THE STABILIZING EFFECT OF SPERMINE AND RELATED AMINES ON MITOCHONDRIA AND PROTOPLASTS

Celia White Tabor

National Institute of Arthritis & Metabolic Diseases
National Institutes of Health
Bethesda 14, Maryland

Received January 20, 1960

Putrescine, spermidine, and spermine are widely distributed in natural materials (Rosenthal, et al., 1956; Tabor, et al., 1958; Herbst, et al., 1958), but their function remains largely unknown. In this paper we are reporting on the stabilizing effect of these amines on mitochondrial and protoplast suspensions. We are also including some observations on the effect of these amines in causing agglutination of mitochondria and microsomes and in inhibiting the leakage of ultraviolet-absorbing material from mitochondria.

To study the mitochondrial suspensions, we have followed the changes in the optical density at 520 mμ (OD_{520}) since this has been used in several laboratories as an indication of mitochondrial stability. A fall in the OD_{520} has been interpreted to mean mitochondrial swelling (Hunter, et al., 1959; Lehninger, 1959; Lipsett, et al., 1959).

When freshly prepared rat liver mitochondria were suspended at 25° C in 0.33 M sucrose* - 0.025 M Tris HCl, pH 7.5, the addition of sodium succinate (final concentration 3×10^{-3} M) produced a marked fall in the optical density (Hunter, et al., 1959). We observed that this fall was

* The sucrose has been passed through a Dowex 50 (H^+ form) column as described by Fonnesu (Fonnesu, et al., 1956); this treatment has been found to result in a more uniform fall in optical density of the control tubes. We wish to thank Dr. Marie Lipsett for suggesting this procedure.

completely inhibited by 3×10^{-4} M spermine** or spermidine; putrescine or cadaverine had a moderate effect at 3×10^{-3} M (Figure 1A). In a comparable experiment, the closely related dibasic amino acids, L-lysine and L-ornithine, as well as NaCl and NH_4Cl had no effect, even at 3×10^{-2} M; a small effect was seen with KCl (Figure 1B). $MgCl_2$ (3×10^{-3} M) inhibited the succinate-induced fall in OD_{520}, as reported by Hunter (Hunter, et al., 1959). The amines had little or no effect on the fall in OD_{520} induced by phosphate or ascorbate in Hunter's medium, or by thyroxine in the medium used by Lehninger (Lehninger, 1959).

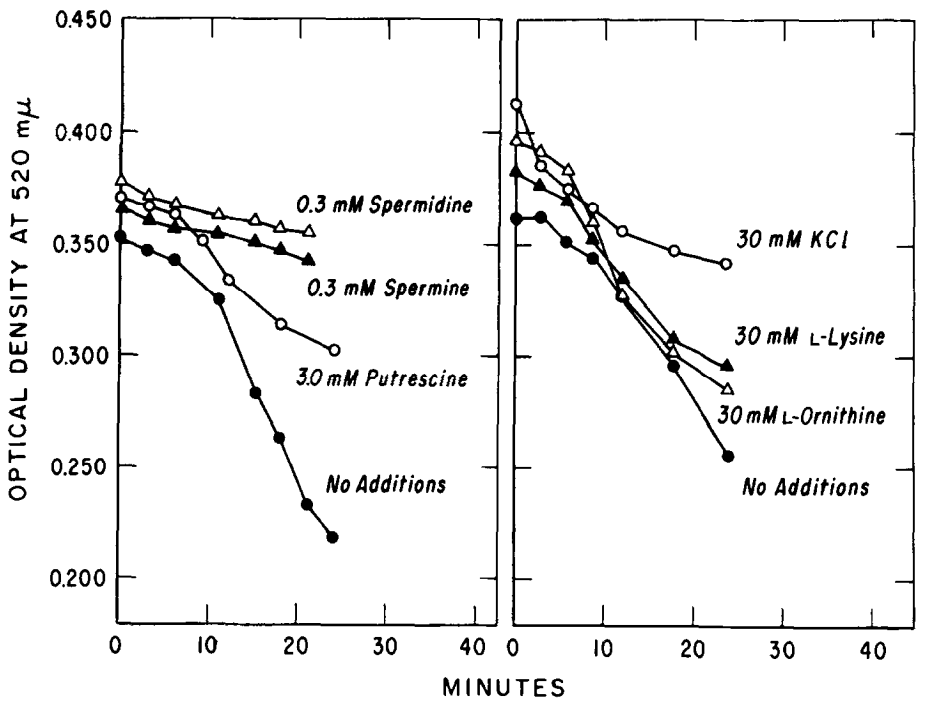

Fig. 1A. Effect of amines on the succinate-induced fall in optical density of a suspension of rat liver mitochondria. The effect of 3 mM cadaverine was identical with that of putrescine.

Fig. 1B. Effect of amino acids and salts on the succinate-induced fall in optical density of a suspension of rat liver mitochondria.

** All salts were in the HCl form.

Lipsett, et al. have reported that the OD_{520} falls when mitochondria are suspended in a hypotonic medium (0.1 M sucrose - 0.01 M Tris HCl, pH 7.5) at 25° C (Lipsett, et al., 1959). We observed that this fall in optical density was inhibited by 3×10^{-4} M spermine or spermidine, or by 3×10^{-3} M putrescine or cadaverine. There was no inhibition by 3×10^{-3} M L-lysine, L-ornithine, NaCl, KCl, or NH_4Cl; at 3×10^{-2} M, these have only a moderate effect.

Mitochondria in isotonic sucrose lose 260 mμ absorbing material to the suspending medium (Siekevitz, et al., 1955). Without spermine, the OD_{260} of the supernatant fluid of mitochondria suspended in 0.33 M sucrose - 0.025 M Tris HCl, pH 7.5, at 0° for 18 hours was 0.29. When spermine was present at 3×10^{-3} M, the OD_{260} was 0.19. Further experiments are now being carried out to study whether these amines have an effect on various enzymatic activities in mitochondria.

Mitochondria and microsomes remain in a state of dispersion in 0.25 M sucrose for at least 18 hours. The presence of spermine or spermidine at 3×10^{-4} M caused aggregation and settling of these dispersions. Cadaverine and putrescine at 3×10^{-3} M produced aggregation of mitochondria, but not of microsomes. NaCl, KCl, and NH_4Cl at 3×10^{-2} M had no effect on aggregation. $MgCl_2$ at 3×10^{-3} M was not effective; however, at 3×10^{-2} M, $MgCl_2$ produced aggregation of mitochondria.

These data on mitochondria may be related to the findings of Mager that spermine and other amines prevented osmotic lysis of Pasteurella tularensis, Neisseria perflava, and Achromobacter fischeri (Mager, 1955, 1959a), as well as protoplasts of Escherichia coli, (Mager, 1959b). Likewise the effect of the amines on the leakage of 260 mμ absorbing material observed for Hemophilus parainfluenzae (Herbst, et al., 1958) and for Pasteurella tularensis (Mager, 1959b), may be similar to the effect on the leakage of 260 mμ absorbing materials from mitochondria.

In this laboratory we have also found that spermine prevents lysis of E. coli protoplasts, confirming Mager's observations. The OD_{660} of a suspension of E. coli cells in 0.5 M sucrose was 1.08; when these cells were

suspended in water the OD_{660} was 0.96. A comparable suspension of protoplasts prepared by the lysozyme-EDTA procedure (Mahler, et al., 1956) had an OD_{660} of 1.08 in sucrose, and 0.10 in an equal volume of water. A comparable suspension of protoplasts in 10^{-3} M spermine, spermidine, putrescine or cadaverine gave an OD_{660} of 0.80-0.87. This effect was not seen with 10^{-2} M NaCl, KCl, NH_4Cl, L-lysine, or L-ornithine.

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