 HCl

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b) solution: $1{,}25.10^{-4}$M spermidine, the same conc. Na^+ as at a,

or $2{,}35.10^{-4}$M " , $1{,}6.10^{-2}$M Na^+

c) a form containing $2{,}35.10^{-4}$M spermidine and $9{,}2.10^{-3}$M Na^+ respectively, differing by lower transparency within the range of 320 - 350 nm. No other

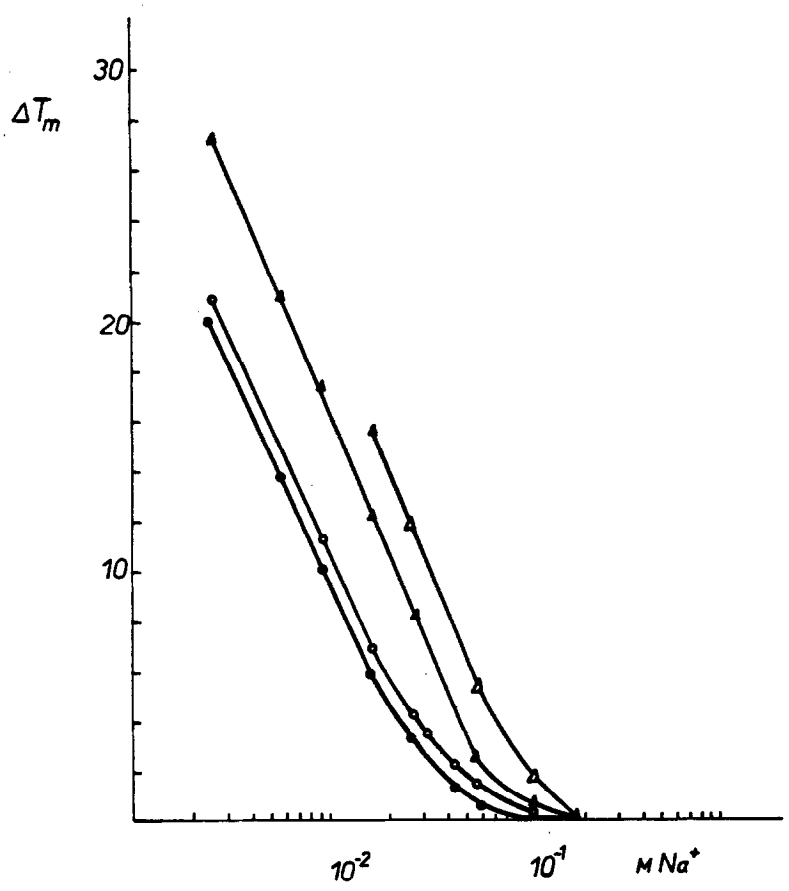

Fig. 3. Effect of Na^+ concentration on ΔTm for various amines.

▲ Tm = Tm of DNA + polyamine minus Tm of DNA alone.

△ 2,35.10^{-4}M spermidine.3 HCl

▲ 8,05.10^{-5}M spermidine.3 HCl

○ hexandiamine - 1,6.2 HCl

● putrescine.2 HCl

Concentrations of hexandiamine.2 HCl and putrescine.2 HCl were 2,35.10^{-4}M. DNA concentration 25 µg/ml.

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differences could be observed between b and c. The c form changes to the b form when increasing NaCl conc. up to 1,6.10^{-2}M or setting the temperature to approximately 2°C below that the expected Tm.

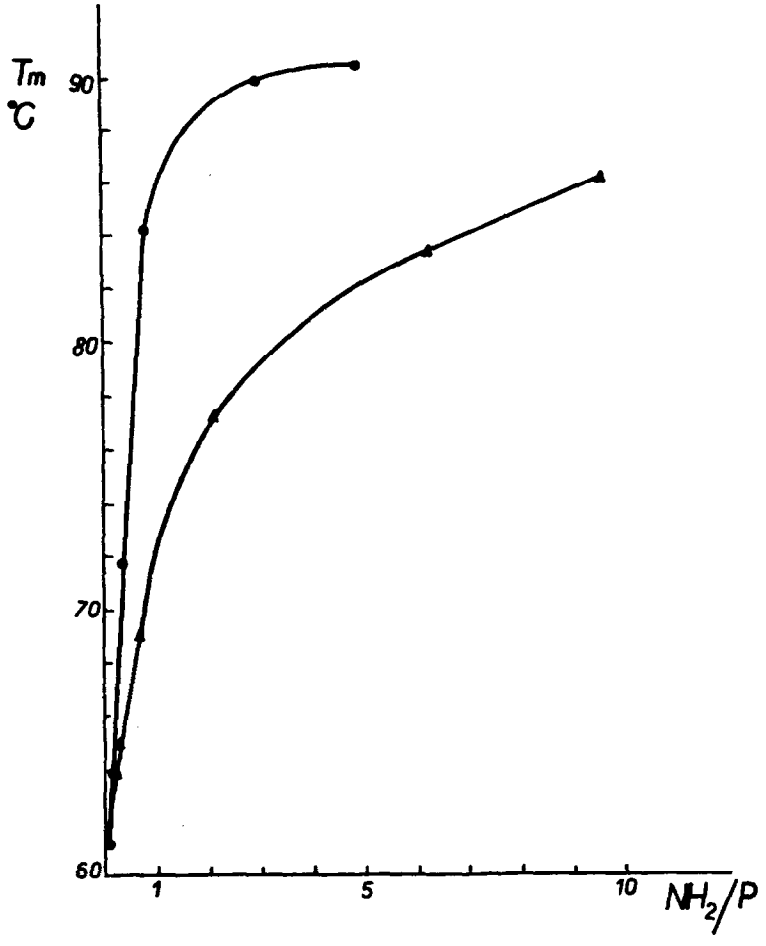

Fig. 4. Dependence of Tm of calf-thymus DNA on the ratio equivalent of polyamines/DNA phosphate in $2,2.10^{-3}$M NaCl + $2,2.10^{-4}$M Na citrate.
● spermidine.3 HCl ▲ putrescine.2 HCl.

Conclusions

According to the model of Liquori et al. (1967) the stabilization of DNA by polyamines is a result of the formation of cross-links, when the aminogroups of polyamines are bound to DNA phosphate by electrostatic linkages. It is obvious from our results, that there is a competition between Na^+ and polyamine NH_3^+ for the DNA phosphate. This is illustrated

in Fig. 2. The curves represent a sum of two opposite processes: destabilization due to the dissociation of the DNA-polyamine complex, and stabilization due to the increase of $[Na^+]$. The ratio of both processes determines the changes of Tm. Polyamines do not exhibit any effect on Tm of DNA when the Na^+/NH_2 ratio reaches the value of $1,9.10^2$, $2,3.10^2$ and $2,7 - 4,5.10^2$ for putrescine, hexandiamine and spermidine, respectively in the presence of the used concentrations of polyamines.

It appears that two factors are responsible for the more considerable increase of Tm in the environment of low ionic strength in the presence of very low concentrations of polyamine. The first is the low $[Na^+]$; due to this, equilibrium between Na+ and NH_3^+ of amines is shifted in favour of the polyamine complex. The second is the higher NH_2 ionisation due to lower Tm (Hirshman, Leng and Felsenfeld, 1967).

Further work on the indicated problems is in progress.

References.

1. Ames, B. N. and Dubin, D. T., J. Mol. Biol. 235, 769,(1960).
2. Hirschman, S. Z., Leng, M., Felsenfeld, G., Biopolymers 5, 227, (1967).
3. Kaiser, D., Tabor, H., Tabor, C. W., J. Mol. Biol. 6, 141, (1963).
4. Leibo, S. P. and Mazur, P., Biophys. J. 6, 747, (1966).
5. Liquori, A. M., Constantino, L., Crescenzi, V., Elia, V., Giglio, E., Puliti, R., De Santis Savino, M., Vitagliano, V., J. Mol. Biol. 24, 113,(1967).
6. Mahler, H. R.,and Mehrotra, B. D., Biochem. Biophys. Acta 68, 211, (1963).
7. Mehrotra, B. D., and Mahler, H. R., Biochem. Biophys. Acta 91, 78, (1964).
8. Tabor, H., Biochem. 1, 496, (1962).
9. Zamenhof S., in C.S. Vestling, Biochemical Preparations, Vol. 6, 1958, p.8.
10. Zárybnický, V., Czechoslovak J. Phys. B, 16, 66, (1966).
11. Zárybnický, V. and Horáček, P., Folia Microbiol. No5, (1968), in press.