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Divalent Cation Reversal of Tetracycline-Inhibited Respiration of *Klebsiella pneumoniae*

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Magnesium and ferrous compounds reversed tetracycline inhibition of respiration in *Klebsiella pneumoniae*, and the two compounds together had greater reversing ability than either alone.

The mutual effects of tetracycline and metallic cations for various biological systems have been reported (2, 3). An interest in the carbohydrate and protein metabolism of *Klebsiella pneumoniae* led to an investigation of the ability of certain divalent cations to reverse tetracycline-inhibited respiration in this microorganism. The findings are presented in this note.

K. pneumoniae ATCC 8052 was chosen as the test organism for this study. Respiration was expressed as oxygen uptake, which was determined by the standard manometric techniques of Umbreit et al. (1). Cultures (24 h) of cells grown in nutrient broth (Difco) were harvested by centrifugation and washed twice with Burk salts medium (4). A saline suspension of the cells was then adjusted to an optical density of 1.5 at 450 nm on a photometer (Bausch and Lomb Spectronic 20). Oxygen uptake at 37 C was recorded at 15-min intervals for a period of 3 h. The cells were allowed to respire for 15 min before tetracycline was added. A control flask was used to measure the endogenous respiration of the cells. Another flask (also a control) contained cells and glucose substrate, but neither tetracycline nor metallic cations. Tetracycline solutions were made just before use by dissolving tetracycline hydrochloride (Lederle Laboratories) in one-half of the desired volume of distilled water, adjusting the pH to 7.5 with 0.5 N NaOH, and adjusting to final volume by the addition of distilled water. Stock solutions of $MgSO_4$ and $FeSO_4$ were sterilized by filtration through a membrane filter (Millipore) in a Swinney adapter.

The results of three trials are shown in Table 1. Respiration, inhibited by 10^{-3} M tetracycline, was shown to be partially reversed by 10^{-2} M Mg^{2+} and 10^{-3} M Fe^{2+} and was even more reversed by a combination of the two metallic cations at these same concentrations. Assuming complete dissociation of the tetracycline molecule, the ratio of ferrous cation to tetracycline molecule needed to give this amount of reversal was 1:1, whereas the ratio of magnesium cation to tetracycline molecule was 10:1 for approximately the same degree of reversal. This relationship would indicate that the ferrous cation can more readily reverse tetracycline inhibition of respiration in *K. pneumoniae* than can the magnesium cation.

The results presented here are in agreement with those found in previous investigations. Weinberg (3) showed that ferrous and ferric cations more readily counteracted tetracycline inhibition of growth of *Pseudomonas aeruginosa* than did the magnesium cation. Van Meter et

TABLE 1. *Effect of divalent cations on tetracycline-inhibited respiration of Klebsiella pneumoniae*

Incubation condition	Total oxygen uptake (μliters/3 h)		
	Trial 1	Trial 2	Trial 3
No addition	371	365	383
10^{-3} M tetracycline	123	120	125
10^{-3} M tetracycline + 10^{-2} M Mg^{2+}	228	225	247
10^{-3} M tetracycline + 10^{-3} M Fe^{2+}	211	214	229
10^{-3} M tetracycline + 10^{-2} M Mg^{2+} + 10^{-3} M Fe^{2+}	306	316	336

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al. (2) reported the inhibition of oxidation by chlortetracycline in rat liver mitochondria and found that inhibition occurred in the presence of magnesium ion, low phosphate concentrations, and adenosine triphosphate. The introduction of added magnesium reversed this inhibition. The interpretation offered to explain this finding was that there was a direct reaction on the oxidative enzymes by the tetracyclines, thus producing the observed inhibition. The reversal of this inhibition by magnesium reflects a removal of the inhibition by the formation of a metal complex. It is hoped that future studies to investigate the kinetics of the formation of this cation-antibiotic complex in *K. pneumoniae* can be carried out.

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