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Influence of Magnesium and Manganese on Some Biological and Physical Properties of Tetracycline

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Accumulation of ^{3}H-tetracycline in nonproliferating cells of susceptible and resistant strains of *Escherichia coli* and *Staphylococcus aureus* in tris(hydroxymethyl)aminomethane (Tris) buffer (10 mM, *p*H 7.5) was significantly decreased in the presence of 5 to 40 mM $MgCl_2$ and increased in the presence of 5 to 10 mM $MnCl_2$. When the bacteria first accumulated ^{3}H-tetracycline in plain Tris·HCl, and the metal salts were thereafter added, a prompt decrease or increase in radioactivity of the cells was observed after the addition of Mg^{2+} or Mn^{2+}, respectively. In phosphate buffer (10 mM, *p*H 7.5), the effect of Mg^{2+} was delayed. Three minutes after addition of ^{3}H-tetracycline, uptake was as in the control cell suspension, but thereafter it dropped rapidly. When ^{3}H-tetracycline was incubated with Mg^{2+} before addition to the bacterial suspension, uptake was scarcely measurable. The addition of Mg^{2+} to growing cultures of *S. aureus* and *E. coli* caused a marked decrease in susceptibility; in contrast, no increase in susceptibility could be demonstrated when Mn^{2+} was added. It was also demonstrated that Mg^{2+} and Mn^{2+} had distinct influences on the absorption spectrum, the optical rotatory dispersion, the circular dichroism, and the lipid solubility of tetracycline.

Chelation of tetracyclines with cations of metals has been observed for many years (1, 4, 24, 30). It has also been shown that the inhibition of certain cell-free enzymatic processes by tetracycline is antagonized by some metal salts (3, 25, 31). The explanation for this may be either that the drug acts by removing ions essential for the enzymatic reaction, or that the metal salt of tetracycline is a less effective inhibitor than the free drug. Phosphates and citric acid enhance the intestinal absorption of tetracyclines, probably by precipitating interacting metallic ions (13). It has also been reported that certain cations, particularly Mg^{2+}, Mn^{2+}, Fe^{2+}, and Fe^{3+}, may reduce the antibacterial effect of tetracyclines; older literature on this object has been comprehensively reviewed by Laskin (17). It is now generally accepted that inhibition of protein synthesis is the major mechanism by which tetracyclines exert their bacteriostatic activity (18). The influence of metal ions on tetracycline inhibition of certain enzymatic processes can therefore not adequately explain their reversal of growth inhibition. Recently, Franklin and Higginson (12) have demonstrated that the binding of tetracycline by isolated cell membranes and whole cells of *Escherichia coli* is significantly decreased in the presence of magnesium salt; this important observation led us to perform a series of experiments on the influence of magnesium and manganese ions on the intracellular accumulation of tetracycline in susceptible and resistant strains of *E. coli* and *Staphylococcus aureus*. We have also examined the influence of these ions on the absorption spectrum, the optical rotatory dispersion, the circular dichroism, and the lipid solubility of tetracycline, hoping to find clues to their opposite biological effect.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 W945 (*lac*$^-$, *pro*$^-$, *thi*$^-$) and a chromosomal mutant of this organism (K-12/1), resistant to chloramphenicol and tetracycline (Tc), have been described (28). Another Tc-resistant variant of W945 (K-12/R) was obtained by transfection with a Tc R factor (26). We have also earlier described (2) the *S. aureus* strains used in this study: (i) *S. aureus* 111 with inducible resistance to

Tc by virtue of a plasmid; (ii) a partly derepressed mutant of this strain (now designated 111C); and (iii) a Tc-susceptible variant (111S) obtained by growth of 111 at 44 C.

Nutrient media. *E. coli* was grown in Tryptose phosphate broth (TP; Difco, Detroit) or Davis's minimal medium (7) supplemented with L-proline (50 mg/liter) and thiamine-hydrochloride (1 mg/liter). For growth of *S. aureus*, nutrient broth (NB; Difco) was used throughout. All chemicals were of highest purity grade obtainable commercially.

Optical rotatory dispersion and circular dichroism. Optical rotatory dispersion and circular dichroism of Tc were determined with a Cary 60 UV/ORD/CD spectrophotometer (Varian, Palo Alto, Calif.) at 29 C under constant nitrogen flush. The samples were dissolved in 10 mM tris(hydroxymethyl)aminomethane (Tris) buffer, adjusted to *p*H 7.5 with HCl, with the indicated concentrations of $MgCl_2$ or $MnCl_2$.

Absorption spectrum. The absorption spectrum of Tc was examined with a Cary 14 spectrophotometer with automatic recording. In the range 190 to 250 nm, the spectrum obtained was verified with a Cary 15 spectrophotometer. The same buffer with respective metal salts was used to determine the base line.

Accumulation of Tc. Accumulation of Tc by the bacteria was determined by the membrane filter method described previously (2), with tritiated Tc from New England Nuclear Corp., Boston, Mass. [tetracycline-7-^3H (N) hydrochloride]. Filter membranes from Sartorius, Gottingen, were found to have markedly lower self-adsorption of Tc than Millipore membranes. The cells for uptake experiments were obtained from cultures in the mid-log growth phase. The cells were gathered on membrane filters, washed once with the medium to be used in the uptake experiment (10 mM Tris·HCl at *p*H 7.5, 10 mM sodium phosphate buffer at the same *p*H value, or TP with indicated salt concentrations), and thereupon suspended in this medium at an optical density of 0.1 to 0.2 at 540 nm. The cells were preincubated for 15 min at 37 C with slight aeration before ^{3}H-Tc was added. Variations of this procedure will be indicated below.

Distribution of Tc between nonpolar solvents and water. Relative values for solubility of Tc in nonpolar solvents were obtained by determination of the apparent partition coefficients of the drug in the systems: chloroform-aqueous Tris·HCl (10 mM, *p*H 7.5) and olive oil-aqueous Tris·HCl. The influence of Mg^{2+} and Mn^{2+} on the distribution was examined by the addition of 10 mM $MgCl_2$ or $MnCl_2$ to the aqueous buffer.

Fifteen milliliters of a 10 μg/ml ^{3}H-Tc solution in Tris·HCl with or without 10 mM $MgCl_2$ or $MnCl_2$ was incubated in an Erlenmeyer flask for 15 min at 37 C. The radioactivity of ^{3}H-Tc corresponded to about 4×10^4 dpm/ml. Chloroform (15 ml) was added, and the flask was stoppered and shaken mechanically for 1 hr at 25 C. The mixture was then centrifuged at 25 C for 1 hr (30,000 $\times$ *g*). From the aqueous layer, three 1-ml portions were transferred to separate scintillation vials, the residual aqueous buffer was siphoned off, and three 1-ml portions of the chloroform layer were also sampled for determination of radioactivity. The apparent partition coefficient was calculated as [dpm of chloroform (3 ml)] /[dpm of water (3 ml)].

Each examination was repeated six to nine times, and mean values and standard errors were calculated.

Examination of the distribution of Tc between olive oil and buffer was performed in a similar way. After addition of olive oil (Shemen Zait, Israel) the two phases were mixed by sonic treatment for 10 min (Branson sonifier B12, microtip limit). Centrifugation was for 2 hr (30,000 $\times$ *g*) at 25 C in polyethylene tubes. The oily phase was sampled from above and the watery phase by puncture of the bottom of the test tube with an injection needle.

Counting procedure. Radioactivity was determined with a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Corrections for quenching and for the efficiency of the counting were made automatically with a Tri-Carb Absolute Activity Analyzer, model 544. If background exceeded 1% of the total count, the necessary correction was applied.

The scintillation fluid as well as other details concerning materials and methods have been described (2, 27, 29).

RESULTS

Effect of Mg^{2+} and Mn^{2+} on uptake of Tc by E. coli. The influence of Mg and Mn ions, supplied as chloride salts, on the intracellular accumulation of Tc in the susceptible *E. coli* K-12 is depicted on Fig. 1. The cells in these experiments were suspended in Tris·HCl (10 mM, *p*H 7.5) with the indicated concentrations of metal salts 15 min before addition of the drug. With Tc at a concentration of 10 μg/ml (0.0225 mM), a marked influence on uptake was exhibited by 5 mM $MgCl_2$ or $MnCl_2$, the former decreasing it and the latter increasing it. Uptake was not only increased but it also continued for a longer period in the presence of 5 to 10 mM Mn^{2+}. Mn^{2+} (20 mM) had no greater influence than 10 mM, and with 40 mM the effect of this ion was abolished. With 1 mM Mg^{2+} or Mn^{2+}, the effect was very slight, and lower concentrations were of no demonstrable effect. When both Mg^{2+} and Mn^{2+} were added, the influence of Mg^{2+} seemed to dominate. With 10 mM Mg^{2+} plus 10 mM Mn^{2+}, Tc accumulation was approximately as with 5 mM Mg^{2+}.

When the bacteria accumulated ^{3}H-Tc in plain Tris·HCl buffer for 30 min, and thereupon Mg^{2+} or Mn^{2+} was added, the effect of these ions appeared promptly. As shown in Fig. 2, uptake was halved 3 min after the addition of Mg^{2+}, and doubled in the same length of time if Mn^{2+} was added.

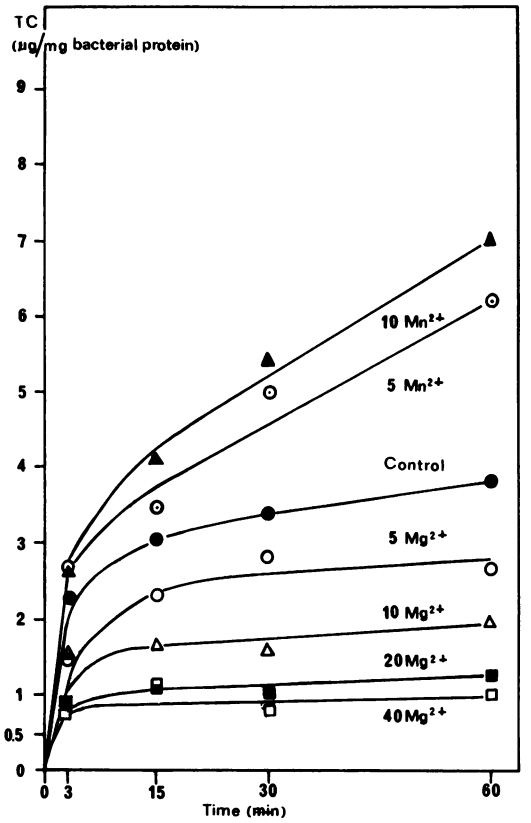

FIG. 1. *Uptake of tetracycline by a nonproliferating cell suspension of Escherichia coli. The cells were preincubated for 15 min at 37 C in Tris·HCl without or with $MnCl_2$ or $MgCl_2$ (5 to 10 mM Mn^{2+} or 5 to 40 mM Mg^{2+}). Thereafter, 10 μg of ^{3}H-tetracycline per ml was added, and samples were collected at the indicated time intervals.*

On the other hand, when bacteria were preincubated in Tris·HCl with the metal salts, and thereafter washed and transferred to plain Tris with ^{3}H-Tc, the uptake was not influenced significantly by the kind of metallic ion present during the preincubation period. We have not examined the influence of the preincubation conditions on the intracellular content of metallic ions in the bacteria.

Decreased uptake of Tc in presence of Mg^{2+} might be due to a direct influence on the bacteria, to an effect on the drug molecules, or to both. If the effect was essentially due to an influence on the drug molecules, the prompt exit of Tc already accumulated (Fig. 2) would indicate a rapid turnover of Tc bound to the cells. We were able to exclude the first of these possibilities in uptake experiments, by using TP broth or sodium phosphate buffer (10 mM, pH 7.5) as suspending media; in both cases, the effect of Mg^{2+} on uptake appeared with a delay of several minutes after the addition of Tc (Fig. 3). This must be due to a delayed interaction between Mg^{2+} and Tc, since uptake was negligible from the start, when Tc was preincubated with Mg^{2+} for 15 min before addition to the bacteria.

Figure 1 indicates that Mg^{2+} and Mn^{2+} exhibit their influence both on the uptake rate and on the capacity of the bacteria for Tc. Under the experimental conditions used, the initial uptake was, however, very rapid, and the determination by sampling after 3 min was not very exact. This point was therefore studied at 25 C with 0.2 μg of Tc per ml with or without preincubation of the drug with the metal salts. Under these conditions, 1 mM Mg^{2+} and Mn^{2+} had a significant influence on the uptake rate, and with 10 mM this effect was increased (Fig. 4).

Resistant strains of *E. coli* concentrate Tc to a significantly lower degree than susceptible ones (8, 11, 15, 16). Nevertheless, the influence of Mg^{2+} and Mn^{2+} in the resistant strains K-

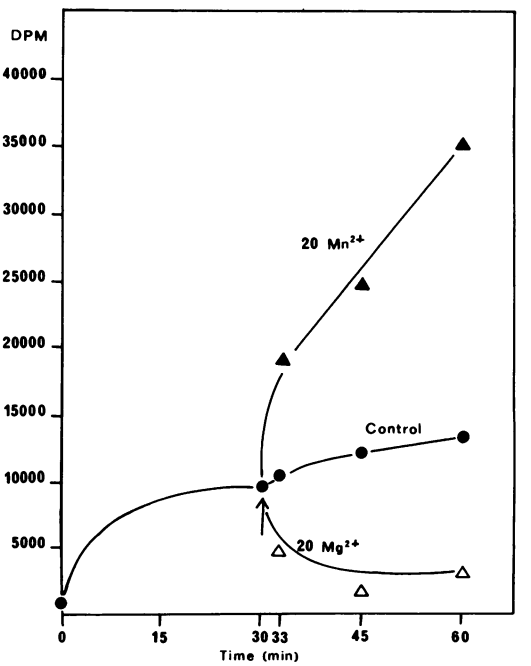

FIG. 2. *Uptake of tetracycline by nonproliferating Escherichia coli. The cells were suspended in 10 mM Tris·HCl (pH 7.5) with 10 μg of ^{3}H-tetracycline per ml. After 30 min, 20 mM Mn^{2+} or Mg^{2+} was added to portions of the bacteria. Radioactivity of the washed bacteria was determined by liquid scintillation (see Materials and Methods) and is indicated on the ordinate per milligram of bacterial protein.*

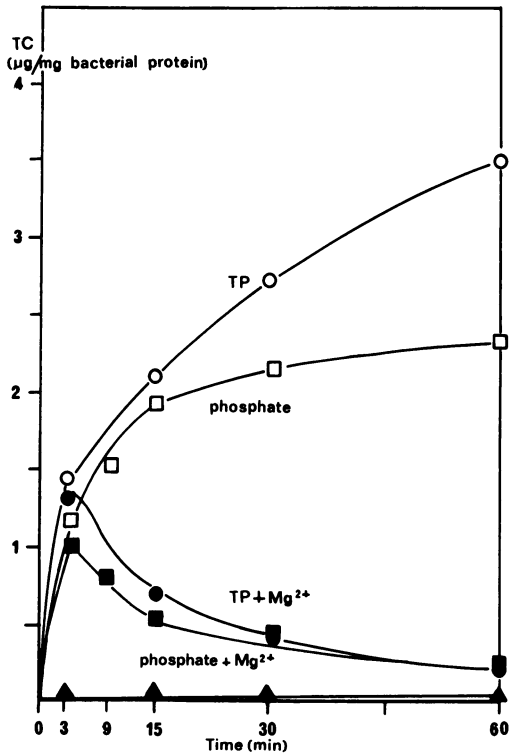

FIG. 3. *Influence of Mg^{2+} on uptake of tetracycline (Tc) by nonproliferating cells of Escherichia coli K-12 in the presence of phosphate. Suspending media were plain TP (○) or phosphate buffer (10 mM, pH 7.5) (□), and TP + 40 mM Mg^{2+} (●) or phosphate buffer + 40 mM Mg^{2+} (■). In ▲, TP + 40 mM Mg^{2+} + 10 μg of ^{3}H-Tc per ml was preincubated for 15 min at 37 C; Thereafter the bacteria were added from a mid-log culture, and samples were collected at the indicated times.*

12/1 and K-12/R was analogous to that observed with the susceptible strain K-12.

Effect of metallic ions on uptake of Tc by S. aureus. In principle, the influence of Mg and Mn ions on Tc uptake by nonproliferating suspensions of *S. aureus* 111S was quite similar to their effect on *E. coli*. In some of the experiments, Tc was supplied at a final concentration of 95 μg/ml, in others at 10 and 0.1 μg/ml, but in all cases 10 mM Mg^{2+} decreased uptake significantly. As was the case with *E. coli* (Fig. 3), the influence of 10 mM Mg^{2+} on accumulation of Tc by staphylococci was delayed several minutes when the bacteria were suspended in TP. Also, when Tc was preincubated with 10 mM $MgCl_2$, uptake was negligible. Furthermore, when Mg^{2+} or Mn^{2+} was added to staphylococci exposed to Tc for 30 min, i.e., cells which had accumulated Tc to equilibrium, 10 mM Mn^{2+} caused an additional 60 to 70% net increase in accumulated Tc, and 10 mM Mg^{2+} caused a threefold decrease in net Tc retained by the cells. These results were similar to those shown in Fig. 2 for *E. coli*.

All these experiments were repeated with the resistant inducible 111 and partly derepressed 111C strains (2, 27, 29). The results will not be discussed in detail since they were similar to those obtained with the susceptible 111S.

Resistance to Tc in media containing Mg^{2+} and Mn^{2+}. The decreased accumulation of Tc in the presence of Mg^{2+} allowed growth of *E. coli* K-12 (Fig. 5a) and *S. aureus* 111S (Fig. 5b) in otherwise inhibitory concentrations

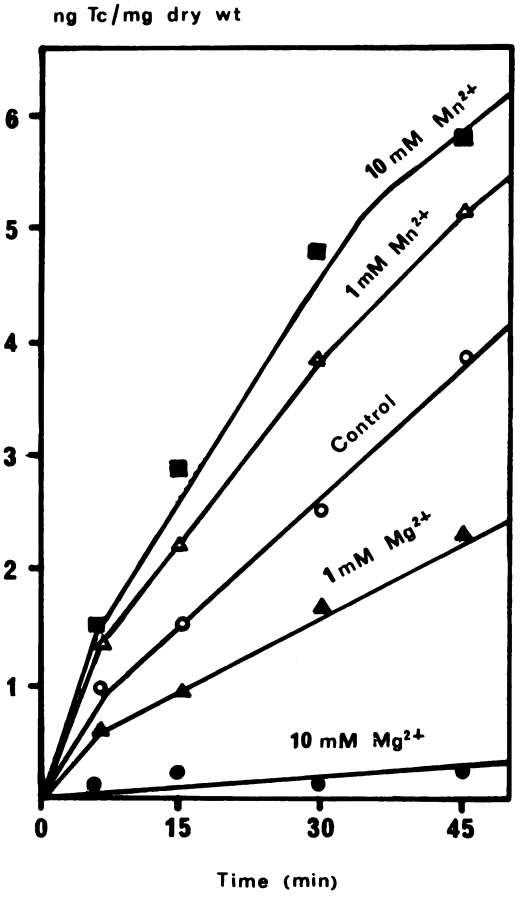

FIG. 4. *Uptake of tetracycline by a nonproliferating suspension of Escherichia coli. Tris·HCl (10 mM, pH 7.5) was incubated for 30 min at 37 C with 0.2 μg of ^{3}H-tetracycline per ml, without metal salts or with 1 mM $MnCl_2$, 10 mM $MnCl_2$, 1 mM $MgCl_2$, or 10 mM $MgCl_2$. Thereafter the solutions were transferred to 25 C, a washed suspension of bacteria was added, and portions were sampled for examination of radioactivity at the indicated periods.*

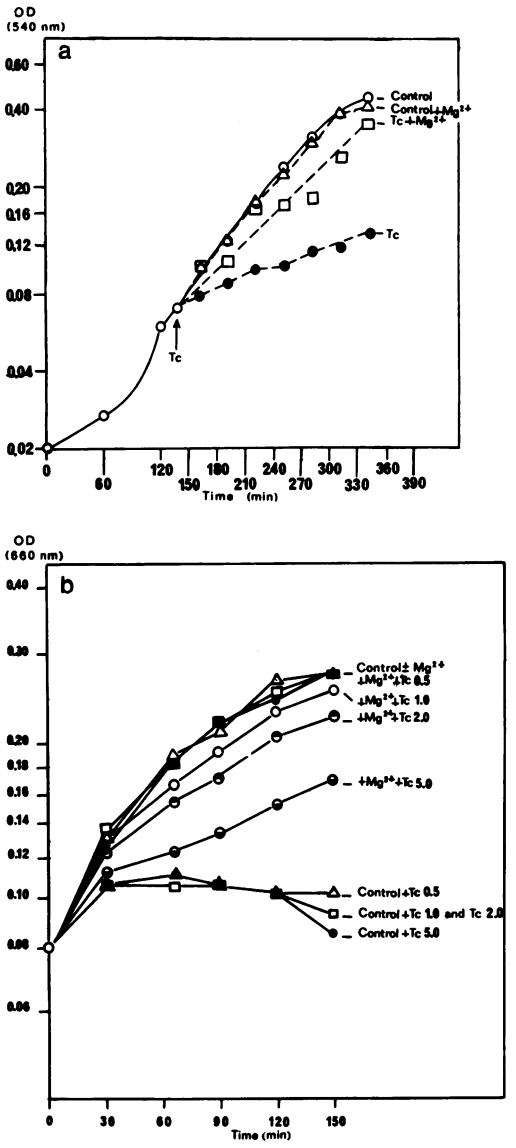

FIG. 5. *Inhibition of bacterial growth by tetracycline in presence of 10 mM $MgCl_2$. (a) Escherichia coli K-12 was grown in Davis and Mingioli's salt medium with or without 10 mM $MgCl_2$. Arrow indicates the time when 0.5 μg of tetracycline per ml was added to samples of the culture. (b) Staphylococcus aureus 111 S was grown in nutrient broth with or without 10 mM $MgCl_2$. To samples of the culture was added 0.5, 1.0, 2.0, and 5.0 μg of tetracycline per ml at time 0.*

of the drug; 5 μg of Tc per ml in the presence of Mg^{2+} was indeed much less bacteriostatic than 0.5 μg of Tc per ml when Mg^{2+} was not added (Fig. 5b).

On the other hand, Mn^{2+} did not increase susceptibility. We have as yet no plausible explanation for this.

Influence of Mg^{2+} and Mn^{2+} on the partition of Tc in olive oil-water and in chloroform-water. The influence of Mg^{2+} and Mn^{2+} on the rate of Tc uptake and the capacity for Tc in the bacteria could indicate that these metals alter the affinity of the drug molecules for a specific uptake system. Alternatively, Tc might be regarded as a lipid-soluble compound whose uptake by the cells occurs in response to a membrane potential. Mg^{2+} might combine with Tc under building of a complex that is less, and Mn^{2+} one that is more, lipid-soluble than "free" Tc. These ions might thus shift the equilibrium between the cell interior and exterior in opposite directions. A correlation of Tc resistance with the quantity and composition of bacterial lipid bas been reported (9, 23). We have therefore studied the influence of these ions on the distribution of Tc between water and nonpolar liquids. As shown in Table 1, the apparent partition coefficient of Tc in olive oil-aqueous buffer was 0.064 and in chloroform-aqueous buffer 0.109. Both Mg^{2+} and Mn^{2+} increased this coefficient moderately. The results seem therefore to contradict the assumption that the opposite influence of Mg^{2+} and Mn^{2+} on bacterial uptake of Tc is due simply to their effect on lipid solubility of the drug.

Influence of Mg^{2+} and Mn^{2+} on the absorption spectrum, circular dichroism (CD), and optical rotatory dispersion (ORD) of Tc. Under the conditions used in most of the accu-

TABLE 1. *Apparent partition coefficients (nonpolar liquids-aqueous buffer) of ^{3}H-tetracycline[a] in the presence and absence of Mg^{2+} or Mn^{2+}*

System	Apparent partition coefficient[b]
Olive oil-aqueous Tris·HCl	0.064 ± 0.0018
Olive oil-aqueous Tris·HCl with 10 mM $MgCl_2$	0.070 ± 0.0011
Olive oil-aqueous Tris·HCl with 10 mM $MnCl_2$	0.081 ± 0.0011
Chloroform-aqueous Tris·HCl	0.109 ± 0.0024
Chloroform-aqueous Tris·HCl with 10 mM $MgCl_2$	0.128 ± 0.0028
Chloroform-aqueous Tris·HCl with 10 mM $MnCl_2$	0.128 ± 0.0007

[a] To the aqueous phase was added 10 μg of ^{3}H-tetracycline per ml with about 4×10^4 dpm/ml.

[b] Mean plus or minus standard error.

mulation experiments, with about 500× molar excess of Mg^{2+} and Mn^{2+}, spectral shifts were seen in the CD of Tc (Fig. 6a). This was particularly pronounced in the region of 230 to 260 nm. The influence of these two ions was in general similar, although not identical. ORD (Fig. 6b) was similarly influenced. The effect of these cations on the absorption spectrum of Tc was also considerable (Fig. 7). The detailed interpretation of the spectral shifts requires consideration of which of the chromophores of the four-ringed Tc molecule (Fig. 8) contribute to the different parts of the spectra (19, 21, 22). It should be pointed out that the observed effect of chelation on CD in the 250 to 260 nm region was opposite to that reported by Mitscher et al. (21, 22) for Mg^{2+}. This may be due to the much higher concentrations of cations and lower concentration of Tc used by us, since it was our aim to examine the influence of the ions under conditions in which a clear-cut effect on bacterial Tc uptake was observed.

DISCUSSION

The antibacterial function of Tc depends on its binding to the bacterial ribosomes (6) and

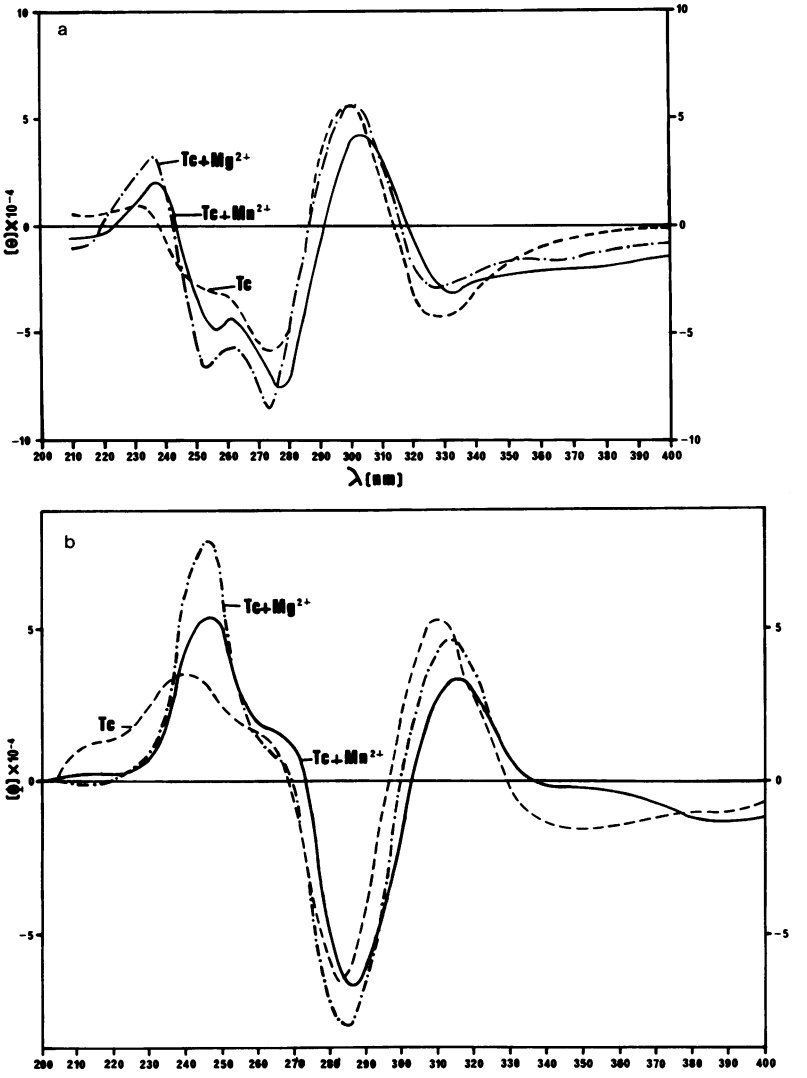

FIG. 6. *(a) Circular dichroism of tetracycline (10 μg/ml) in plain Tris·HCl (10 mM, pH 7.5) and Tris·HCl + 10 mM $MgCl_2$ or $MnCl_2$. (b) Optical rotatory dispersion of tetracycline in plain Tris·HCl (10 mM, pH 7.5) and Tris·HCl + 10 mM $MgCl_2$ or $MnCl_2$.*

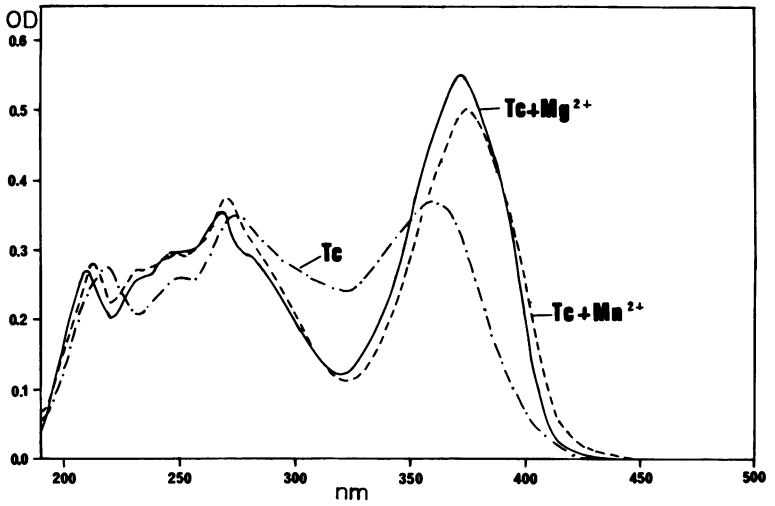

FIG. 7. *Absorption spectrum of tetracycline (10 μg/ml) in plain Tris buffer (10 mM, pH 7.5) and Tris buffer + 10 mM $MgCl_2$ or $MnCl_2$. The "blank" cuvette contained the same buffer and metal salt as the sample.*

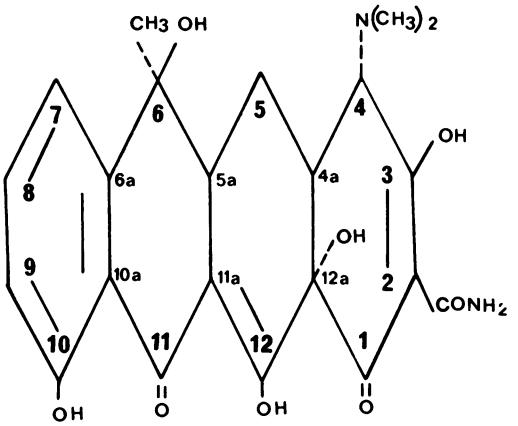

FIG. 8. *Tetracycline.*

the degree of its intracellular concentration. This latter factor depends probably on the degree to which the drug is bound to the bacterial cell, a factor that can be drastically altered when Mg^{2+} or Mn^{2+} is added to the bacteria. Though the existence of a specific bacterial carrier for Tc has still to be proved, this possibility seems to be the most plausible explanation of the concentrative, energy-dependent intracellular accumulation of the drug in a nonmetabolized form, as well as of the striking influence of slight molecular modifications on uptake (29). Intestinal absorption and renal clearance of Tc might well depend on their lipid solubility (5), but there seems not to be any correspondence between this property and susceptibility of sensitive staphylococci (Sompolinsky and Kraus, *manuscript in preparation*). At pH 7.5 the drug is partly anionic, and it is therefore not surprising that Mn^{2+} and Mg^{2+} increased lipid solubility (Table 1), but this effect of Mg^{2+} is opposite to that expected if the decreased uptake was due to altered lipid solubility. The decreased susceptibility to Tc that is obtained when Mg^{2+} is added to nutrient broth indicates that this ion has the same effect on the cells of a growing culture as on nonproliferating cell suspensions.

Izaki and Arima (15) reported increased accumulation of oxytetracycline by *E. coli* when glucose and $MgSO_4$ or $MnSO_4$ were added, but only when high concentrations of the drug were used (100 to 400 μg/ml), and the metallic ions were added to a concentration of 0.4 mM. Franklin and Higginson (12) found a definite decrease in Tc binding by *E. coli* incubated in Tris buffer with 0.06 μg of Tc per ml and 10 mM $MgCl_2$.

Resistance to Tc in *S. aureus* has been found to correlate with the synthesis of a specific resistance protein (2) that probably causes a decreased intracellular concentration of the drug. A priori, it was possible that metallic ions would influence the affinity to this resistance mechanism in such a way that their effect on Tc accumulation would be different in resistant and susceptible cultures, but this was not the case. However, it is possible that the addition of Mg^{2+} and Mn^{2+} in our experiments did not raise the intracellular concentration of these ions, and therefore did not affect transport of Tc out of the cells.

Our results indicate, furthermore, that the accumulated Tc has a considerable turnover

rate. This is not demonstrable by adding large amounts of nonlabeled Tc to bacteria incubated with ^{3}H-Tc or vice versa, in analogy to convenient technique in many uptake systems, since bacterial uptake of Tc seems not to be saturated even at very high concentrations of the drug (12, 14). The rate of exchange of Tc can easily be calculated from experiments as those of Fig. 3, and in *E. coli* incubated with 10 μg/ml it reaches values of more than 0.04 μg of the drug per mg of dry weight per min, or about 10^7 molecules per bacterium per min, if the dry weight of one bacterium is set at 2×10^{-7} mg.

The delayed influence of Mg^{2+} on Tc uptake which was observed in TP may be due to the high phosphate content—about 10 mM equivalents of inorganic P and 3 of organic P, determined as described by Fiske and SubbaRow (10). Phosphates have been added to therapeutic Tc preparations in order to avoid chelation with metallic ions in the intestine. In our system the effect of Mg^{2+} was only delayed a few minutes by phosphate buffer.

A practical aspect of our results might be the relation to Tc therapy of urinary tract infections—the concentration of Mg^{2+} in urine might be high enough to void the efficacy of the drug even in the case when the infecting microorganism is found susceptible by the usual laboratory criteria. This problem shall be dealt with elsewhere.

One of the most interesting objectives of this study was to investigate the possible relation between molecular conformation in solution and specific uptake of Tc. The relation between uptake and molecular conformation has great interest because uptake is the first step in the interaction between the drug and the microorganism and a condition for susceptibility. It may also be of some interest to study the influence of Mg^{2+} on Tc inhibition of an in vitro ribosomal protein-synthesizing system, but this might give results difficult to interpret, since such a system is in itself highly sensitive to variations in Mg^{2+} concentration. The resistant microorganism is distinguished by at least two proteins with affinity for Tc, and their functional efficacy might vary with the concentration of metallic ions: (i) the repressor of Tc resistance (2, 27); and (ii) the resistance protein (2). In spite of this, our results showed that Mg^{2+} and Mn^{2+} exhibited, in principle, identical influences on uptake of Tc in susceptible and resistant organisms. By the use of spectroscopic methods we hoped to obtain some information concerning the influence of the metallic ions on the conformation of Tc in solution. The Tc molecule contains two chromophoric regions, one consisting of the carbonyl, or potential carbonyl, groups C_1–C_3 of the A ring, the other of C_{10}–C_{12} of the B, C, and D rings (Fig. 8). In the absorption spectrum of Tc in 0.1 N HCl, peaks are encountered at approximately 220, 275, 320, and 360 nm. The 320-nm band is too weak to be seen with the concentration of Tc used by us, and the other bands are somewhat translocated at higher *p*H (Fig. 7). Whereas the BCD chromophore contributes to all these absorption bands, the A ring influences only the ultraviolet peak at 275 nm (19). The 275-nm band is thus of a composite nature, and in the CD spectrum it is dissociated into a negative peak at approximately 262 nm and a positive one at 292 nm, the former representing the contribution of the highly enolic A ring. Mitscher et al. (20–22) found that the addition of Mg^{2+} causes a substantial diminution of absorption in the 260 nm region, when the examination was performed in buffered alkaline solution, and they assumed this to be due to an alteration of the chirality of the molecule, particularly through influence on the A ring. Figure 6a shows a quite opposite effect of the addition of Mg^{2+}. The discrepancy might possibly be due to the much higher concentration of Mg^{2+} used by us and probably chelation at different sites of the Tc molecules. It is therefore not easy to interpret the results in exact terms of a relation between molecular structure of Tc and binding to the bacteria. It might seem disappointing that the influences of Mg^{2+} and Mn^{2+} on the absorption spectrum, CD, and ORD of Tc, though distinct, are far from being so different as might possibly have been expected from the biological data. It should, however, be recalled that if Tc uptake depends on the interaction with a protein in the same way that a substrate interacts with an enzyme, then rather delicate variations in the nature of complex formation or degree of molecular twisting may be of opposite consequences. Further studies should attempt to reveal more information of this aspect—which would facilitate the prediction of activity in relation to molecular structure of different tetracyclines.

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